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Jatropha, Challenges for a New Energy Crop

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Jatropha, Challenges for a New Energy Crop

Volume 2: Genetic Improvement and Biotechnology

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Preface

Jatropha, Challenges for a New Energy Crop – Volume 2 aims to report on the state of the art of scientific investigations that were made during the past 10 years on the new crop *Jatropha curcas*. The progresses obtained on the knowledge of this abstemious, semi-wild species are already impressive and were mainly achieved in just a decade (2001–2011). This knowledge extends from basic Jatropha physiology and biological reproduction to the basic agronomic practices and systems for its productive management, but also the complete set of biotechnological tools, such as in vitro culture, genetic transformation, genome sequencing, genetic maps, and marker-assisted selection that are necessary for its selective breeding. These scientific and technological achievements paved the way for the future technological management and domestication of Jatropha as an industrial oilseed crop able to contribute to the feeding of the transport system.

In view of the importance that Jatropha demonstrated worldwide by its large-scale cultivation and emerging value for energy business as a biofuel, we felt the necessity of this first comprehensive compilation by global experts. The access to objective information may be difficult to people not directly involved with Jatropha because it is scattered among science media eventually written in different languages. Thus, we gathered the information scattered worldwide in a sort of summary or general agreement of what is known on Jatropha at the moment. This form of a compilation was also necessary because the knowledge on Jatropha is shared over the tropical belt also called *Jatropha Belt* by different teams, in different politico-economic realities and with different technological and scientific backgrounds. A compilation was the best way to faithfully transmit the point of view of these experts with as few biases as possible. We believe and hope that this compilation will be a valuable source of inspiration for next-generation scientists investigating this new crop, for technologists invested in improving its profitability as well as for decision makers and policy implementers, and politicians, economists, environmentalists or social management who are thinking and acting for the development of a world based on sustainability.

In Volume 1, we outlined the whole *Productive Chain* of Jatropha including the worldwide economic importance of Jatropha as well as its physiology, farming, oil

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processing, by-products, biodiesel, and biofuel combustion in order to provide a general picture of the Jatropha's potential to the readers. Volume 2 is presented in 4 units comprising 31 chapters covering the main aspects of its biology and reproduction, genetic diversity and domestication, germplasm, and biotechnology. It aims to give a kind of comprehensive picture on Jatropha as a *Biological System* with the purpose to understand what can be improved in Jatropha and how such improvement can be achieved.

We wish to express our gratitude to all the contributors from all over the world for readily accepting our invitations for not only sharing their knowledge, but for admirably integrating their expertise on scattered information from diverse fields in composing the chapters and enduring editorial suggestions to finally produce this venture that we hope will be a success. We greatly appreciate their commitment.

We also acknowledge the support received from many colleagues in the preparation of the manuscripts as well as thank our spouses and relatives for bearing with us, our commitment to the book.

We thank Hannah Smith, Associate Editor, Springer Science, USA, and her staff for their unstinted cooperation at every stage of the book production.

Finally, we apologize for any mistakes, omissions or failures that may subsist in this work.

Warangal, India — Bir Bahadur
Hyderabad, India — Mulpuri Sujatha
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About the Editors

Bir Bahadur
Graduated from Nizam College and holds a postgraduate degree from University college, Osmania University, Hyderabad, India. He obtained his Ph.D. in plant genetics from Osmania University and was closely associated with Prof. J.B.S. Haldane, FRS, the renowned geneticist of the last century. He advised, guided and encouraged Bahadur to study heterostyly and incompatibility in Indian plant species, a subject first studied by Charles Darwin in England about 160 years back. He made significant contributions in several areas of plant biology especially in incompatibility, mutagenesis, morphogenesis, tissue culture, and organism asymmetry in relation to yield and application of SEM in plant sciences. He published over 250 research papers, which are well received and quoted in national and international journals, including a number of theses and several publications on Jatropha and Castor. He served Osmania and Kakatiya Universities as lecturer, reader and professor; he also served as chairman, head of department, dean of the Faculty of Science, Kakatiya University, Warangal. He has taught genetics, biotechnology and reproduction of plants for over 40 years and accumulated research experience in these areas for about 50 years. He was a post-doctoral Fellow at the Institute of Genetics of Hungarian Academy (Budapest); recipient of the Royal Society Bursary, London; and Honorary Research Fellow at the Birmingham University (UK). He has been invited speaker of over 100 conferences including Max Planck Institute, Koln (Germany); Institute of Genetics (Budapest); Birmingham (UK); University Texas, Houston (USA); Missouri University, St Louis (USA), Sabrao Conference, Szukoba, Tokyo (Japan); Indian Science Congress; etc. He has authored/edited eight books and was editor-in-chief of both *Proceedings of Andhra Pradesh Akademi of Sciences* (Hyderabad, India) and *Journal of Palynology* (Lucknow, India). He is on the editorial boards of several journals in India. He is recipient of Best Teacher Award by Andhra Pradesh state Government and Prof. Vishwamber Puri, Gold Medal of Indian Botanical Society for his original contributions in various aspects of plant sciences. He is fellow of over a dozen professional bodies in India and abroad including the Fellow of Linnean Society, London; Fellow of Institute of Biology, and Chartered Biologist, London; and member of New York Academy of Sciences.

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He has been recently awarded the Bharath Jyoti Award for his sustained academic and research career at New Delhi. Presently he is on the Board of Directors of Sribiotech, Hyderabad, India, Emeritus Professor, Genetics Department, Shadan Post Graduate Centre, Osmania University, Hyderabad.

Mulpuri Sujatha
Graduated in plant sciences from the University of Hyderabad (UoH), India. She has a Ph.D. in genetics from Osmania University (OU), Hyderabad, and worked on intergeneric and interspecific affinities between Ricinus and Jatropha. She made significant contributions for the genetic improvement of oilseed crops through genetics, tissue culture and biotechnological tools. Her important achievements include development of male sterility systems in safflower, sunflower and niger and reliable and efficient tissue culture and transformation protocols for sunflower, castor, niger, safflower and Jatropha. The genetic transformation protocols developed are being used for the development of insect-resistant transgenics through the deployment of suitable cry genes in castor, development of transgenic male sterility and fertility restoration system in safflower and development of transgenics for resistance to necrosis disease in sunflower. Her experience in molecular markers resulted in mapping of downy mildew resistance gene (*Pl13*) in sunflower besides development of appropriate molecular markers for distinguishing the toxic and non-toxic accessions of *Jatropha curcas*.

Nicolas Carels
Graduated in agronomy in Belgium and did a Ph.D. in plant pathology at *Faculté des Sciences Agronomique de Gembloux* (FSAGx, Gembloux) prior to working as a scientist on the elaboration of the first genetic map of sugar beet at the end of the 1980s (ICIseed-SES, Belgium). He then moved to Paris at *Institut Jacques Monod* (IJM, CNRS, France) where he did a Ph.D. on the genome organization in plants. He continued his work on genomics in Italy at *Stazione Zoologica 'Anton Dohrn'* (SZN, Naples) and Spain at the *Centro de Astrobiología* of *Instituto Nacional de Técnica Aeroespacial* (INTA-CAB, Madrid, Torrejon de Ardoz) prior to moving to Brazil at *Universidade Estadual de Santa Cruz* (UESC, Ilhéus, Bahia) where he contributed to the application of bioinformatics and genomics to the improvement of the resistance of cacao and rubber tree to fungal diseases. He took Jatropha at its beginning when it was declared a strategic crop for the Brazilian economy by President Lula. His investigations covered the measure of the genome size by flow cytometry and the application of reverse genetics to detect QTLs for oil production with the purpose of breeding Jatropha for this trait. He also published an extensive review (Advanced Botanical Review - ABR) on Jatropha and more recently an overview on bioenergies (InTech) with special concern for climate change mitigation and biodiversity preservation. He is now a Federal Officer of *Fundação Oswaldo Cruz* (Fiocruz, Rio de Janeiro, Brazil) and is interested by the exploration of genomics, bioinformatics, and natural products for the human health benefit.

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Contents

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Part II Genetic Diversity of Jatropha and Domestication

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Part I
Biology and Reproduction

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Chapter 1
Laticifers of *Jatropha*

K.V. Krishnamurthy, Padma Venkatasubramanian, and S. Lalitha

Introduction

Laticifers are cells or series of connected cells containing a fluid called latex in suspension emulsion state. Laticifers often form a system that permeates various tissues of plants body. Latex containing plants include some 12,500 species under 900 genera and 22 families; excepting *Gnetum*, which is a gymnosperm and *Regnellidium*, which is a water fern while all the rest are angiosperms (Metcalfe 1967, 1983; Evert 2006). Among the angiosperms, Euphorbiaceae forms very important latex bearing family, containing ca. 6,300 species, under 245 genera and 37 tribes (Govaerts et al. 2000; Radcliffe-Smith 2001). *Jatropha* is an important genus of Euphorbiaceae in view of its great biodiesel potential. This paper deals with the laticifers and latex of *Jatropha*.

Types of Laticifers

Laticifers are generally classified into three broad categories: **Non-articulated**, **articulated** and **idioblastic.** The non-articulated laticifers are single cells that through continued growth develop into tube like structures. They may undergo branching to various degrees (equal to non-articulated branched type) or remain unbranched (non-articulated unbranched type). The articulated laticifers are made up of more than one cell that are placed one over the other. The number of cells forming single laticifers may vary from one laticifer to another. All the constituent cells are invariably more elongated than adjacent parenchyma cells although it is not

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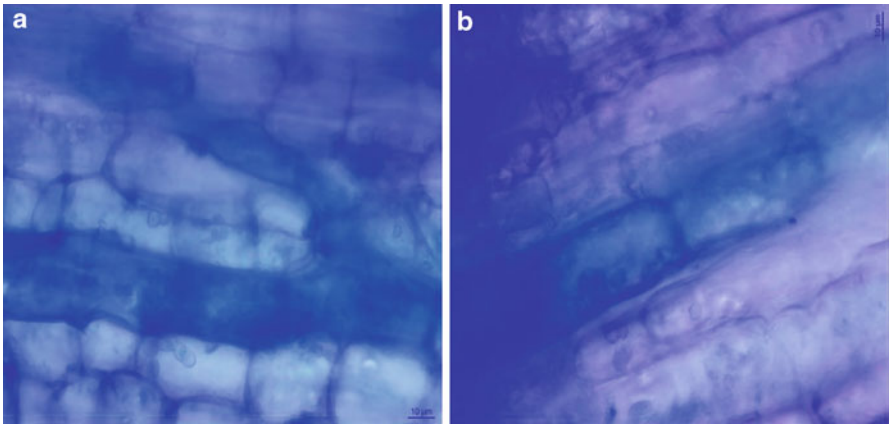

Fig. 1.1 (**a** and **b**) Non-articulated branched and articulated unbranched laticifers respectively of *J. gossypiifolia* and *J. tanjorensis* stained with Toluidine Blue O

a rule. The cross wall between successive cells may remain intact; possess one or more pores of various sizes (perforated) or in extreme instances the cross walls may be totally dissolved to form a long duct. The articulated laticifers may remain unbranched or may get anastamosed to various extents through lateral union with similar laticifers. The third category of laticifers are usually isodiametric cells of parenchyma type filled with latex. Such cells are dispersed in the other tissues of plant organs as idioblasts. Otherwise, these idioblastic laticifers, also called as **simple laticifers,** share most other features of typical laticifers. There is often a series of integrating laticifer cell type from simple ones to very complex categories of laticiferous tubes.

The genus *Jatropha* is reported to possess all the three categories of laticifers (Dehgan and Craig 1978) although Pax (1884), Scott (1886) and Rao and Malaviya (1964) reported only non-articulated laticifers in the species of *Jatropha* that they have studied. The length to which non-articulated laticifers may grow varies from species to species and the growth may be straight or bent to various extents. The following species of *Jatropha* possess exclusively non-articulated laticifers: *J. augustii* and *J. fremontioides* (Dehgan and Craig 1978). By contrast, the following species of *Jatropha* exclusively have the articulated laticifers: *J. multifida*, *J. cathartica*, *J. capensis, J. lobata*, *J. integerrima*, *J. hernandifolia*, *J. curcas*, *J. malacophylla*, *J. platyphylla*, *J. moranii*, *J. ciliata* (Dehgan and Craig 1978). We have found that in *J. tanjorensis* also the laticifers belong to the articulated type with cross walls intact between cells (Fig. 1.1a, b). Deghan and Craig (1978) also reported that the articulated laticifers vary in length depending on the species with short or long cells arranged end to end, but all with distinct cross walls. Probably in all species of *Jatropha* that have articulated laticifers the cross walls remain intact. Many species of *Jatropha* have both articulated and non-articulated laticifers (Dehgan and Craig 1978) *viz., J. gossypiifolia*, *J. excisa* var. *pubescens*, *J. paradoxa*, *J. marginata*, *J. fissipina*, *J. ferox*, *J. podagrica, J. trieronymii*, *J. unicostata*, *J. gallabatensis*,

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J. lagarinthoides, *J. macvaughii, J. cordata*, *J. verrucosa*, *J. standleyi*, *J. canescens*, *J.cinerea*, *J. giffordiana*, *J. neopauciflora, J. cardiophylla*, *J. cuneata*, *J. dioica* and probably *J. velntina* (where non-articulated type is to be verified). Kakkar and Paliwal (1972) earlier reported articulated and non-articulated types in *J. gossypiifolia,* a fact confirmed by Dehgan and Craig (1978).

Idioblastic laticifers are also present in some species of *Jatropha* (Deghan and Craig 1978). According to these authors, this type of laticifer occurs only in the leaves, but not in petioles, stems and roots. They are of variable shapes and occur in mesophyll of the leaves often towards the leaf margins. According to these authors since the idioblastic laticifers integrate with certain other idioblasts that contain tannins, mucilage, proteinaceous material and other compounds they cannot be delimited precisely. In fact they refrain from calling them laticifers and refer to them as idioblasts, although they are convinced on evidences that these are laticiferous in nature.

Origin and Distribution of Laticifers

Laticifers in *Jatropha* occur both in primary and secondary bodies of the plant. It is generally believed that articulated laticifers originate both in primary and secondary tissues, whereas the non-articulated nearly and exclusively originate in primary tissues (Rudall 1987, 1994; Wurdack et al. 2005). Popham (1947) observed laticifers in the embryonic stems and root of *J. cordata* and these differentiate successively in cortical parenchyma, xylem and phloem. Further, a few larger, thick walled mostly empty cells that become filled with latex during the first 3–4 days after germination differentiate adjacent to phloem in the hypocotyl. They appear in the stem soon after differentiation of cortical parenchyma. In the root, laticiferous cells differentiate into xylem, phloem and tissues central of cork cambium during 2nd or 3rd week of germination. In the hypocotyls, laticifers differentiate in cortical parenchyma and in phloem during the 5th or 6th day of germination while in xylem between the 1st and 3rd weeks. In the stem, they are mostly formed in the cortical parenchyma. Cass (1985) made a detailed study of the origin and distribution of laticifers in *J. dioica*. The non-articulated laticifers of these species become recognizable when the embryo is approximately 0.3 mm long, following the initiation of cotyledons. A transection (TS) near the cotyledon node showed a ring of 5–7 laticiferous initials with large nuclei. These cells are up to 24 μm long and are observed outside procambium. These cells extend bidirectionally along with procambium both in hypocotyl and into cotyledons. Soon the arrangement of laticifers in the hypocotyl becomes complicated by the random branching of laticiferous initials and a large ring of up to 70 laticifer branches are observed by the time the embryo matures. Finally, laticifers get themselves arranged in two rings through an inward branching of the original ring. The two rings are separated by the developing phloem. The branches of the outer ring sub-divide and extend into the cortex and no laticifers are observed in the pith. The distribution of laticifers in cotyledonary

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and embryonic leaves differs somewhat from that in the hypocotyl. Each cotyledonary vascular trace is in close association with abaxial and adaxial laticifer extensions. Similarly, branching of cotyledonary laticifers parallels that of cotyledonary vascular bundles. A similar pattern of development of laticifers is seen in the embryonic leaf. In the mature embryo of this species, two concentric rings of laticifers extend through most of the length of hypocotyl (2.5 mm) blindly ending near the bases of four branch root primordials. Laticifer extends from the outer ring near the nodal region and enters into cotyledons and embryonic leaf. Centripedal extensions of the outer ring form a complex system of laticifers surrounding the apical meristem of the embryo. The tips of laticiferous initials remain adjacent to the apical meristematic tissue and become incorporated in the new tissues as the epicotyl elongates. During germination of embryo, mitotic divisions commence in the large nucleus of the initials to produce multinucleate and cynocytic laticifers. Thus, in this species the origin and differentiation of laticifers both temporally and spatially are similar to that observed in *Nerium oleander* (Mahlberg 1961). While Mahlberg (1961) located the origin of laticifer initial in procambium, Cass (1985) believed that it is issued from phloem parenchyma. The latter author also found a close distributional relationship between laticifers and phloem in *J. dioica*.

Deghan and Craig (1978) have noted that the branches of non-articulated laticifers are frequently in continuation of the tubes associated with vascular tissue, but permeate in intercellular spaces forming a network in the mesophyll. These workers found the articulated laticifers also most often on the periphery of the vascular tissue, but also occasionally within them. Non-articulated laticifers according to these authors originate in the protophloem and are in general more abundant in the phloem region in common with articulated laticifers.

We have made a detailed study on the pattern of distribution of laticifers in stems, petioles and leaves of three species of *J. tanjorensis, J. multifida* and *J. gossypiifolia* (Figs. 1.2, 1.3, and 1.4). We noted that articulated type predominates in the first two species and non-articulated in the third species. In all the three species, laticifers are not found in the pith of the stem and in the parenchyma inside the petiole vascularization. A careful study indicates that the first few laticifers are initiated in the peripheral cells of phloem, but perhaps through their chemical influence a larger number of laticifers differentiated in the cortical parenchyma of stem and peripheral parenchyma outside the vascularization of petioles and midribs of leaves.

It appears to be true that idioblastic laticifers are scarce/absent in stems and petioles and are restricted to lamella as Deghan and Craig (1978) have shown for some species of *Jatropha*. They may differentiate from the cells of the palisade mesophyll or spongy mesophyll depending on the species. In a few species they are specifically restricted to the mesophyll of leaf margins.

Once initiated, laticiferous initials may grow by apical intrusive growth, by symplastic growth or by both. In most if not all the cases, non-articulated laticifers growth occurs by apical intrusion. If the organ growth is unidirectional, then the apical intrusive growth of laticifers is often seen only in the direction of the organ growth. If the organ growth is bidirectional, then the apical intrusive growth of

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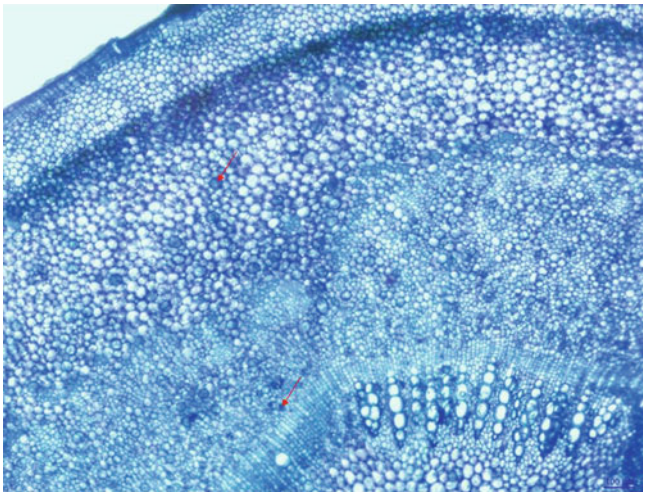

Fig. 1.2 TS of portion of the stem of *J. tanjorensis* stained with Toluidine Blue O showing laticifers (*red arrows*) in the phloem and cortical regions

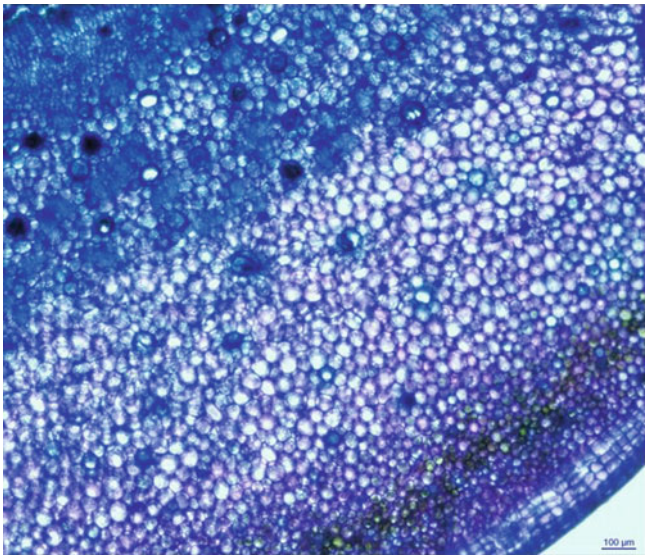

Fig. 1.3 TS of portion of stem of *J. multifida* stained with Toluidine Blue O showing laticifers (*darkly* stained cells) distributed in the phloem and cortical regions

laticifers can happen in both the tips. However, in embryonic organs, non-articulated laticifers associated with vascular strands often have an initial phase of symplastic growth followed by a pronounced apical intrusive growth. In most of the articulated laticifers growth is invariably symplastic. In the case of idioblastic laticifers, either there is no growth or it takes places all around the cell either

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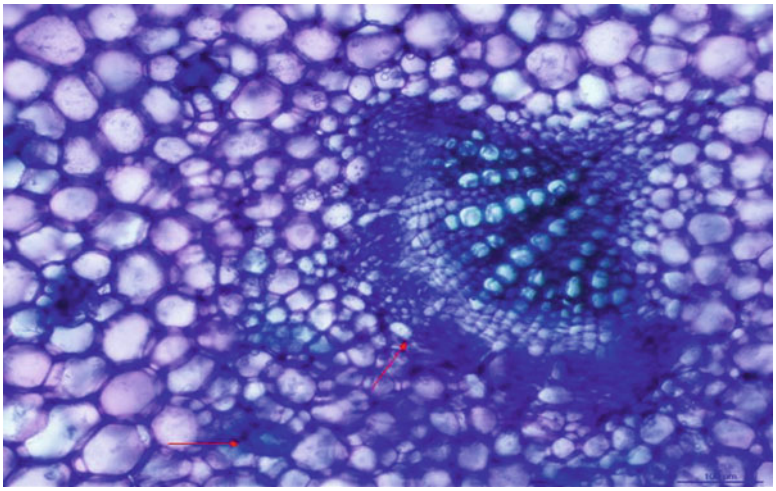

Fig. 1.4 TS of portion of petiole of *J. tanjorensis* showing laticifers (*red arrows*) in peripheral parenchyma and phloem regions

uniformly or at specific loci so that the mature idioblast may have an even or uneven outline**.**

Structure of Laticifers

The laticifers are generally tubular both in articulated and non-articulated categories, while it is variously shaped in case of idioblasts. Liu et al. (2006) have studied the distribution and size of laticifers in various plant parts of *J. curcas.* However, in most other respects the structure of laticifers is similar in all three categories. Milky latex is absent in species of *Jatropha* and in most species latex is not coloured and more or less sticky. In that respect, latex of this genus differs from many other genera where it is milky. The contents of non-articulated laticifers of *Jatropha* were described as generally quite granular in nature and saffranin positive (Dehgan and Craig 1978). Our studies show that the latex contains starch grains, lipids, mucilage, and predominantly phenolics. Work from other laboratories have indicated the presence of the two novel lathyrane in *J. curcas* (Naengehomnong et al. 1970), curcain, a protease (enzyme) from the latex of *J. curcas* (Nath and Dutta 1991) and curcaycline A, a novel cyclic octapeptide from the latex of *J. curcas* (van den Berg et al. 1995).

The wall of the laticifers is very thick and often made up of concentric layers of cellulose. The cellulose wall layers also contain phenolic acid, perhaps ferulic acids as judged from TBO staining. In some of the articulated laticifers, the lateral walls are frequently ridged irregularly (Dehgan and Craig 1978), a fact that we have also observed in the laticifers of *J. multifida.*

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Taxonomic Considerations and Conclusions

It is already well known that at the higher taxon level laticifers are almost restricted to angiosperms (except in *Gnetum* and *Regnellidium*). It is also well known that only about 22 families of angiosperms have laticifers. Within Euphorbiaceae *sensu lato* to which *Jatropha* belongs, laticifers are restricted with one or two exceptions to subfamilies Crotonoidae and Euphorbioideae. While Acalyphoideae and Phyllanthoideae lack laticifers, at the exception of being *Dalechampia* (Hayden and Hayden 2000), *Macaranga*, *Kirganelia reticulata* (Balaji et al. 1996), *Dicoelia* sp. (Hayden and Hayden 2000) and *Omphalea* sp. (Wurdack et al. 2005). Thus the distribution of laticifers is a useful character that can be used in the broad classification of Euphorbiaceae.

It is generally believed that the type of laticifer is not constant to the given family and the types have evolved independently of one another. Metcalfe (1967) has shown that in Euphorbiaceae, many species have non-articulated type of laticifers, but only species like *Hevea* and *Manihot* have articulated type. However, Dehgan and Craig's (1978) work has clearly shown the occurrence of both types in the genus *Jatropha* and that many species have both types as already indicated. In the 37 species investigated by Dehgan and Craig (1978) articulated laticifers occur in two clades again emphasizing their independent origin even within a genus like *Jatropha*. But neither occurrence is in a supported position within non-articulated clade (Wurdack et al. 2005).

The work of Dehgan and Craig (1978), excluding few exceptions indicate that laticifers of leaves broadly limit taxonomic boundaries. Both articulated and non-articulated laticifers occur in the same leaf in section *Tuberosae*, Sub-section *Tuberosae* as well as in Section *Collenucia*, *Spinosae* and *Jatropha*. Sections *Curcas* and *Mozinna* in the subgenus also show both types of laticifers. In addition, section *Loureria* (except *J. fremonitoides*) and section *Mozinna* show the idioblasts. *J. dioica* shows both articulated and non-articulated as also irregularly shaped idioblasts near leaf margin. Section Polymorphae (possibly except *J. macrorhiza*), *Tuberosae* (sub-section *Capenses*) in subgenus *Jatropha*, and section *Platyphyllae* in subgenus *Curcas* lack non-articulated laticifers and idioblasts that show the articulated type. Of the five species examined in section *Peltatae*, only *J. podagrica* and *J. hieronymii* show both the articulated and non-articulated types. *J. multifida* and *J. cathartica* lack the non-articulated laticifers and idioblastic laticifers, but not the articulated ones. The most striking feature of Dehgan and Craig (1978) study perhaps is the occurrence of idioblasts in the most primitive sections of the subgenus *Curcas*, namely *Loureria* and *Mozinna.* The origin of these idioblasts is probably independent of those found in *J. augustii* because in the latter, they are derived from the mesophyll cells whereas those of *J. cardiophylla* and others in the subgenus *Curcas* restricted to the periphery of the vascular tissue. According to these authors the presence idioblastic (laticifers) cells is assumed to be an apomorphe character.

According to Rudall (1994) many genera of Euphorbiaceae without laticifers have elongated, highly branched sclerides in the mesophyll, which may in some

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instances be homologous with laticifers. However, the putative homology between laticifers and branched, foliar sclereides offers a very confused picture given the differences between these structures. In the absence of credible evidences, the suggestion of Rudall should be accepted only with reserve.

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Chapter 2
Wood Anatomy of Indian Jatrophas

Bir Bahadur, K.V. Krishnamurthy, and E. Chamundeswari

Introduction

Euphorbiaceae includes a large number of woody taxa, shrubs, trees, a few lianas and some plants with an unusual habit like the Candelabra, Euphorbias and the Ericoid *Cluytia*. The wood structure of these taxa shows great variation making it difficult to present a general diagnosis of the euphorbiaceous wood. Pax (1884) was the first to study wood anatomy in Euphorbiaceae and asserted that the wood is not of any systematic value. Earlier literature on wood anatomy has been reviewed by Solereder (1899) and by Metcalfe and Chalk (1950). Solereder (1899) recorded the presence of thick-walled wood fibers in the Euphorbiaceae and noted that they sometimes possess gelatinous wall, but such a character was not observed by Bamber (1974) who investigated 44 taxa of Crotonoideae and 33 taxa of Phyllanthoideae. However, Bamber (1974) distinguished two types of fibers, i.e., (1) those with moderately thick walls, small lumen and birefringence patterns, and (2) those with thick wall, without lumen cavity and birefringence pattern suggesting the absence of third secondary wall layer in Phyllanthoideae. Pax and Hoffman (1931) studied the wood anatomy of 22 taxa comprising 17 genera of the tribe Cluytieae belonging to 6 sub-tribes, i.e., Codiaeinae, Jatrophinae, Cluytinae, Galeariinae, Acidocrotoninae and Ricinodendinae. Record (1938) investigated the woods of some taxa of American Cluytieae, while Heimsch (1942) studied the LM and SEM of xylem of Euphorbiaceae and noted that both primitive and specialized woods occur in the family. Stern (1967) studied the wood anatomy of

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Kleinodendron and supported its inclusion in the tribe Cluytieae on the basis of the similarities with other members. Bamber (1974) and Barajas–Morales (1985; 1987) provided additional information on the wood of *J. chamelensis, J. malacophylla* and *J. platyphylla* from Mexico in respect to various characters like vessels, fibre sizes, presence of resin, crystals, etc. Ibarra (1985) made a detailed study of the woods of several Mexican Euphorbiaceae. Mennega (1987) studied extensively the wood anatomy of biovulate taxa of Phyllanthoideae (35 genera and 116 species) and distinguished two wood types on the basis of wood anatomy i.e., (a) *Aporosa* type with great number of primitive characters especially the perforation plates, (b) *Glochidion* type with absence of scalariform vessels/perforation plates.

Recently, Wiedenhoeft (2008) investigated the comparative ecological wood anatomy of several taxa of Crotineae while Ibarra (1985) made a detailed study of the woods of several Mexican Euphorbiaceae. Webster (1975) recognized five subfamilies in Euphorbiaceae, i.e., Phyllanthoideae, Oldfieldoideae, Acalyphoideae, Crotonoideae and Euphorbioideae. The first two comprise the Biovulate taxa and the latter three sub-families comprise the Uniovulate taxa. According to this author, the basic pattern of wood is similar in taxa belonging to all the Uniovulate subfamilies characterized by the absence of scalariform vessels, perforation plates, presence of medium to very large inter-vascular pitting and similar vessel ray pitting, presence of apotracheal, diffuse, banded parenchyma and numerous narrow heterocellular rays. Hayden and Brandt (1984) studied the wood anatomy of three specimens of *Neowawraea phyllanthoides,* a rare and endangered taxon endemic to Hawaiin Islands, and compared it with the woods of other Euphorbiaceae. Wiedenhoeft (2008) investigated the comparative ecological wood anatomy of several taxa of Crotonoideae.

It is thus obvious that though various aspects, mostly of systematic interest, have been investigated in considerable detail, the genus *Jatropha* still offers scope for further work on wood anatomy.

Wood Characteristics of *Jatropha* L.

The genus *Jatropha* L. is represented by about 175 species (Dehgan and Webster 1979) distributed mostly in American and African continents while in India its distribution is limited to about a dozen species. Data on wood anatomy of 10 *Jatropha* species investigated earlier by Chamundeswari et al. (2005) has been extended by a recent study of *J. heynei* from Dr. Venu Madhav (Karimnagar, Andhra Pradesh, India).

The wood characteristics described below were obtained by sectioning wood blocks in slides of 20–25 μm thick at the Centre for Drugs Research from Osmania University (Hyderabad, India). The sections were stained, dehydrated and mounted following standard methods (Jane 1970). Free hand sections were also taken and processed similarly. Measurements (20–25) were taken for each

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of the quantitative wood characters studied under a standard binocular. The terminology used is in accordance with that prescribed by the committee on Nomenclature (IAWA 1964) and the Committee on Standardization of terms of cell size (IAWA 1937, 1939). Vouchers of microtome slides of all the species are being maintained in the Department of Botany, Osmania University (Hyderabad) and Kakatiya University (Warangal, India). The wood features of the *Jatropha* species studied earlier are also described. Some species in particular *J. curcas* has been reinvestigated in addition to *J. heynei,* which was not studied earlier. By contrast, *J. maheshwarii* has not been investigated as the wood was not fully mature.

J. gossypiifolia var. *gossypiifolia*

J. gossypiifolia var. *gossypiifolia* is characterized by a diffuse and porous wood with growth rings. Pores are rarely solitary and are commonly organized in radial groups of 2–8. Radial groups are more common than solitary vessels. Solitary pores are circular to oval while pores in radial groups are flattened with tangential walls. Vessels of radial groups are long (375 μm), narrow (54–64 μm in diameter), with simple perforations mostly oblique. Intervessel pits are alternate, crowded, contiguous, angular with lenticular apertures and aligned horizontally. Parenchyma is scanty in paratracheal xylem, but abundant in apotracheal xylem; it is organized in numerous uniseriate tangential bands often alternating with fibers. Wood space is filled up more by parenchyma than by fibres, which gives it a lax structure. The mean diameter of axial parenchyma is 18.3 μm. Xylem rays are overwhelmingly uniseriate, rarely partially biseriate with 2–35 cells in length, homocellular and made of upright or vertical or square type cells forming a homogeneous ray tissue filled with starch grains. Many axial parenchyma cells also have starch although less abundant than in ray cells. On average, ray cells are 18.3 μm in diameter and 1,100 μm in length. Fibers are non-libriform, non-septate, 18.3–22 μm wide, 146.5 μm long and 4 μm in wall thickness (Fig. 2.1a–c).

J. gossypiifolia var. elegans

Wood diffuse, porous, pores solitary or in radial groups of 2–10, radial groups more common than solitary vessels. Solitary pores mostly oval. Vessel members long, narrow, perforations simple, oblique, pore diameter 56–73 μm and mean vessel length 475 μm. Intervessel pits alternate, crowded, contiguous, angular apertures lenticular, horizontally aligned, 3–7 μm in diameter. Axial parenchyma is paratracheal and apotracheal. Paratracheal parenchyma are meager in the form of scanty vasicentric sheaths; apotracheal parenchyma abundant, in numerous uniseriate tangential rows alternating with the rows of fibres, 18.3 μm in diameter. Some

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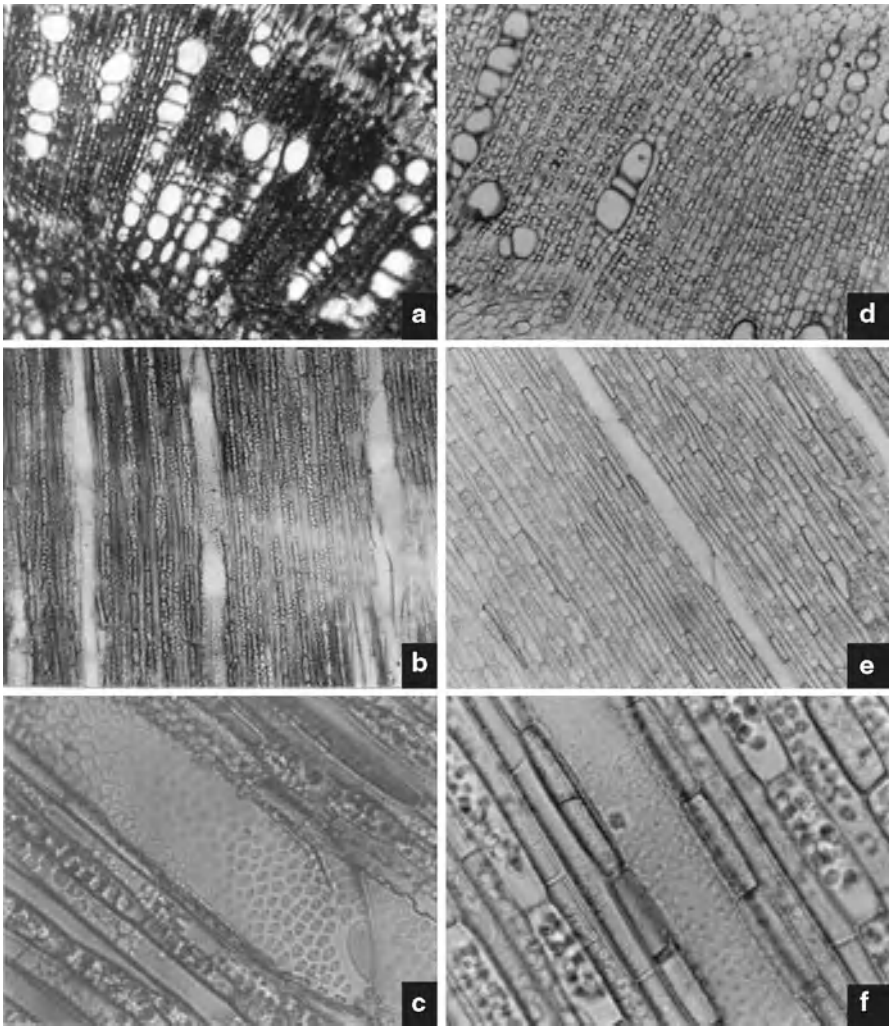

Fig. 2.1 (**a**) TS of *J. gossypiifolia* var. *gossypiifolia* wood (× 500), (**b**) TLS of *J. gossypiifolia* var. *gossypiifolia* wood (× 70), (**c**) vessel of *J. gossypiifolia* var. *gossypiifolia* (× 2000), (**d**) TS of *J. gossypiifolia* var. *elegans* (× 600), (**e**) TLS of *J. gossypiifolia* var. *elegans* (× 76), (**f**) vessel element (enlarged) of wood of *J. gossypiifolia* var. *elegans* (× 2000)

of the axial parenchyma cells contain starch grains. Xylem rays overwhelmingly uniseriate, locally biseriate, 2–20 cells high, homocellular, of upright (vertical) or square type, ray tissue homogeneous with abundant starch grains. Ray cells 25.6 μm wide and has a mean length of 1,000 μm. Fibres non-libriform, angular in TS; non-septate, occupies less space than that of xylem parenchyma. Fibres 14–25 μm across, 165 μm long (mean length) and wall thickness is 3–5 μm (Fig. 2.1d–f).

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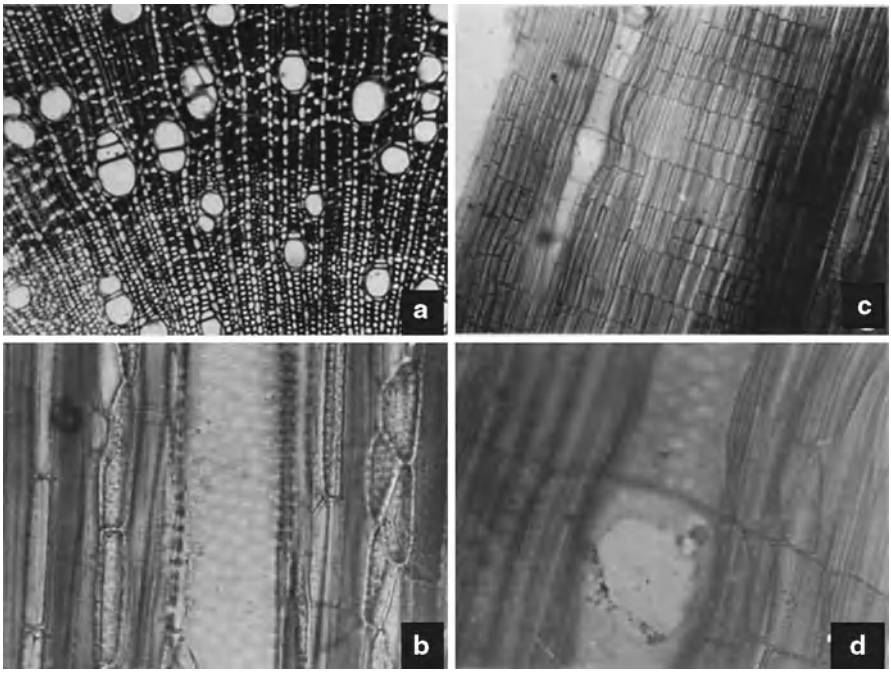

Fig. 2.2 (**a**) TS of *J. integerrima* with solitary vessels (× 700), (**b**) TLS of *J. integerrima* (× 80), (**c**) RLS of *J. integerrima* (× 500), (**d**) *J. integerrima* wood showing details of vessel element structure (× 1600)

J. integerrima

Wood diffuse, porous, pores predominantly solitary, rarely in radial pairs, or multiples of 3, solitary pores rounded to occasionally oval. Vessel diameter 51–73 μm, mean length 540 μm. Intervessel pits simple, 7.3 μm in diameter. Perforations simple with oblique plates, intervessel pits multiseriate, crowded, angular with transverse lenticular apertures. Axial parenchyma fairly abundant, predominantly apotracheal and aligned in numerous uniseriate tangential rows alternating with rows of fibres. One or two rows of fibres present in between parenchyma rows. Diameter of xylem parenchyma is 18.3 μm. Xylem rays overwhelmingly uniseriate, rarely partially biseriate, 3 or more than 50 cells high, rays homocellular, of upright vertical or squarish type, ray tissue homogeneous. Fibres squarish to rectangular in TS, non-libriform, non-septate, 11–15 μm across and have a mean length of 400 μm long and a wall thickness of 3.5 μm (Fig. 2.2a–d).

J. panduraefolia

Wood diffuse, porous, pores solitary or in radial pairs of 2–10; radial multiples more frequent than solitary pores; solitary pores rounded to oval; vessel elements long,

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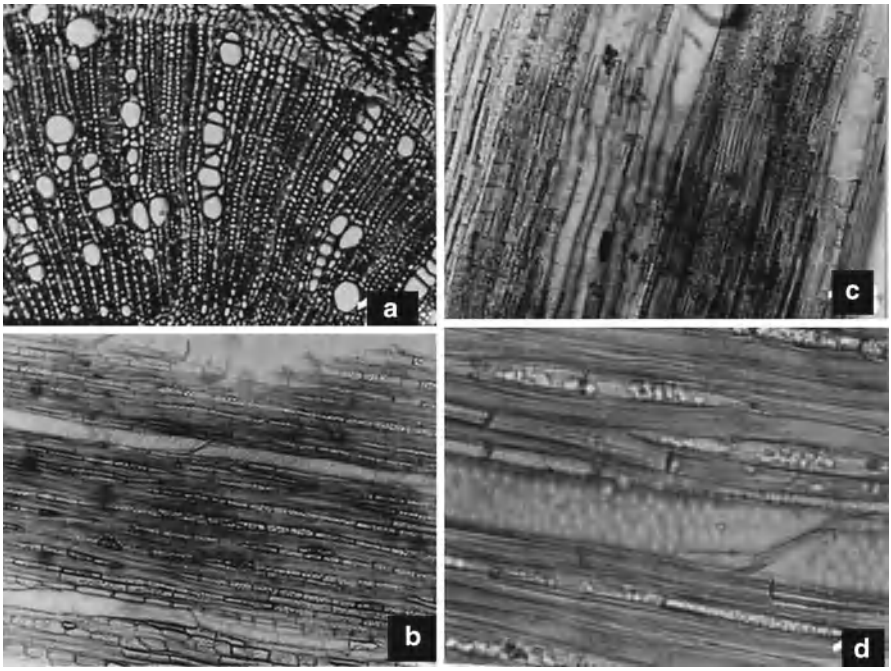

Fig. 2.3 (**a**) TS of wood of *J. panduraefolia* with vessels in distinct radial multiples of 4–5 (× 1600), (**b**) TLS of wood of *J. panduraefolia* (× 60), (**c**) RLS of the wood of *J. panduraefolia* (× 600), (**d**) TLS of *J. panduraefolia* (× 2000)

narrow, mean vessel length 440 μm, vessel diameter 50–73 μm, perforations simple and oblique, inter-vessel pits crowded, angular with transversely lenticular apertures, mean diameter 7.3 μm. Xylem parenchyma fairly abundant, predominantly apotracheal and aligned in numerous uniseriate tangential rows, alternating with 1–2 seriate fibre rows; solitary or pairs of diffuse parenchyma cells also seen, paratracheal parenchyma in scanty vasicentric sheaths. Axial parenchyma 18.3 μm across, xylem rays overwhelmingly uniseriate, rarely locally biseriate filled up with starch grains, ray tissue 30–50 cells high, homocellular, homogenous; cells of upright (vertical) or square type; fibres squarish to rectangular or somewhat irregular in TS, non-libriform, non-septate, 11–15 μm across, with a mean length of 400 μm, and a wall thickness 3.5 μm (Fig. 2.3a–d).

J. glandulifera

Wood diffuse, porous, pores solitary or more commonly in radial multiples; each radial multiple with 2–6 pores, pores have 60–90 μm mean diameter; vessel elements have 400 μm mean length perforations of vessel elements simple, intervessel

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pits about 7 μm in diameter. Axial parenchyma abundant, paratracheal and apotracheal, paratracheal parenchyma is scanty, vasicentric, forming 1–2 seriate sheaths continuous with the pore contour of vessels; apotracheal parenchyma abundant, in numerous uniseriate tangential rows, often wavy, between adjacent rays, two or three parenchyma cells in each parenchyma strip. Parenchyma cells ovoid to irregular, often of the same size. Apotracheal parenchyma also seen as individual diffuse cells or groups of 2–3 cells, axial parenchyma occupies more space than fibres in the wood forming an appreciable volume of the wood. Rays uniseriate, homocellular, homogenous; cells upright or vertical or squarish, filled with starch grains. Ray cells have a width of 20 μm and mean length of 200 μm. Fibres squarish to rectangular, non-libriform, 11–15 μm across and 500 μm long. Fibre wall is about 3.6 μm thick (Fig. 2.4a–c).

J. multifida

Wood diffuse, porous with growth rings. Pores solitary, or in radial pairs or in radial multiples of 3–8, solitary vessels more common, rounded to oval. Vessels occasionally with tyloses. Vessel elements long, narrow, perforations simple, oblique; mean vessel length 585 μm, vessel diameter 69–85 μm, traversed pits alternate, crowded, contiguous, angular, apertures lenticular, horizontally aligned, pit diameter 10–11 μm. Axial parenchyma very abundant, occupies more space than the fibres in the wood, both paratracheal and apotracheal. Paratracheal type in scanty, vasicentric sheaths completely or partially surrounding vessels, apotracheal ones very abundant, closely spaced in uniseriate tangential rows, more or less regularly alternating with uniseriate tangential, much narrow rows of fibres forming a fine reticulum with rays. Parenchyma cells much larger and thinner than fibres; diameter 18.3 μm. Xylem rays numerous, closely spaced, mostly separated by two or three parenchyma cells and 2–5 fibres. Rays overwhelmingly uniseriate, rarely biseriate, 30–40 cells high, ray cells larger in diameter than in other species, filled with many simple starch grains; rays homocellular, cells upright (vertical) or squarish. Rays have a mean length of 292.8 μm and a mean width of 29.2 μm. Fibres squarish to rectangular, locally irregular, occupy less space than parenchyma in the wood, non-libriform, non-septate, has a mean length of 550 μm, 11–15 μm in diameter and mean wall thickness of 3 μm (Fig. 2.4d–f).

J. tanjorensis

Wood diffuse, porous, vessels solitary or in radial multiples, with 3–6 pores in each radial multiple. Solitary pores more common than of radial multiples, pores rounded to oval. Vessels often with tyloses. Perforations simple, oblique. Vessel diameter ranges from 62–70 μm, mean vessel length is 475 μm. Intervessel pits alternate,

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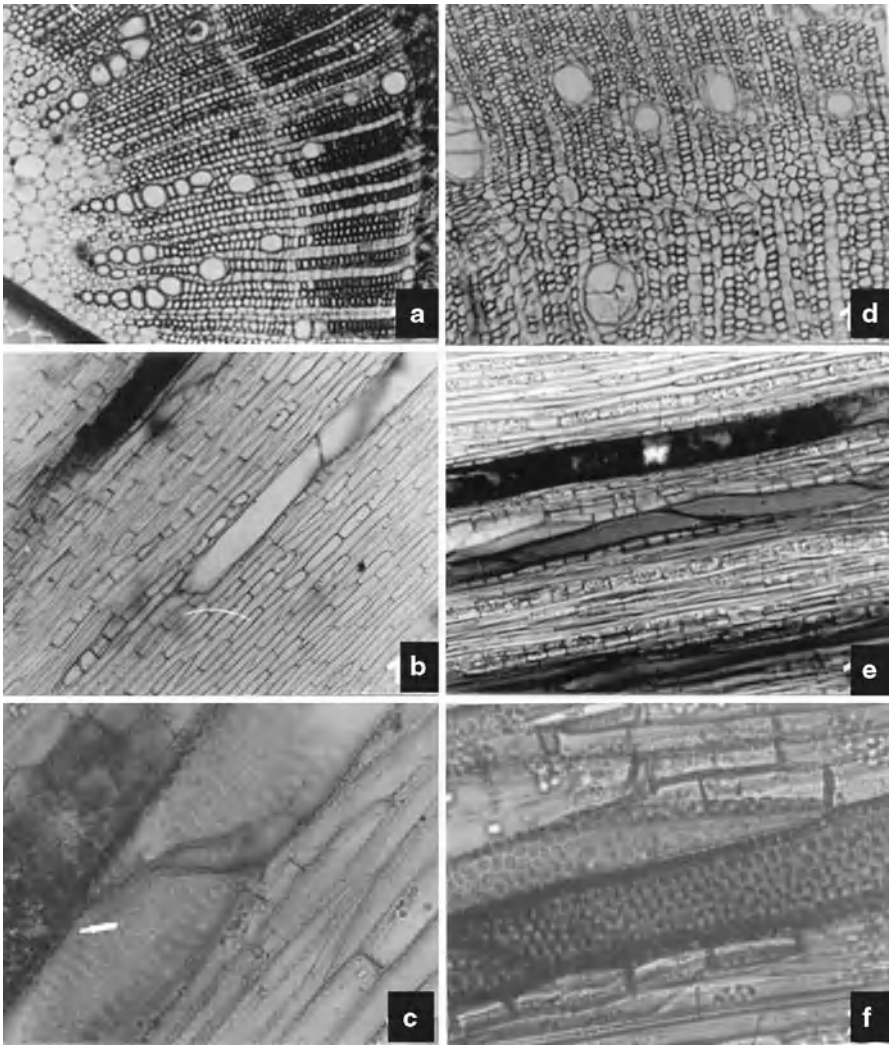

Fig. 2.4 (**a**) TS of wood of *J. glandulifera* with solitary and radial multiples of 2–3 vessels (× 440), (**b**) TLS of wood of *J. glandulifera* (× 80), (**c**) TLS of wood of *J. glandulifera* showing details of vessel element structure (× 2200), (**d**) TS of *J. multifida* with tyloses and large ray cells (× 700), (**e**) TLS of *J. multifida* showing tyloses and large cells (× 70), (**f**) TLS of wood of *J. multifida* showing details of vessel elements (× 2000)

crowded, contiguous, becoming angular hexa to polygonal, often horizontally aligned, lenticular, local coalescence of apertures seen. Intervessel pits have 7.3 μm as mean diameter. Axial parenchyma abundant, mostly apotracheal, in uniseriate tangential rows, vasicentric paratracheal type as scanty sheaths. Parenchyma in contact with vessels is tubular, those in uniseriate rows are rounded to irregular. Parenchyma simple pitted, beaded or narrowly bordered in appearance. Pits large, transversely

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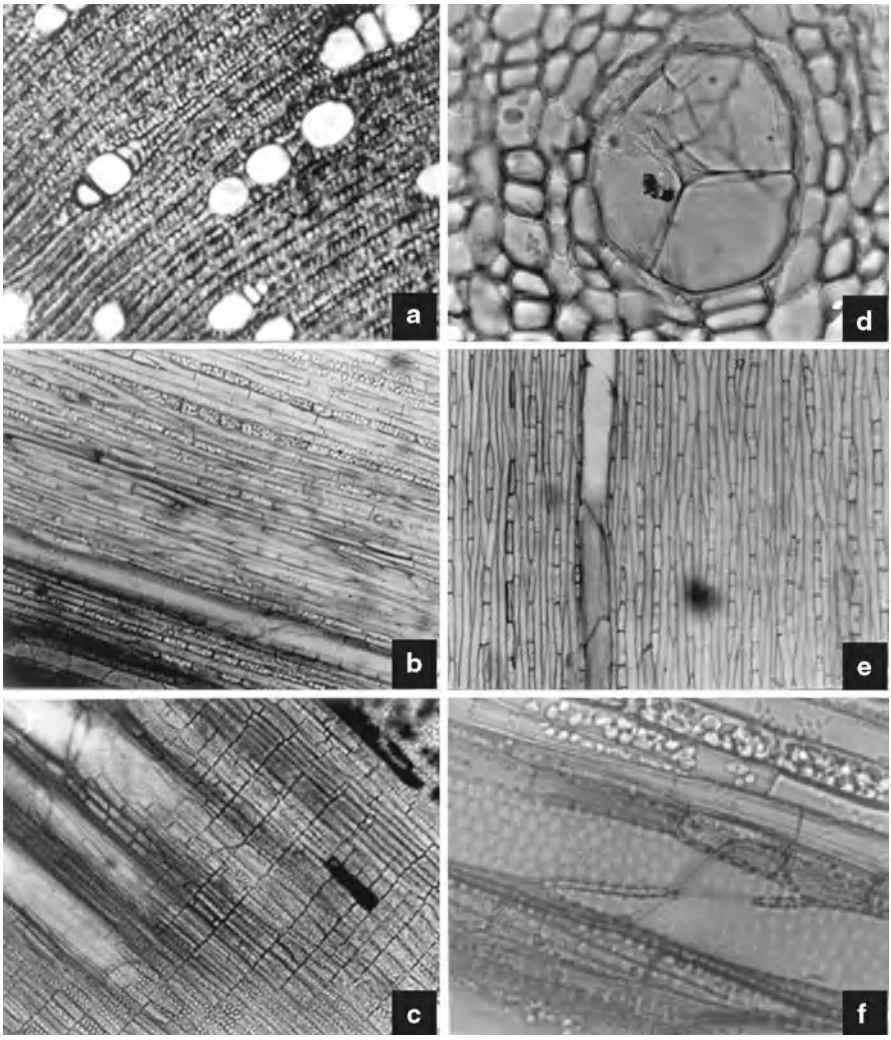

Fig. 2.5 (**a**) TS of wood of *J. tanjorensis* with vessels in radial multiples (× 700), (**b**) TLS of wood of *J. tanjorensis* (× 70), (**c**) RLS of wood of *J. tanjorensis* (× 600), (**d**) TS of wood of *J. curcas* showing tyloses in the vessel element (× 600), (**e**) TLS of wood of *J. curcas* (× 70), (**f**) a portion of TLS of wood of *J. curcas* (× 1800)

elongated, narrowly bordered or almost simple. Vessel parenchyma pits similar, 18.3 μm in diameter. Xylem rays 3–25 cells in height, overwhelmingly uniseriate, rarely partially iseriate, (biseriate portion confined to a few cells in the centre of the ray – wherever it is seen), homocellular, elongated in TLS, upright (vertical) or squarish in RLS. Ray tissue homogenous, ray cells profusely filled up with starch grains. Fibres thick walled, squarish to rectangular, libriform and non-septate. Fibres 11–15 μm across with a mean length 50 μm, wall thickness 3–4 μm (Fig. 2.5a–c).

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J. curcas

Wood diffuse, porous with faint growth rings. Pores solitary or in radial multiples of 2–10. Solitary vessels are common. The mean vessel element length is 586 μm and the width ranges from 51 to 62 μm across. Pores more or less rounded to oval, perforations simple, oblique, inter vessel pits numerous, alternate, contiguous, becoming hexagonal to polygonal, pit apertures lenticular, horizontally aligned. Vessel-ray pits large, transversely elongated, simple or narrowly bordered. Vessel-axial parenchyma pits large, transversely elongated, simple, narrowly bordered. Size of intervessel pits 14.6 μm, tyloses often present in vessels. Axial parenchyma fairly abundant, mostly apotracheal, as uniseriate tangential rows, occasionally paratracheal as scanty vasicentric sheaths. Parenchyma cells abutting on vessels tubular elsewhere rounded to irregular in cross sections. Parenchyma seems to occupy more space in the wood tissue than fibres. The mean diameter of xylem parenchyma cells is 18.3 μm. Wood rays numerous, overwhelmingly uniseriate, rarely locally partially biseriate, 2–18 cells in height, homocellular, cells filled with starch grains, ray cells almost all of upright (vertical) or occasionally square type. Ray cells have a mean width of 25.6 μm and a mean length of 220 μm. Fibres non-libriform, moderately thick walled, mostly rectangular in cross section and non-septate. Fibres measure 14.6–22 μm across and have a mean length of 550 μm. Wall thickness is about 3.66 μm (Fig. 2.5d–f).

J. podagrica

Wood diffuse, porous, pores solitary or in radial multiples, with 2–8 pores in each radial multiples; solitary pores common; abundantly tylosed, more or less rounded to oval; Perforations simple, end walls oblique, vessel diameter 69.5–84 μm, mean length of vessel 402.6 μm; intervessel pits alternate, angular, contiguous, apertures horizontal, lenticular; coalescence of apertures frequently seen, intervessel pits 7.32 μm diameter. Vessel ray pits large, transversely elongated, simple, vessel parenchyma simple, vessel parenchyma pits same as vessel ray pits. Axial parenchyma abundant, mostly apotracheal as uniseriate tangential rows alternating with fibre rows forming a reticulum with rays. Paratracheal parenchyma meager, in the form of scanty vasicentric sheaths, axial parenchyma cells abutting on vessels tabular, elsewhere rounded to irregular, axial parenchyma occupies more space than fibres. Xylem rays numerous, overwhelmingly uniseriate, rarely locally partially biseriate, 20–22 cells in height, homocellular, cells with starch grains, almost all of upright (vertical) or square type. Fibres non-libriform, non-septate, moderately thick-walled, mostly rectangular or squarish in TS (Fig. 2.6a–b).

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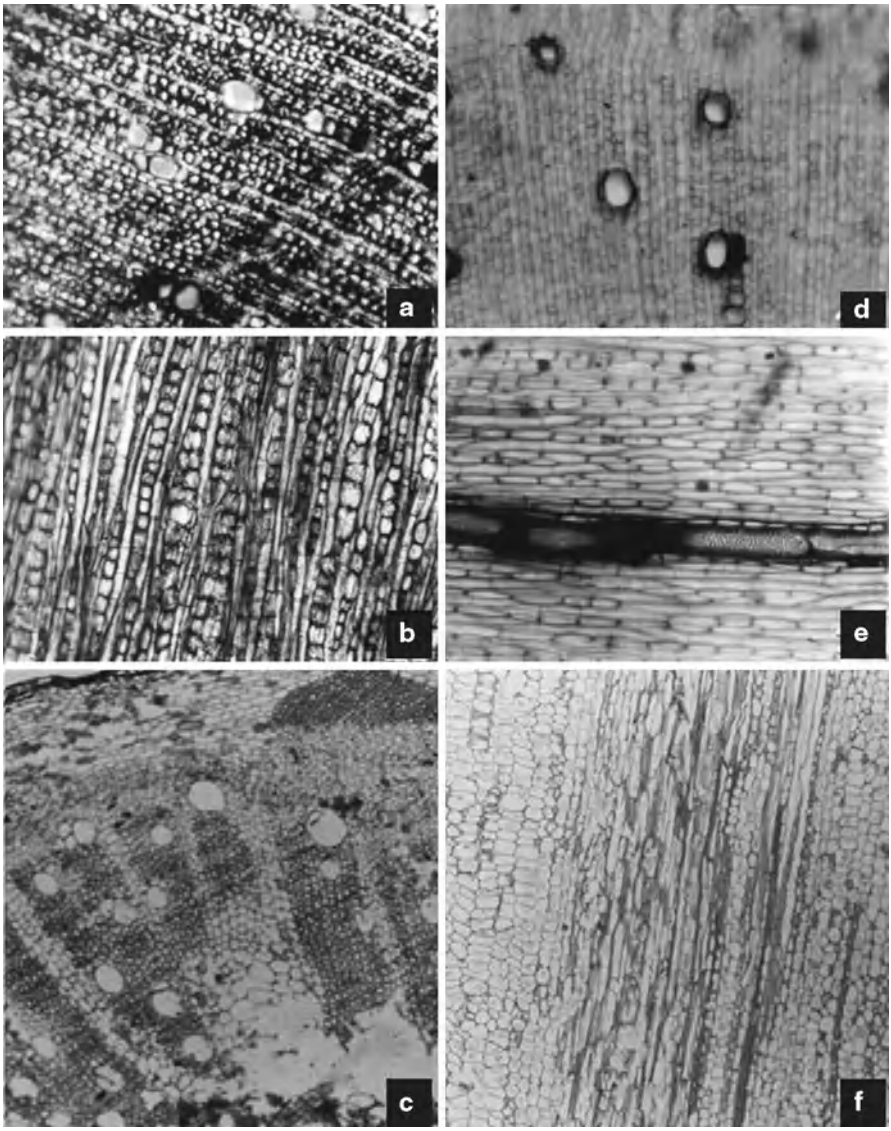

Fig. 2.6 (**a**) TS of wood of *J. podagrica* (× 470), (**b**) TLS of wood of *J. podagrica* (× 470), (**c**) TS of wood of *J. villosa* (× 500), (**d**) TLS of wood of *J. villosa* (×80), (**e**) TS of wood of *J. heynei* (× 80), (**f**) TLS of wood of *J. heynei* (× 500)

J. villosa

Wood diffuse, porous, growth rings indistinct, pores small to medium, radial diameter 62–64 μm, solitary or in radial chains of 2–6, unequally distributed. Vessel

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elements short to moderately long, 405 μm long, perforations simple, oblique; intervessel pits bordered, angular, alternate, 3–4 μm. Axial parenchyma scanty, paratracheal and apotracheal; paratracheal as thin (one or partially two rows) sheaths, around pores apotracheal mostly diffuse. Xylem rays mostly uniseriate, locally partially biseriate, usually 5–15 or rarely even up to 20 cells high, cells elongated, 250–270 μm long with a mean diameter of 26 μm, rays homocellular, made up entirely either of upright or square cells. Fibres non-libriform, rather thin walled septate, have a mean length of 53 and 3.5 μm broad (Fig. 2.6c–d).

J. heynei

Wood diffuse, porous, growth rings indistinct, pores mostly solitary or in radial rows (2–3) or multiples of 3–4; lumina locally filled by tyloses, pores more or less rounded to polygonal, medium to small, pore diameter 50–80 μm in diameter, perforation simple, horizontal to oblique, intervessel pits numerous, bordered, alternate, transversely elongated, hexa to polygonal. Axial parenchyma scanty; elements medium to long (250–500 μm), paratracheal and apotracheal; paratracheal parenchyma mostly vasicentric in one to two cell thick sheaths, apotracheal parenchyma with uni-to bi-seriate tangential rows, occasionally distributed among the fibres. Xylem rays numerous, mostly 3–5 seriate locally uni-or bi-seriate sparsely distributed, rays are homocellular made of procumbent cells, have 20–25 μm width and up to 100–150 μm length, non-septate, 8–12 μm in diameter and 250–350 μm long, fibre wall has a mean thickness of 3.5 μm (Fig. 2.6e–f).

Xylotomical Features

The important xylotomical features noted in different species of *Jatropha* reported here are as below. All the species of *Jatropha* exhibit similarities in the following features (Chamundeswari et al. 2005). In that sense one can say that these common features represent the plesiomorphic (or primitive) characters of the genus *Jatropha*, i.e.:

1. Diffuse, porous nature of the wood;
2. Presence of solitary radial multiples of vessels;
3. Vessel diameter of very small type (55–85 μm);
4. Perforations simple and oblique;
5. Intervessel pits numerous, alternate and contiguous;
6. Xylem parenchyma abundant, mostly uniseriate, apotracheal and rarely paratracheal;
7. Xylem rays overwhelmingly uniseriate, homocellular filled with starch grains;
8. Fibres are non-libriform and non-septate;
9. Laticifers are totally absent in the wood.

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These features vary quantitatively in value among species, which can be considered as apomorphic characters (or derived) specific to the species. The quantitative measurements of the value associated to these specific characters among the various species in the genus *Jatropha* show that according to transport structures (1) vessels are similar across species being only differing by the diameter, which is ranging from 55 to 84 μm, hence, these vessels are classified as from the very small type (Reyes 1938); (2) vessel elements are considerably long varying in length from 350 to 500 μm; (3) intervessel pits ranges from 4 to 10 μm in diameter; (4) fiber length ranges from 150 to 550 μm and width from 11 to 25 μm; (5) wall thickness is about 4.0 μm; (6) ray length ranges from 300 to 1,100 μm and ray width from 20 to 30 μm; (7) ray cells are considerably elongated in *J. gossypiifolia* varieties; (8) tyloses are present distinctly in *J. curcas, J. podagrica* and *J. tanjorensis.*

In *J. multifida,* the ray cells are larger in diameter than in other species. The cultivated species, *J. panduraefolia* and *J. integerrima* resemble each other in several morphological features. However, the pores of *J. integerrima* are mostly solitary and only occasionally in radial bundles of 2–4, whereas in *J. panduraefolia* there are radial bundles of 2–10 vessels, a feature more commonly found than solitary ones. The other two cultivated species, *J. multifida* and *J. podagrica,* likewise are xylotomically similar to each other and differ only in minor details. In the case of the two wild varieties, *J. gossypiifolia* var. *elegans* and *J. gossypiifolia* var. *gossypiifolia* the rays are relatively very tall. In *J. gossypiifolia* var. *gossypiifolia,* the rays are 3–35 cells high and in *J. gossypiifolia* var. *elegans* the rays are 2–18 cells high. It is of interest to note that *Cnidoscolus bellator, J. curcas* and *J. cardiophylla* are characterized by simple vessel perforations, thin walled imperforate tracheary elements, absence of crystals in ray cells and axial parenchyma (Pax and Hoffman 1931). These observations are in conformity with that reported earlier by Chamundeswari et al. (2005).

The *Jatropha* species studied here are either shrubby or herbaceous taxa, which are reflected in their wood anatomy. It is well known that less woody growth forms tend to have a wood that can be called juvenilistic (Carlquist 1975, 2001). According to that concept also known as paedomorphosis, primary xylem particularly metaxylem is noticed in secondary xylem or wood. The juvenile wood characters, particularly obvious in the woods of *Jatropha* species studied here, include upright to square ray cells, lateral walls, pitting of vessels with wider than normal pit apertures as well as simple perforation plates, which is characteristic of the majority of paedomorphic woods. Yet, another important feature to be mentioned is the predominance of axial parenchyma cells in fibres of paedomorphic wood compared to common tree woods. This is a familiar feature seen in many succulent angiosperms; a feature associated with water storage. Incidentally many *Jatropha* species share this feature.

Barajas-Morales (1985, 1987) investigated the specific gravity of woods of various tropical taxa including several Euphorbiaceae and took into account the quantitative characteristics of wood (studied about 12 parameters) of three *Jatropha* species from Mexico, i.e., *J. chamelensis, J. platyphylla* and *J. malacophylla* and noted differences in specific gravity in these species ranging from 0.15 to 0.47 while

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other species of Euphorbiaceae, such as *Adelia oaxacana, Celaenodendron mexicanum* showed higher specific gravities (0.87–0.47); a fact that has been correlated with the various xylotomical features *vis-a-vis* their ecological adaptations (arid conditions). Barajas-Morales (1985, 1987) also noted high correlation between humidity content of wood and its density or specific gravity. Hooda and Rawat (2006) reported the wood density of *J. curcas* to range from 0.33 to 0.37 while Sotolongo et al (2009) reported the value as 0.35, meaning that this wood can be classified as light, porous and soft. The latter authors concluded that the wood of J.curcas to be not good for fuel as it burns quickly with energy content of 15.5 MJ Kg^{-1} commonly used as main energy source in developing countries and has the potential to supply 1.3 PJ energy.

Selective pressure during speciation is the mechanism that has been used to explain the differences of wood characteristics between the mesic species and comparative ecological anatomy of Crotoneae has been tentatively correlated with cladistic analysis (Wiedenhoeft 2008). It is a common observation that wood anatomy lacks physiological background, i.e., relationship between habit and the various ecological factors of the population habitat where speciation occurs. According to Fritts (1976), one of the most significant effects of water stress may be the reduction of cell size and the increase of cell wall thickness. This author stated that the moisture content of the cambium would be the direct limiting factor for reduced cell elongation and division under water stress (Barajas-Morales 1987). *J. curcas* assumes attention for its ability to thrive in a wide range of ecological conditions throughout the tropical and sub-tropical regions of the world. Thus, a similar study to the one by Barajas-Morales (1987) on various accessions of *J. curcas* known to be elite in different geographic regions has to be undertaken in addition to the characterization of tissue adaptations to saline and alkaline habitats in order to proceed with successful domestication of *J. curcas*.

Conclusions

From the foregoing, it is obvious that a high degree of overlapping and uniformity in the diverse xylotomical characters exist between different *Jatropha* species, which makes rather difficult to precisely classify the various species of this taxon based only on wood anatomy. There are, however, certain features that seem to be of taxonomic importance in the classification of closely allied species, *viz.,* solitary or radial multiples of vessels, presence or absence of tyloses, and average height of xylem rays. Further work on American and African species concerning the division of the genus *Jatropha* in sections and sub-sections is still necessary before valid conclusions on the taxonomy and phylogeny of this genus can be drawn.

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Chapter 3
Breeding System and Pollination in *Jatropha curcas*: An Overview

A.J. Solomon Raju and Bir Bahadur

Introduction

Euphorbiaceae is a large family of flowering plants with 300 genera and around 7,500 species consisting mostly of herbs and a few shrubs and trees. The family is mainly tropical with the majority of species distributed in the Indo-Malayan region and tropical America. Flowers vary widely in their structure and are usually unisexual with the male and female flowers occurring in a same inflorescence or on a same plant (Watson and Dallwitz 1992). Monoecy, the production of separate male and female flowers on a same plant is a sexual system relatively common among angiosperms and is widely prevalent in the members of Euphorbiaceae (Solomon Raju and Ezradanam 2002a). This sexual system has been divided into *strict monoecy* and *male-biased monoecy* by Gross (2005). Strict monoecy, with equal ratio of male and female flowers, is not the common form of monoecy while male-biased monoecy, with more male and few female flowers, is the most common form, especially in Euphorbiaceae. Monoecy has been reported in species of *Euphorbia, Phyllanthus, Argythamnia, Chamaesyce, Cnidoscolus, Croton,* and *Jatropha* (Bullock 1985), such as *Jatropha gossypiifolia* (Reddi and Subba Reddi 1983). *Croton bonplandianum* (Reddi and Subba Reddi 1985), but also in *Cicca acida* and *Emblica officinalis* (Subba Reddi and Reddi 1984). These authors also reported that the species of *Jatropha* are insect-pollinated while the species of *Croton* and *Phyllanthus* are wind- and insect-pollinated. Species *Cicca* and *Emblica* are exclusively wind-pollinated. Some species, such as *Euphorbia antiquorum* and *E. tortilis*, can be temporally dioecious and insect-pollinated (Subba Reddi et al. 1995)

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with the effect that the flowering sequence within a single inflorescence ensures a temporal separation of sexual function in favour of protogyny (Cruden 1988). Protogyny has been reported to be associated with all monoecious species mentioned above. Altogether, it appears that the members of Euphorbiaceae with monoecious sexual system are either anemophilous or entomophilous or both.

In this paper, we provide a synthesis of relevant information on the reproductive biology and pollination ecology with reference to the sexual system and pollinators of *Jatropha curcas* L.

The Genus *Jatropha*

Jatropha L. is a genus of approximately 175 succulent shrubs or trees, which contain phorbol esters and other compounds that are highly toxic. All species in this genus show similar flower structure and on this count it has been suggested that they have the same common ancestor, which might be a primitive *Euphorbia*. *J. curcas* has received considerable attention of scientists worldwide due to its seeds being a source of oil suitable for biofuel production and of its various compounds with medicinal activities, such as anti-cancerous and anti-microbial properties, etc. Keeping this in view, the potentialities of this species have been and are being carried out to investigate the suitability of its cultivation on marginal and waste lands with low potential for food crops. Other species of the genus *Jatropha*, such as *J. glandulifera, J. gossypiifolia, J. tanjorensis, J. heynei, J. maheshwarii, J. nana, J. villosa* (wild species), *J. multifida, J. integerrima, J. panduraefolia* and *J. podagrica* (cultivated species) are also found in India. *J. nana*, a bushy species endemic to Maharashtra, is cultivated for its seed oil and used as na energy source (Fairless 2007). *J. gossypiifolia*, a widely grown shrub with crimson reddish leaves and flowers with glandular hairs all over the plant body, produces small brown seeds, which oil is used to treat rheumatism and paralytic affections (Anonymous 1965). *J. glandulifera*, a small bushy shrub with greenish-yellow flowers and glandular hairs only in the axils of leaves, is valued for its seed oil, which has been shown to be useful for treating certain human ailments in ayurvedic literature (Ambasta 1992).

Interspecific hybridization studies on *Jatropha* species have been carried out by a few workers to develop genetically stable and viable hybrids for introgression of interesting agronomical traits from wild relatives into *J. curcas*. Following Dehgan (1984) reporting sterile pollen in the progeny of crosses involving *J. curcas* x *J. cathartica* and *J. curcas* x *J. podagrica*, Reddy et al. (1987) investigated self and cross-incompatibility in *Ricinus* and various *Jatropha* species. Later on, crosses between *J. curcas* and *J. multifida, J. podagrica, J. gossypiifolia* or *J. glandulifera* were found to be fertile while *J. curcas* x *J. integerrima* was partially sterile and *J. curcas* x *J. tanjorensis* was completely sterile (Sujatha and Prabakaran 1997). Interestingly, complete male and female sterility has been found by investigating the floral biology among some clones of *J. tanjorensis* (Sahai and Rawat 2009). In that regards, *J. villosa* var. *villosa, J. multifida*, *J. podagrica, J. maheshwarii,*

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J. glandulifera, J. gossypiifolia, J. integerrima and *J. curcas*, are the other species of *Jatropha* that produce the highest percentage of fertile pollen (Sasikala et al. 2009). Hence, it is important to consider these species as parents for interspecific crosses in *Jatropha*. *J. curcas* has been and is being worked out worldwide for improvement of seed oil quality (low phorbol ester toxicity and better fatty acid balance) and content.

Pollination Ecology of *J. curcas*

J. curcas is native to Mexico and Central America, but is presently found throughout the arid and semi-arid regions of the tropical to subtropical belt worldwide. It is cultivated in many Latin American, Asian and African countries (Chen and Zhenb 1986) and occurs mainly at lower altitudes (0–500 m) in areas with an annual temperature well above 20°C (Chen and Zhenb 1986; Hikwa 1995; Henning 1996; Makkar et al. 1997). There are several publications describing floral biology, pollination biology, breeding systems, reproductive phenology (Bhattacharya et al. 2005; Luo et al. 2007a, b; Qing et al. 2007; Prakash et al. 2007; Dhillon et al. 2006; Banjo et al. 2006; Rao et al. 2008; Campa et al. 2008; Solomon Raju and Ezradanam 2002a, 2003; Atmowidi et al. 2008; Puji et al. 2010) that were reviewed by Divakara et al. (2010). After the studies of Chaturvedi and Jehan (1982) on the pollen of *J. integerrima* and *J. panduraefolia*, Bahadur et al. (1997, 2000, 2012) made comprehensive and comparative studies of pollen in *Jatropha* species found not only in India but from outside India also, including *J. curcas*, and showed the pollen to be oval to spheroidal, inaperturate with exine showing a crotonoid pattern with clavae. Mature pollen is binucleate in *J. curcas, J. gossypiifolia, J. podagrica* and *J. integerrima* although cases of trinucleate pollens have also been reported in the closely related *Cnidoscolus* species (Webster and Rupert 1973). Ultrastructure of microspore development, microsporogenesis and male gametogenesis of *J. curcas* were investigated by *transmission electron microscopy* (TEM) (Liu et al. 2007a, b). These authors studied the microsporogenesis and male gametogenesis; and described the anthers as tetrasporangiate; development of anther wall to be dicotyledonous type. Pollen grains are two-celled; the exine is thicker than intine. Liu et al. (2008) studied comparative floral organogenesis in *J. curcas, J. podagrica* and *J. gossypiifolia* under *scanning electron microscopy* (SEM) employing various stages of flower development and noted the development of sepals from a floral primordia; 2/5 sequence, which could be clockwise or counter clockwise, five petals initiate simultaneously; the stamens are dicyclic, obdiplostemenous, the carpel primordia also arise simultaneously. In the female flowers the ovary bulges out and stamen primordia though present degenerate as the flowers matures while in male flowers primordia for carpels are absent. Luo et al. (2007a, b) reported floral display and breeding system of *J. curcas* in Yunnan Province, China and commented that the fruits are formed as a result of cross pollination, but could be due to apomixis and noted a large number of female flowers to open first after third to fifth day suggesting xenogamy.

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They also studied pollen viability, after staining with triphenyl tetrazolium chloride and reported higher pollen viability in flowers that bloom after 9 h, the pollen viability is reduced after 33 h and viability lost after 40 h. Further, their observations on stigma receptivity are interesting: The stigmatic secretions are not obvious; instead they noted that larger ratios of green stigma to whole stigma are in favor of larger pollen receptivity. On the other hand, Lyra et al. (2011) investigated the pollen viability of *J. ribifolia* and *J. mollissima* by comparing its *in vitro* germination in various concentrations of sucrose as well as its staining in acetocarmine, aceto-orcein and cotton blue.

By reference to the floral development established in *Arabidopsis* (Smyth et al. 1990), it has been possible to recognize 12 phases by SEM in *J. curcas,* according to the early or late developmental time of calyces, petals, glands, stamens and pistil (Wu et al. 2011). Up to stage VI, Wu et al. (2011) did not find any obvious difference between male and female flowers. Sex differentiation only occurred in the floral meristem after stage VII and male flowers developed as unisexual flowers only while female flowers developed as a bisexual tissue until the moment where male structures (stamen) selectively aborted and were undergoing degradation. It may be relevant to point out that the floral meristem consists of three stages that include a vegetative stage, a transition from vegetative to floral stage and the development of floral parts. The meristem transition stage is recognized at day 6 (Noor et al. 2011).

J. curcas is a perennial, deciduous shrub growing up to 3–10 m that blooms in March to April (in India). It is monoecious with male and female flowers on a same plant and in a same inflorescence that can be characterized as an axillary, paniculate or dichasial to polychasial cyme (Solomon Raju and Ezradanam 2002a, b). The fact that the number of flowers vary considerably among dichasial cymes is a natural consequence of their variation in size. For example, up to six compound cymes, i.e., inflorescence has been reported (Noor et al. 2011).

Wu et al. (2011) noted on the basis of an over 2 year study, three types of inflorescences, which differed significantly according to growing seasons, i.e., (1) the *female type* with 65,295 male flowers and 7,425 female flowers over 450 inflorescences, which means a 79 (♂) to 8 (♀) ratio; (2) the *middle type* with 674,100 male flowers and 25,020 female flowers over 3,476 inflorescences, which means a 94 (♂) to 26 (♀) ratio; and (3) the *male type* inflorescence with 218,225 male flowers and no female flowers over 1,075 inflorescences. The flower ratio correlates with seasons according to rainfall and temperature regimes. The first type occurs just after the blossom period from February to March after the end of the previous year when the temperature is low. The third type occurs when temperatures start to drop during October and November (for details the reader may refer to the original, but excellent contribution of Wu et al. (2011)).

The male-to-female flower ratio is very similar among reports carried out in different parts of India. According to Bhattacharya et al. (2005), the male-to-female flower ratio is 24.5 (♂) to 1 (♀), while Solomon Raju and Ezradanam (2002a) found 29 (♂) to 1 (♀). Similarly, according to the period of the year, the ratio was 22 (♂) to 1 (♀) in December and 27 (♂) to 1 (♀) in April, under Malaysian climate (Noor

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et al. 2011). By contrast, the male type ratio just described was found to be biased toward a balance favoring female flowers in China according to the middle type: ratio of 94 (♂) to 26 (♀) ratio or even the female type: ratio of 8 (♂) to 79 (♀) (Wu et al. 2011). Normally, inflorescences produce a central female flower surrounded by a group of male flowers. However, in a few cases, it may occur that some expected female flowers are substituted by male flowers. An inflorescence normally produces 1–5 female flowers and 25–93 male flowers. Large numbers of male flowers tend to increase the attraction of pollinators because of their larger chemical attractiveness and larger visibility for pollinators (Tcherkez 2004). In an inflorescence, the male flowers anthese first and show daily anthesis until all male buds are exhausted. The female flowers bloom between the second and sixth day (Solomon Raju and Ezradanam 2002a). A somewhat similar condition exists in herbaceous *Cnidoscolus urens* native of Central America (Bawa et al. 1982).

Male flowers are small, odourless and salver-shaped. The five sepals are small and free while the five petals are contort to the left or right (Bahadur and Venkateshwarlu 1976) and are free, but connivent at the flower base, forming a short tube. Ten diadelphous stamens are arranged in two tiers of five each. The lower tier is free, while the upper tier is united at the base. The anthers are yellow, dithecous and dorsifixed. According to Puji et al. (2010), pollen production in lower anthers is 220 pollen grains per anther and 1,100 pollen grains per flower while it is 435 pollen production per anther and 2,175 pollen production per flower in long anthers. Since an insect comes in contact more easily with the long anthers it is possible that pollen of long anthers brings about cross pollination to a greater extent than the pollen of lower anthers. To date, no such study has been conducted on anther dimorphism and accompanied pollen dimorphism in relation to fruit or seed yield. The anthers dehisce 1 h after flower opening by longitudinal slits. The pollen grains are spheroidal to globular, yellow and measure from 81 to 89 μm.

Female and male flowers are somewhat similar, but the former are relatively oval, and larger than the round male flowers. The flowers in most *Jatropha* species contain three styles with three bifid wet stigmas. According to Heslop-Harrison and Shivanna (1977), there is a relationship between pollen nuclear condition, wet stigmas show gametophytic self incompatibility systems (Brewbaker 1967). The ovary is tricarpellate, each with a single locule producing one ovule. The floral base is nectariferous with five yellow elliptic nectary glands around the ovary that secretes nectar. The wet stigmas are receptive during 3 days after flower opening. Solomon Raju and Ezradanam (2002a) reported that male and female flowers secrete the same quantities of nectar volume (0.3 μl) while Bhattacharya et al. (2005) and Puji et al. (2011) observed higher nectar production in female flowers than in male flowers. In native areas of *J. curcas* in Mexico and Southwestern Nigeria, but outside the area where it is actually found, insects were concluded to be important for its pollination (Banjo et al. 2006; Paiva Neto et al. 2010). Investigations carried out in India (Solomon Raju and Ezradanam 2002a; Bhattacharya et al. 2005) and China (Luo et al. 2007), but outside the natural area of *J. curcas* led to similar conclusions. The flower visitation for foraging and pollination activities by *Apis* species was found to be influenced by the sucrose level of nectar (Bhattacharya et al. 2005).

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Among sugars, glucose, sucrose and fructose are in equal ratio apart from alpha amino acids in *J. glandulifera, J. podagrica* and *J. panduraefolia* (Bahadur et al. 1986). These authors correlated the chemical composition of nectar to specific pollinators and their pollination energetics.

J. curcas shows only a weak protandry, which seems to have no appreciable role to promote geitonogamy or xenogamy (Solomon Raju and Ezradanam 2002a; Luo et al. 2007a). In India and other parts of the world, many insects are reported (Solomon Raju and Ezradanam 2002a); among which bees, ants, flies and thrips are found to be effective pollinators of *J. curcas* (Fig. 3.1a–e). Ants and thrips collect nectar and pollen from male flowers or nectar from female flowers mostly in the same visit. Thrips being tiny in size move across freely in the flowers, but mostly stay on the same plant if not on the flowers of the same inflorescence due to the availability of rich quantities of floral rewards and, hence, have little role in cross-pollination. Ants also exhibit more or less such a foraging behaviour and hence, contribute mostly to self-pollination. On the contrary, bees and flies collect only nectar from both male and female flowers. Bees and flies are continuously in quest of more forage, flying across male and female flowers, and therefore affect both self as well as cross-pollination. Flies generally visit the flowers in swarms and the standing nectar reward does not satisfy their requirement; in search of more nectar, they fly to different male and female plants effecting both self and cross-pollination. Luo et al. (2011) have made interesting observations on the contribution of pollination in *J. curcas* in southwestern China. They observed diurnal pollinators to be bees and flies and the nocturnal pollinators to be moths. They noted freshly opened flowers receive more insects by diurnal insects than nocturnal and hence contribute to good seed production. In addition they also observed female flowers to open first than male flowers in rare cases, showing tendency to promote xenogamy and minimize geitnogamy. We have no idea about the pollination dynamics that operates in some locations where *J. curcas* grows wild and also there are plants that produce bisexual or pistillate flowers. This indeed is of special significance in terms of *J. curcas* improvement program and deserve immediate attention by Mexican researchers (see Ovando–Medina et al. 2012). These observations suggest that bees, especially honey bees, and flies could be used to enhance pollination rate to achieve higher fruit yield in *J. curcas* plantations. In India (Lucknow, Uttar Pradesh), honeybees and wasps were reported to be pollinators of *J. curcas* (Bhattacharya et al. 2005), while, in China, the pollinators of this species were flies, bees, beetles and butterflies (Luo et al. 2007a, b). More precisely, Puji et al. (2010) reported that ants, bees, butterflies and flies visit *J. curcas* flowers, but only the bees *Xylocopa confusa, Apis cerana* and *Apis dorsata* are effective pollinators of this species. These studies strongly suggest that *J. curcas* is exclusively entomophilous, which is supported by the low adhesiveness of stigma for pollen that does not permit anemophily (Achten et al. 2010). Insects are expected to be the major pollination agents due to (1) the bright yellow anther colour of male flowers evenly spread over the inflorescence life span; (2) the mild fragrance and nectar availability of both male and female flowers; and (3) the large pollen size and many exine ‘verrucae’ favoring adhesion. Actually, *J. curcas* plants grown in isolation

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Fig. 3.1 *Jatropha curcas*: (**a**) Inflorescence, (**b**) *Apis florea,* (**c**) *Trigora iridipennis,* (**d**) *Camponotus* sp., (**e**) *Chrysomya megacephala*, (**f**) fleshy fruits, (**g**) dry and dehiscing fruits

waste the male flowers on the first day, but this does not occur in plant communities (Solomon Raju and Ezradanam 2002b).

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The level of genetic diversity and genetic differentiation in *J. curcas* populations deserves attention due to its long history of introduction as an exotic species not only in India, but also in several other countries. In such a situation, plant populations may result in a complex genetic history including several potential genetic bottlenecks (Kjaer and Siegismund 1996; Lengkeek et al. 2005). The plant can set seeds after both insect and self-pollination. But in general, fruits from self-pollinated flowers are lighter and the progeny of selfed plants had shorter roots than those of cross pollinated plants (Abdelgadir et al. 2008; Puji et al. 2010). In addition, fruits from self-pollinated flowers abort before maturation in 25% of cases and show lower seed weight as well as lower germination rate (Solomon Raju and Ezradanam 2002b; Puji et al. 2010). This is probably due to early acting inbreeding depression and thus may reflect a natural trend towards out-crossing. *J. curcas* is able to reproduce via selfing, but it has also been suggested to reproduce through apomixis (Luo et al. 2007a). Bhattacharya et al. (2005) also reported that *J. curcas* produces fruits through apomixis to as much as 32%. According to Paiva Neto et al. (2010), apomixis would be absent in *J. curcas* in its natural area. Further, Solomon Raju and Ezradanam (2002b) also suggested apomixis to be absent at least when *J. curcas* is distributed in areas outside its natural habitat. The plant would not require the complex monoecious sexual system with copious pollen production and moderate volume of nectar production (mechanisms that are energetically expensive) to set seeds through sexual reproduction if apomixis would be functional. Each female flower should produce fruit if apomixis was functional, but it is not what is observed in *J. curcas*. Therefore, the function of apomictic mode of reproduction in *J. curcas* is ruled out in both its native habitat and outside its areas of distribution.

Research on the existence of a system to promote out-crossing and minimize self-pollination in *J. curcas* is still scarce (Achten et al. 2010). Temporal dioecism often seen in monoecious plants with unisexual flowers does not exist in this species. Luo et al. (2007a) described the opening sequence of the male and female flowers at inflorescence level and hence at plant level. The flowering pattern reported by them suggests a sort of temporal dioecism that promotes out-crossing while minimizing self-pollination via geitonogamy. This breeding system suggests a self-compatible out-crossing mode requiring pollen vectors for pollination. However, the existence of temporal dioecism has been contested since male flowers open daily at inflorescence level until all male flowers are exhausted and female flowers open intermittently during the same period (Solomon Raju and Ezradanam 2002a). In addition, most of the pollen that germinates on self-pollinated stigma, enters through the stylar canal and finally ovary within 1 h after pollination (Parthiban et al. 2009). This entire process suggests that there is no hindrance for the self-pollen tube growth, development and entry through the entire length of the style prior to fusion within ovule in the ovary. Thus, one may conclude that *J. curcas* has functional self-compatibility to set fruits and seeds through self-pollination. Such a breeding system ensures seed set when the cross-pollen flow does not reach the stigma during the latter's receptive period. In *J. curcas*, the function of self-compatibility and hence the ability to have both self and cross-pollination ensures the success of sexual reproduction, but seed set is dependent on the availability of

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pollinators in its growing areas. Germplasm improvement by "smart" intraspecific and interspecific crossing programme between landraces from Asia, Africa and new introduction from native Americas is also needed to widen the existing gene pool of *J. curcas* accessions (Achten et al. 2007). We believe that the understanding of pollination biology of such intraspecific and interspecific *Jatropha* hybrids is also necessary before one goes for their mass propagation (Basha and Sujatha 2009; Parthiban et al. 2009).

Inbreeding depression has been reported in *J. curcas* by different investigators. Surveys based on genetic markers uncovered surprisingly low levels of genetic diversity in *J. curcas* landraces from China (Sun et al. 2008) and only modest levels of diversity in India (Basha et al. 2009; Ranade et al. 2008), showing that the large scale gene pool may rest on a fairly fragile genetic foundation. Crosses between elite Asian individuals with new accessions introduced from American landraces with larger genetic diversity may allow overcoming any inbreeding depression that could possibly be associated to Asian accessions because of their low genetic variability and thereby increase their vigour and fruit production (Achten et al. 2010). The understanding of the breeding pattern in *Jatropha* is central for the design of domestication strategies in *J. curcas*. Breeding, large-scale mass propagation and distribution across landscapes will obviously be much easier if the species reproduces by natural selfing without inbreeding depression or, reproduces by apomixis (Poehlman and Sleper 1995). Knowledge about the degree of genetic diversity among and within natural populations in and outside the center of origin is a prerequisite to gain information on where to find the genetic material necessary to breed high-yielding *J. curcas* cultivars. A recent study in India and Indonesia indicated that hermaphrodite flowers may occur in *J. curcas* and these flowers are also able to set seeds (Sujatha, personal communication; Noor et al. 2011). Further studies are being carried out in this direction. Hermaphrodite flowers would also throw more light on the evolution of sexual system in the *Jatropha* genus in general and *J. curcas* in particular.

In India, Kumar et al. (2008) based on 27 accessions of *J. curcas* collected from Madhya Pradesh, Gujarat, Rajasthan, Maharashtra, Andhra Pradesh, Chhattisgarh and Uttar Pradesh, reported that there is a large extent of variation in these accessions with reference to morphological and nutritional traits as well as different enzymes produced by these plants. They found positive correlations between all morphological traits, such as plant height, collar diameter, number of branches and branch length. There was also variation in polyphenol oxidase and peroxidase enzymatic activities; this variation would enable to estimate the genetic relationships in the germplasm and to evaluate the tolerance to drought as well as flowering and fruiting, among other parameters. Furthermore, these authors suggested that quantification of peroxidase and polyphenol oxidase enzymes can be used as markers for the estimation of genetic variability and also for the screening of breeding lines with high yielding potential. The estimation of these enzyme activities would help in improving the nutritive value, as both enzymes show positive correlation with nutritive content. Saikia et al. (2009) screened and evaluated a total of 34 sources of *J. curcas* from Gujarat, Orissa, Andhra Pradesh, Uttarakhand, Manipur,

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Haryana, Arunachal Pradesh, Nagaland, Tripura, Meghalaya, West Bengal, Tamil Nadu, Kerala, Jharkhand, Mizoram and Assam, representing the promising *Jatropha* growing belt of India. They assessed the magnitude of genetic variation in growth, behaviour and adaptability in North East India to identify the best sources to utilize for reforestation and future genetic improvement work. They observed variation in accessions with respect to morpho-physiological characters and growth performance; they attributed these variations to the fact that *J. curcas* grows over a wide range of rainfall, temperature and soil types. They also stated that crown exposure, genotype of mother tree, soil and climate are important factors affecting the morpho-physiological characters and growth performance. Higher photosynthetic rate, stomatal conductance and leaf area have a relationship to high seed weight. Mixed clay and sandy soils offer a texture that promotes a better aeration that facilitates gas exchange and increases photosynthetic activity.

J. curcas is toxic due to the presence of phorbol esters in high concentrations in its seeds (Makkar et al. 1997; Adolf et al. 1984). Phorbol esters are known to cause several biological effects (Haas et al. 2002; Goel et al. 2007). Removal of phorbol esters during processing is possible (Makkar et al. 2008), but is not an easy task and the presence of toxic phorbol ester degradation products after treatment cannot be ruled out (Rakshit et al. 2008). Phorbol esters may confer protection to the plants against pests, and hence are an important issue when testing whether phorbol ester free plants are, indeed, more or less susceptible to damage from pests compared to accessions containing phorbol esters. Plants from some provenances in Mexico contain very low or non-detectable levels of phorbol esters (Basha et al. 2009; Makkar et al. 1997, 2008). The presence of naturally occurring plants with low levels of phorbol esters is interesting in the context of domestication, because it makes likely that plant accessions without phorbol esters can easily be developed without the use of either sophisticated molecular breeding technologies or transgenic modifications. Introducing non-toxic material may raise complications, as non-toxic and toxic plants are morphologically alike but for variation in fruit shape and size (Martinez et al. 2012). Additionally, close proximity of toxic and non-toxic plants can trigger unexpected traits through cross-pollination (Achten et al. 2010). Phorbol and phorbol-free plants or landraces may also end up modified concerning chemical composition of nectar and thus may affect pollinator activity and behaviour, which in turn may have side effects on pollinator ecology and fruiting behaviour. Despite the promising results that can be expected from selective breeding for non-toxic *J. curcas* varieties (Achten et al. 2007), pollination ecology of phorbol-free accessions will need to be investigated before exploitation on commercial scale.

Fruiting Ecology of *J. curcas*

Fruit production is important, because it provides the plant with the prominent functions of escape, genetic recombination and dispersal for colonization (Janzen 1969). Each seed is an independent colonizer and seeds are adapted in many ways for

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dispersal (Ingrouille 1992). The conditions of exposure of a plant during the flowering and fruiting phase determine the quality of fruits produced (Ayodele 1999). Flowering and fruiting phases of a plant interact with each other (Burit 1975). If seed or fruit set cannot be promoted by hand-pollination, natural pollination is presumed to be a limiting factor of fruit production (Zimmerman and Aide 1989). Therefore, pollination and nutrient resource limitation are mutually exclusive for the observed pattern of seed or fruit production. Fruit set by hand-pollination may come at the expense of future growth, reproduction, or survival, indicating that plants are resource-limited over their lifetime (Janzen et al. 1980; Montalve and Ackerman 1987). This may enable to understand the interaction between resource limitation and fruit growth. Fruits do not develop immediately after pollination and fertilization in most plant species. The length of time from pollination to fruit maturation varies from a few days to a year (Janzen 1978; Baker et al. 1983). In some plants, fruits develop rapidly up to a certain point, suspend their development for some time and then come back to develop until maturity (Baker et al. 1983). These observations suggest that the timing of fruit development and maturation as well as natural fruit set rate vary among plant species, indicating that the role of intrinsic factors within a plant is regulated or triggered by extrinsic factors prevailing in its habitat.

Flowering is not always followed by fruit and seed maturation in tropical plants (Baker et al. 1983). Different species take varying amount of time to develop their fruits following flower pollination and fruit maturation may be delayed in some plants for several months (Koptur et al. 1988). The duration of fruit maturation varies from a few days to a year (Janzen 1978). In *J. curcas,* flowering is immediately followed by fruit and seed maturation. Fruits mature within 8 weeks during normal rainy years and within 11 weeks during drought years. Furthermore, fruit size varies between normal and subnormal rainy years. This suggests that rainfall has direct effects on fruit maturation and size in *J. curcas.* Since plant roots develop mainly in the shallow zone, a severe water stress may affect water availability for fruiting during drought years and nutrient resources available to the plant may become limited due to low nutrient solubility. Therefore, *J. curcas* requires unlimited water availability in soil for rapid fruit maturation and healthy fruit set. There is a selective elimination of certain growing fruits by the plant in relation to the available energy resources. Controlled experimentation carried out by hand-pollination showed that the fruits that first aborted were from geitonogamy, which is a negative selection against selfing. Thus, fruit set is larger when carried out by hand-pollination than by natural pollination because hand-pollination may artificially favour out-crossing (Solomon Raju and Ezradanam 2003). In addition, it is the nutrient limitation that is the limiting factor for high rate of fruit setting when open-pollination is considered, which comes in disagreement with the generalization that pollen ecology is usually the causal factor of low natural fruit set (Zimmerman and Aide 1989).

The rate of natural fruit setting indicates that the plant does not suffer seriously from under-pollination. The production of female flowers in small number, surrounded by a large number of male flowers in *J. curcas* seems to be a strategy to

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ensure excess pollination. The stigma receptivity lasting for as much as 3 days is another guarantee for successful pollination (Chang et al. 2001). However, in plants with pre-dominant xenogamy, high fruit setting will only be achieved with xenogamous pollen because of selective elimination for fruits formed from geitonogamy (Rickert and Francchia 2006). Therefore, the rate of out-crossing pollination has a great effect on the net percentage of natural fruit set. On the same lines, in experiments of seed germination, it has been found that seeds that failed to germinate and also the seedlings that did not survive and perished indicated that they might have come from geitonogamy (Solomon Raju and Ezradanam 2003; Puji et al. 2010).

Of course, fruit production in *J. curcas* can be boosted by manipulating biological processes, such as pollination and use of growth regulators (Luo et al. 2008; Abdelgadir et al. 2008). exogenous applications of cytokinin (6 Benzyladenine 16 mg l^{-1}) to floral shoot apex lead to flower development and promoted substantial increase (threefold to sixfold) in the production of a larger number of flowers, a larger ratio of male to female flowers and a significant number of bisexual flowers resulting in a substantial increase of fruits and seed yield (Bangzhen and Xu 2011).

Development of pistillate lines has been successfully achieved in several crops and heterotic exploitation is yet another option to boost fruit and seed production. Alternately, the application of gametocidal chemicals at various concentrations is known to promote yield in wheat (Mohan Ram and Rustagi 1966). For example, morphactin and pthalimides promote feminization of male flowers in *Cannabis sativa* and the formation of female flowers in castor, respectively (Mohan Ram and Jaiswal 1971; Jaiswal and Madan Lal 1985). Perhaps such experiments need to be conducted to enhance female flower production to boost up fruit and seed yield in *J. curcas*.

Another strategy is to use plant model systems, such as *Arabidopsis* to manipulate genes for flower regulation (Borivetanan and Samipak 2011). Keeping in view the ABC genetic model of flower development, the homeotic gene C (AGAMOUS), which specifies stamen and carpel development (Bowman et al. 1991; Smyth et al. 1990; Jack 2002) can be manipulated to control carpel and seed development (Hong et al. 2003). The successful cloning of *JcAg* (*AGAMOUS*-like gene) involved in the control of flower development in *J. curcas* may open such perspectives (Borivetanan and Samipak 2011). The nucleotide sequence of *JcAg* is 87% similar to *TcAg* (AGAMOUS-like protein) of *Theobroma cacao*. According to phyletic analysis, *JcAg* is firmly situated in the dicotyledon AGAMOUS subclade close to *TcAg* and clearly separated from SHATTERPROOGF (*SHP*) and SEEDSTICK (*STK*) subclades. Thus, *JcAg* is a candidate gene for regulation of flowering in future engineered *J. curcas* transformants. The genetic basis of floral development is now known in many plants and this is referred to as heterochrony, which regulates the conversion of vegetative meristem to floral meristem. The following genes related to floral identity includes *Leafy* (*LFY*), *APl*, *FUL* and *CAL* (Jung and Muller 2009). Factors regulating sex differentiation in *J. curcas* floral meristems could also selectively affect the action of homeotic genes and restrain the initiation of gynoecium meristem in male flowers (Wu et al. 2011) giving credence to the ABC model and

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agamous gene discussed above. Wu et al. (2011) further noted that female and male flowers of *J. curcas* are both located at specific sites with the consequence that female flowers do not appear at male sites, but male flowers may develop at female sites. This justifies why an inflorescence has less female flowers, but more male flowers.

J. curcas shows green, yellow and brown or black fruits at the same time (Fig. 3.1f, g). The yellow fruits are mature, quite fleshy, but do not attract frugivores. Ripe fruits (black) dehisce passively and the seeds fall off to the ground. The inappetence of fleshy fruits for frugivores is advantageous for farmers growing *J. curcas.*

J. curcas is generally known as strictly oviparous; its seeds fail to germinate on the parent plant. However, a viviparous mode of germination has been reported in which seedlings develop before effective seed dispersion from the parent plant (Deore and Johnson 2008). The germination of viviparous seeds, i.e., while the seeds are still on the parent plant, is a relatively unusual phenomenon in angiosperms although common in mangroves. Vivipary in *J. curcas* is an adaptive reproductive strategy that enables seedlings to establish more rapidly *in-situ.* The phenomenon was observed during mass propagation of *J. curcas* under marine humid-pre-humid climate (Mumbai, India). Vivipary in *J. curcas* has been attributed to excessive atmospheric moisture or wet conditions experienced by the plant after seed maturation and ripening. The unusual humid conditions do not normally exist in the natural habitat of *J. curcas* since it is endemic to semi-arid climates. Thus, farmers interested in raising *J. curcas* crop should take into consideration the humid conditions of the area to prevent viviparous mode of seed germination.

In India, fruit production of *J. curcas* is affected by two pests that are emerging as a major problem: the scutellarid bug (*Scutellera nobilis*) that causes flower fall, fruit abortion and malformation of seeds and inflorescences and capsule-borer (*Pempelia morsalis*) that causes economic damage by webbing and feeding on inflorescences and in later stages boring into the capsules (Shanker and Dhyani 2006). Other pests that cause noticeable damage in India include *Stomphastis thraustica, Achaea janata* and *Oxycetonia versicolor.* Intensive investigations are thus, required to assess the economic damages that these pests may cause to *J. curcas* grown on commercial scale. The responses of these pests in relation to phorbol compounds present in *J. curcas* may throw additional light in the direction to the control of these pests.

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Chapter 4
Pollen of *Jatropha* L. Taxonomic and Phylogenetic Considerations

Bir Bahadur, G.V.S. Murthy, and Mulpuri Sujatha

Introduction

Euphorbiaceae is a large and highly eurypalynous family (a glossary for the non-familiar technical terms of this study is available at: http://www.pollen.mtu.edu/glos-gtx/glos-int.htm) comprising of about 300 genera and 8,000 species (Webster 1987). The genus *Jatropha* L. that includes 175 species belonging mostly to the new world (Dehgan and Webster 1979), has not been extensively studied palynologically and has been neglected by systematists (Erdtman 1952; Punt 1987; Kohler 1965, 1967; Khan 1968; Thanikomani et al. 1984). Erdtman (1952) first studied the pollen of *Jatropha* and proposed the descriptive epithet of *crotonoid pattern* for the special type of sculptural exine pattern encountered in some members of Euphorbiaceae (especially Crotonoideae). He also commented on the pollen characters and supported the division of Euphorbiaceae into Platylobeae and Stenolobeae. A *croton sculpture* refers to the presence of polygonal elements, which may or may not have muri and modified columellae. Inaperturate pollen grains have thin foot layers and thin endexine while the colpate grains have both foot layers and thin endexine and such grains are commonly found in the subfamily Crotonoideae (Novicke 1994).

Jatropha curcas L. is gaining global attention as a source of biodiesel. Interspecific breeding among species has been described between *J. curcas* and species from sub-genera I and II, which extends the interest for *J. curcas* to the whole genus as a potential source of genes for the biodiesel business. Accurate species diagnosis and

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classification is therefore necessary. Below, we give a palynodescription by *light microscopy* (LM) and *scanning electron microscopy* (SEM) of over 30 wild and cultivated *Jatropha* species based on the experience accumulated in previous works (Bahadur et al. 1997, 2000, 2012).

Miller and Webster (1962) investigated 11 species of *Jatropha* belonging to four sections, *viz., Polymorphae, Macranthae, Jatropha, Mozinna* and two sub-sections *Eucurcas* and *Mozinna* and commented upon pollen diameter, lack of apertures, verrucate exine, the diversity in the crotonoid pattern. Punt (1962, 1987) made extensive survey of pollen morphology of Euphorbiaceae. Dehgan and Webster (1979) investigated pollen under SEM of 13 *Jatropha* species in both cultivated and wild individuals, i.e., *J. gallabatensis, J. integerrima*, *J. capensis, J. cathartica, J. podagrica, J. augustii, J. multifida*, *J. dioica, J. canescens, J. curcas, J. moranii*, *J. gossypiifolia* and *J. macrorhiza* and briefly commented on their exine patterns with reference to clava as smooth or striated as '**knobs**'.

Literature survey revealed that there are several *Jatropha* species which have not been adequately described palynologically. The species studied so far belong to both sub-genera and their palynodescriptions are also included to make meaningful summary of the *Jatropha* species being studied. However, only few studies do actually exist for Euphorbiaceae. Hesse (1980) investigated the ultrastructure of pollenkit in two species of *Euphorbia* and in *Mercurialis perennis.* Novicke (1994) investigated 69 species among 34 genera and 12 tribes and found a well defined crotonoid pattern with inaperturate pollen characterized by regularly arranged excrecsences of triangular or circular shape supported by baculate, baculoidate or spongy layer.

Takahasi et al. (2000) investigated 30 genera and 96 species of Acalyphoideae LM, SEM and TEM and noted heterogeneity of pollen types. Leticia et al. (2008) have investigated several taxa of Crotonoideae belonging to several sections by LM and SEM. Liu et al. (2007) investigated the ultrastructure of *J. curcas* pollen under SEM and TEM. Punt (1987) investigated pollen of 38 *Phyllanthus* species from New Guineae which he grouped them into various pollen types and commented on their advanced features. Pollen of *J. gossypiifolia* has been studied by Bahadur et al. (1997), *J. panduraefolia* and *J. integerrima* by Chaturvedi and Jehan (1982) and Bahadur et al. (2000). Lynch and Webster's (1975) paper contains some SE micrographs of *Jatropha* pollen but the palynodescriptions are not adequately given. Saad and Ghazaly (1988) made a comparative and comprehensive study (LM, SEM and TEM) on pollen morphology of some Euphorbiaceae of Egpyt including *J. glauca* and commented on inaperturate condition, crotonoid pattern, and clavae as hemispherical and striated. Bahadur et al. (2000, 2012) made a comprehensive light and ultrastructural (SEM) study of several cultivated and wild species of *Jatropha* and made interesting observations on exine features of taxonomic value. Silva Corsa (2008) investigated the pollen morphology of *Croton* species section *Lampricroton* and discussed the taxonomic implications. Recently, Chun et al. (2010) investigated in vitro maturation and germination, and pollen cytology of *J. curcas* pollen.

This paper presents a comprehensive update of analysis of LM/SEM studies of the pollen of all the *Jatropha* species to date apart from those species whose palynodata has not been fully provided by various workers.

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Below, we follow the synoptic classification of *Jatropha* by Dehgan and Webster (1979) to perform the palynodescription of the representative species in *Jatropha* and *Cnidoscolus*.

Genus: ***Jatropha***

Sub-genus: I. *Jatropha* Section: **1. *Jatropha*** Subsect: 1a ***Adenophorae*****:** *J. gossypiifolia*; *J. glandulifera*; *J. tanjorensis*; *J. isabelli*; *J. induta*; *J. maheshwarii*; *J. mutabilis*
Subsect: 1b ***Pubescentes***: No species was investigated.
Section: **2. *Collenucia***: No species was investigated.
Section: **3. *Spinosae***: No species was investigated.
Section: **4. *Peltatae*****:** *J. augustii*; *J. cathartica* (=*J. berlandieri*); *J. mollissima* (=*J. pohliana*); *J. multifida*; *J. podagrica*
Section: **5. *Tuberosae***
Subsect: 5a. ***Tuberosae***: *J. gallabatensis*; *J. heynei*; *J. lagarinthoides*
Subsect: 5b. ***Capenses*****:** *J. capense*
Section**: 6. *Polymorphae***
Subsect: 6a. ***Polymorphae***: *J. integerrima*; *J. panduraefolia*
Subsect: 6b. ***Hernandifoliae***: No species was investigated.
Subsect: 6c. ***Macrorhizae***: *J. macrorhiza*

Sub-genus: II. *Curcas*

Section: **1. *Curcas***: *J. curcas*; *J. villosa* (=*J. wightiana*); *J. rufescens*
Section: **2. *Platyphyllae***: *J. moranii*
Section: **3. *Loureira***
Subsect: 3a. ***Loureira***: No species was investigated.
Subsect: 3b**. *Canescentes***: *J. canescens*; *J. cinerea*
Subsect: 3c. ***Neopauciflorae***: No species was investigated.
Section: **4. *Mozinna***: *J. dioica*

Genus: ***Cnidoscolus quercifolius*** Phol. (= *J. phyllacantha* Mull. Arg); ***C. urens*** (L.) Arthur (= *J. urens longipedenculata* Brandegee).

Observations

To enable proper palynoanalysis and for ease of interpretation as per the existing nomenclature, the palynodata of the species from the literature has been compounded and presented under the heads LM and SEM according to the procedure used for their pollen observation (Erdtman 1952; Dehgan and Webster 1979; Bahadur et al. 2000, 2012). The general aspect of pollen grains as seen under light microscope for the species found in India in the genus *Jatropha* is shown in Fig. 4.1 while their observations are described under the respective species.

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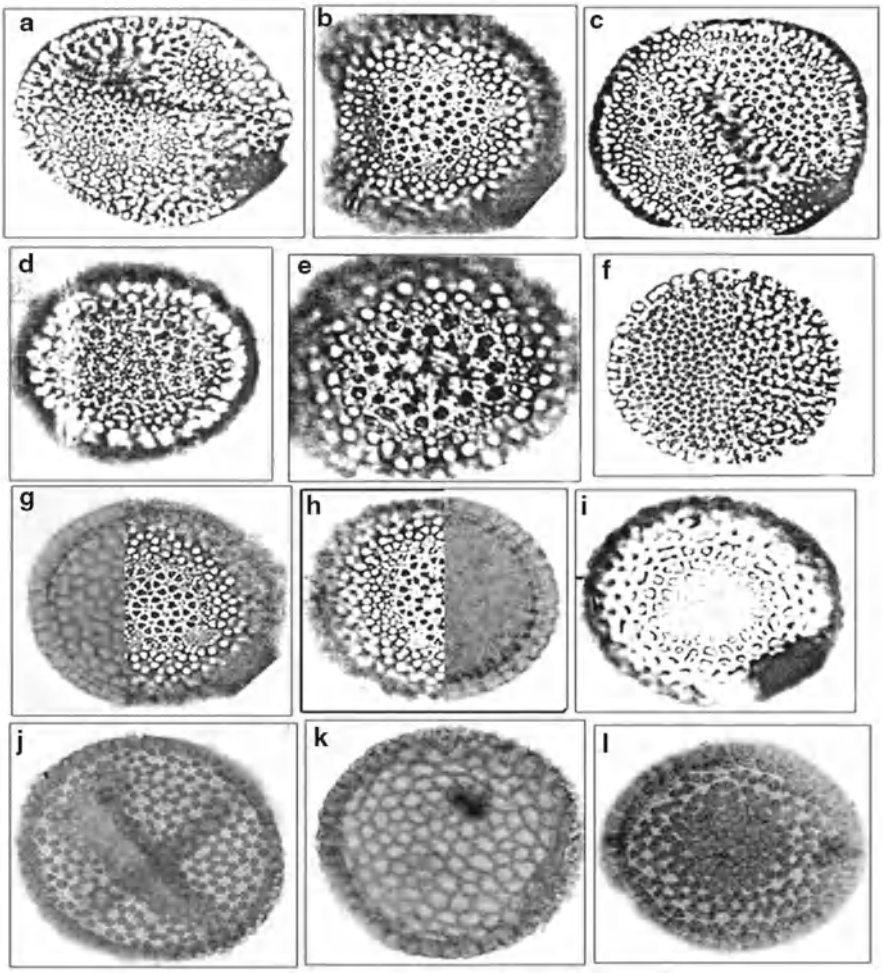

Fig. 4.1 Pollen of Indian *Jatropha* species as seen under LM showing lax or dense crotonoid pattern and clava (**a**) *J. gossypiifolia* var. *elegans* (× 990), (**b**) *J. gossypiifolia* var. *gossypiifolia* (× 990), (**c**) *J. glandulifera* (× 1000), (**d**) *J. heynei* (× 1000), (**e**) *J. tanjorensis* (× 1000), (**f**) *J. podagrica* (× 1000), (**g**) *J. maheshwarii* (× 1450), (**h**) *J. villosa* (× 1000), (**i**) *J. integerrima* (× 1000), (**j**) *J. panduraefolia* (× 1000), (**k**) *J. multifida* (× 1000), (**l**) *J. curcas* (× 1100). Note the Figs. (**d**, **f–h**) shows two segments. The photographs were taken at two different foci and the two sectors are pasted showing variation in exine referred to *Lux obscuritas* (LO analysis). (Source: Bahadur et al. (2000), and unpublished data)

J. curcas L.

LM: Pollen is ± oblate spheroidal to prolate spheroidal, outline circular, radially symmetrical, 61.6 μm in diam., inaperturate (omniaperturate), heavily sculptured with clavate or pilate processes (looking with a rod with swollen head) aligned

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reticulately (hexa or polygonal) to form crotonoid pattern, pattern lax, processes 3–4.5 μm long, heads ± rounded (4.37–5.25 μm diam.), large lumina (4.5–7.5 μm diam.), with groups of 3–6 much smaller free clavae or pila. Ectexine is much thicker (6.0 μm) than endexine (1.9 μm), which is feebly visible (Fig. 4.1e).

SEM: Pollen is ± spheroidal, inaperturate (omniaperturate) heavily sculptured with clavate or pilate processes (Fig. 4.2a) aligned reticulately forming crotonoid pattern. The heads of clavae or pila are more or less rounded with strikingly conspicuous vertical striae (Fig. 4.2b). Pila are confined to angles of penta to hexagonal brochi delimitating a lax crotonoid pattern with thick psilate (smooth) muri demarcating large lumina. Luminal centers are studded with 4–6 (or more) much smaller clava/pila (Fig. 4.2c). Pollen of *J. curcas* has been studied by Dehgan and Webster (1979) and Bahadur et al. (2000). The species *J. curcas* is said to be closely related to *J. afrocurcas* form Africa and several endemic Mexican species, but the material was not available for comparative investigation.

J. cathartica Terran. and Bertand

LM: study was not performed due to paucity of material.

SEM: Pollen is ± spheroidal, inaperturate (omniaperturate) with densely studded clavate processes (Fig. 4.2d) aligned reticulately in a crotonoid pattern (Dehgan and Webster 1979). The pila are balloon shaped apically, but somewhat narrow at base, which are fused with one another (Fig. 4.2e). Pila were reported to be psilate, but they form a cottony mass and are therefore designated as "hirsute". Brochi are penta to hexagonal with muri locally visible, narrow, smooth and irregular (Fig. 4.2f).

J. gallabatensis Schweinf.

LM: Pollen is ± spheroidal, radially symmetrical, 61.6 μm in diam., inaperturate (omniaperturate) heavily sculptured with pilate/clavate processes forming a penta or hexagonal crotonoid pattern moderately dense. Processes are 2.3–3.0 μm long with polygonal heads of 1.5–2.3 μm in diam. distributed according to psilate lumina of 2.3–3 μm in diameter. The ectexine is much thicker than endexine, which is feebly developed. Image not given.

SEM: Pollen is ± spheroidal, inaperturate (omniaperturate), densely studded with clavate processes aligned reticulately in a lax crotonoid pattern. Heads of clava/pila are triangular to ± rounded forming penta-hexagonal brochi whose muri are distinct, locally narrow, smooth and irregular with 1–4 much smaller free clava/pila also with rounded smooth heads (Fig. 4.3a, b).

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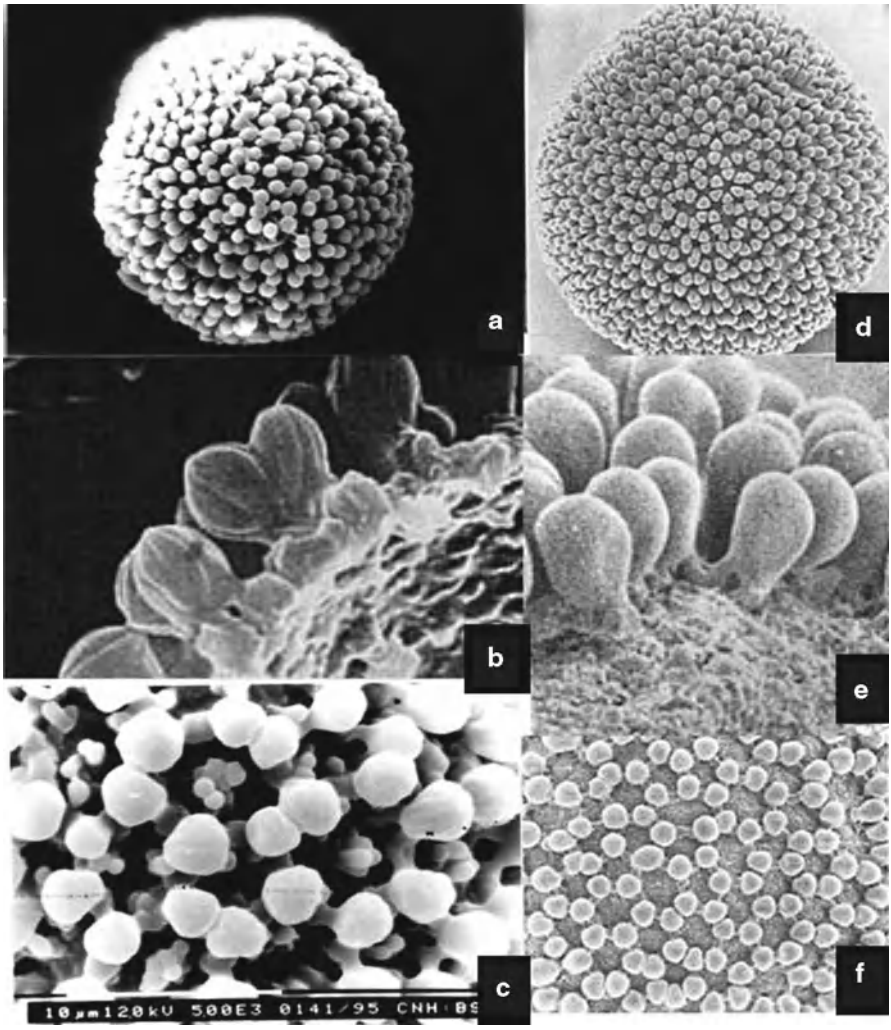

Fig. 4.2 Pollen morphology in (**a**) *J. curcas:* entire pollen grain showing a dense crotonoid pattern (× 1000), (**b**) clavae in a lateral view (× 2750) with conspicuous vertical striae. (**c**) Crotonoid pattern of clava distribution showing thick muroid ridges with luminal bacules in clusters (× 2500), (**d**) *J. cathartica:* entire pollen grain (× 900) showing a crotonoid pattern on the 'hirsute' mode. (**e**) Clavae in lateral view (× 1000) with thin muri and shallow lumen on exine (× 400), (**f**) crotonoid pattern of clava distribution (× 1200) (Source: Figs. (**b**, **d**–**f**) – Dehgan and Webster 1979; Figs. (**a**, **c**) – Bahadur et al. 2000)

J. canescens (Benth.) Muell. Arg.

SEM: Pollen is ± spheroidal, inaperturate (omniaperturate), densely studded with clavate processes aligned relatively in a lax crotonoid pattern. Heads of clava/pila are triangular to ± rounded forming penta-hexagonal brochi whose muri are distinct,

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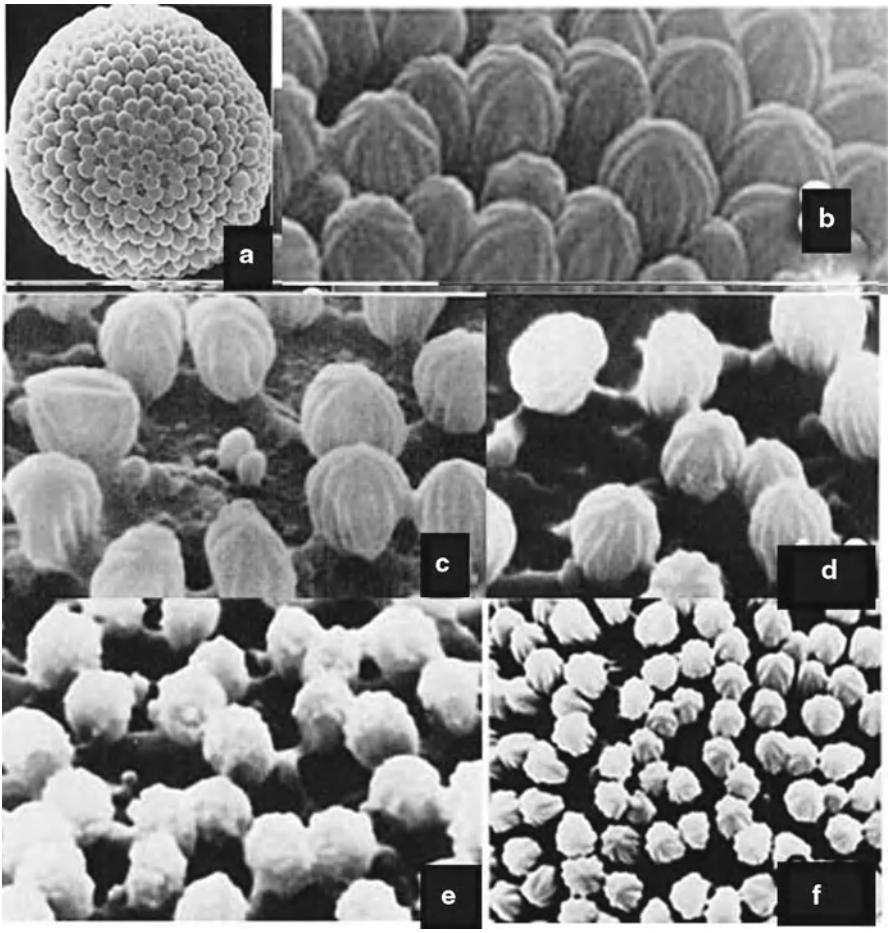

Fig. 4.3 Pollen morphology in (**a**) *J. gallabatensis:* entire pollen grain showing crotonoid pattern (× 1020), (**b**) enlarged portion of exine showing clavae with vertical striae (× 3800), (**c**) *J. canescens:* crotonoid pattern of clava with vertical striae (× 2500), (**d**) *J. macrorhiza:* crotonoid pattern showing clava and thin muri (× 4320), (**e** and **f**) *J. moranii:* crotonoid pattern of exine (× 3500) showing round clava echinate clavae. Crotonoid pattern of clavae (× 2500) (Source: Dehgan and Webster 1979)

locally narrow, smooth and irregular with much smaller free clava/pila also with rounded smooth heads (Fig. 4.3c).

J. macrorhiza Benth.

LM: Pollen ± spheroidal, inaperturate (omniaperturate) densely studded with clavate processes, aligned reticulately in a crotonoid pattern. Crotonoid pattern seems

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lax, heads of clava/pila triangular to ± to rounded. Brochi penta to hexagonal, muri distinct, locally narrow irregular with smaller free clava/pila, heads rounded smooth. Image not given.

SEM: Pollen is ± spheroidal, inaperturate (omniaperturate), densely studded with clavate processes aligned relatively in a lax crotonoid pattern. Heads of clava/pila are triangular to ± rounded forming penta-hexagonal brochi whose muri are distinct, locally narrow, smooth and irregular with much smaller free clava/pila also with rounded smooth heads (Fig. 4.3d).

J. moranii Dehgan and Webster

SEM: Pollen ± spheroidal, inaperturate (omniaperturate), densely studded with clavate processes, aligned relatively in a crotonoid pattern. Crotonoid pattern seems lax, heads of clava/pila triangular to rounded; brochi penta-hexagonal, muri distinct, locally narrow smooth irregular with much smaller free clava/pila, pila heads rounded smooth (Fig. 4.3e, f).

J. dioica Pax and K.

SEM: Pollen ± spheroidal, inaperturate (omniaperturate), densely studded with clavate processes, aligned reticulately in a crotonoid pattern. Crotonoid pattern seems lax, heads of clava/pila triangular to rounded. Brochi penta-hexagonal, muri distinct, locally narrow smooth irregular with much smaller free clava/pila, pila heads rounded smooth (Fig. 4.4a, b).

J. capensis (L.f.) Sond.

LM: Pollen ± spheroidal, radially symmetrical, 61.6 μm in diam., inaperturate (omniaperturate) heavily sculptured with pilate/clavate processes (penta or hexagonal) to form a crotonoid pattern; the pattern moderately dense, processes 2.3–3.0 μm long, heads angular (polygonal), 1.5–2.3 μm diam., lumina 2.3–3 μm in diam., psilate. Ectexine much thicker than endexine which is feebly developed. Image not given.

SEM: Pollen ± spheroidal, inaperturate (omniaperturate) densely studded with clavate processes, aligned reticulately in a crotonoid pattern; crotonoid pattern seems lax, heads of clava/pila triangular to ± rounded. Brochi penta-hexagonal, muri distinct, locally narrow smooth irregular with 1–4 much smaller free clava/pila, pila heads round and smooth (Fig. 4.4c).

This description is based on the image from Dehgan and Webster (1979).

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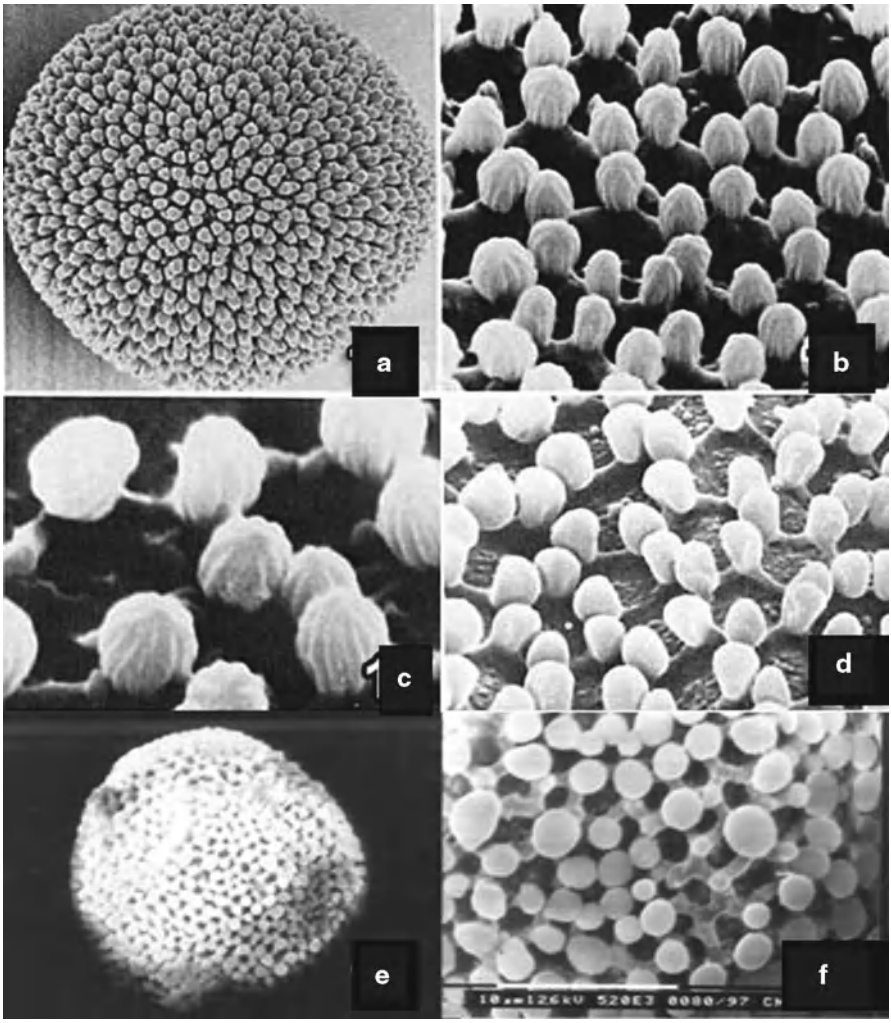

Fig. 4.4 Pollen morphology in (**a**) *J. dioica:* entire pollen grain (× 625*)* with high density crotonoid pattern of clavae. (**b**) small round shape clavae without rods, but with deep vertical striae in the globular heads (× 3100), (**c**) *J. capensis:* crotonoid pattern of exine showing vertically striated pear shaped clavae (× 7700), (**d**) *J. augustii:* crotonoid pattern of exine with shallow lumen and faintly striated clavae (× 950), (**e**) *Cnidoscolus* (= *J. urens*): entire pollen grain showing lax crotonoid pattern (× 550), (**f**) psilate spherical clavae of various sizes and thick muroid ridges (× 2000) (Source: Dehgan and Webster 1979)

J. augustii Pax and Hoffm.

SEM: Pollen ± spheroidal, inaperturate (omniaperturate) moderately densely studded with clavate processes aligned reticulately in a crotonoid pattern. Crotonoid pattern seems lax, heads of clava/pila triangular with vertical striations. Brochi

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penta-hexagonal, muri distinct, locally narrow smooth irregular with much smaller free clava/pila (Fig. 4.4d).

Cnidoscolus urens (L.) Arthur (=J. urens var. longipedenculata Brandegee)

LM: Pollen ± spheroidal, radially symmetrical 61.6 μm in diam., inaperturate (omniaperturate) heavily sculptured with pilate/clavate processes (penta or hexagonal) to form a crotonoid pattern; crotonoid pattern lax, processes 2.3–3.0 μm long, heads angular (polygonal), 1.5–2.3 μm in diam., lumina 2.3–3 μm in diam., psilate, exctexine much thicker than endexine which is feebly developed. Image not given.

SEM: Pollen ± spheroidal, inaperturate (omniaperturate) densely studded with clavate processes, aligned reticulately in a crotonoid pattern. Crotonoid pattern seems lax, heads of clava/pila triangular to ± rounded, brochi penta-hexagonal, muri distinct locally narrow, smooth irregular with 1–3 much smaller free clava/pila, pila heads rounded smooth (Fig. 4.4e, f).

J. gossypiifolia L. var. elegans (Pohl). Muell. Arg

LM: Pollen ± spheroidal to prolate, 82.0–85 × 73 μm, radially symmetrical, 75 μm in diam., inaperturate (omniaperturate), heavily sculptured with pilate/clavate processes aligned reticulately (penta or hexagonally) to form crotonoid pattern, processes 2.3–3.0 μm long, heads ± triangular 2.3 μm in diam., lumina of moderate size, 3–3.8 μm in diam. with a few much smaller free pila/clava. Ectexine thicker than endexine which is feebly developed (Fig. 4.1a).

SEM: Pollen ± spheroidal, inaperturate (omniaperturate), densely sculptured with clavate/pilate processes aligned reticulately in a crotonoid pattern; processes apparently confined to angles of faint brochi, crotonoid pattern with prominently dense heads of clava/pila crowded abutting on each other laterally (Fig. 4.5a); head of processes triangular with crenate margins; clava prominently striate, 12–17 (average 13) ridges and grooves of same width, striae in a radial pattern around a central smooth area on clava/pila heads; lumina small, irregular, smooth with no free processes (Fig. 4.5b). Pollen of *J. gossypiifolia* has been studied by Bhoj Raj (1966), Dehgan and Webster (1979), Bahadur et al. (1997, 2000). It may be of noted that Dehgan and Webster (1979) used pollen from plants of Indian origin.

J. gossypiifolia var. *gossypiifolia* L.

LM: Pollen ± oblate spheroidal to spheroidal radially symmetrical, 55.5 μm in diam., inaperturate (omniaperturate); heavily sculptured with pilate/clavate processes

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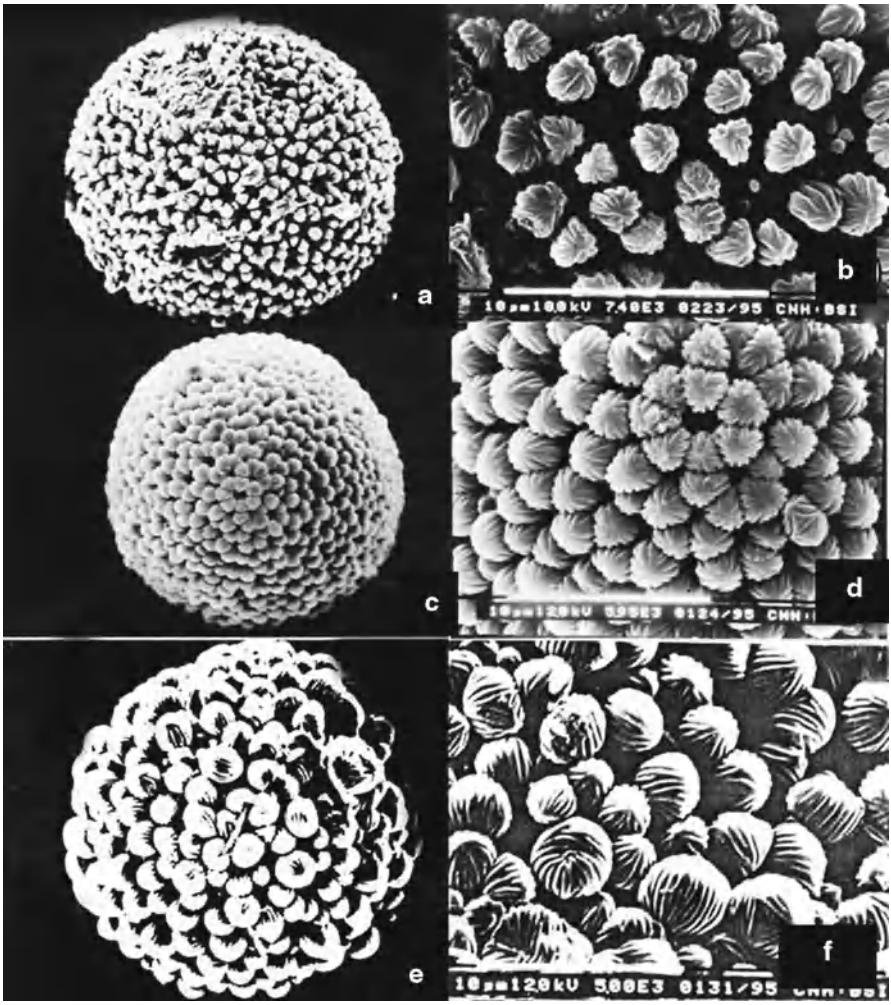

Fig. 4.5 Pollen morphology in (**a**) *J. gossypiifolia* var. *elegans:* entire pollen grain with lax crotonoid pattern (× 550), (**b**) exine showing deep lumen with vertically striated clavae. (**c**) *J. gossypiifolia* var. *gossypiifolia:* entire pollen grain showing crotonoid pattern of high density clavae, (**d**) vertically striated clavae with triangular completely covering lumina, (**e**) *J. tanjorensis:* entire pollen grain with high clava density and unclear pattern of clava distribution. (**f**) Heavily sculptured polymorphic round clavae (× 550) with various transverse patterns of striae (× 2500) (Source: 2000)

aligned reticulately (penta or hexagonally) to form a lax crotonoid pattern, processes 2.3–3.0 μm long, heads ± triangular 1.5 μm in diam., lumina fairly large, psilate. Ectexine thick 6.25 μm, and thicker than endexine which is feebly developed; columella about 1.6 μm long (Fig. 4.1b).

SEM: Pollen ± oblate to spheroidal, inaperturate (omniaperturate), densely sculptured with clavate/pilate processes aligned reticulately in a crotonoid

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pattern; processes confined to angles of faint brochi. Crotonoid pattern lax, muri delimiting lumina faint, heads of clava/pila essentially triangular with prominently crenate margins, 3.2 μm long high, capitate, 2.23 μm in diam. clava prominently striate, average grooves broader or of the striae average, grooves broader or of same width as ridges; strine in radial pattern on heads of processes; lumina large, generally smooth, locally with 1–3 very small free clava/pila (Fig. 4.5c, d). Pollen of this species has been studied by Dehgan and Webster (1979), and Bahadur et al. (1997, 2000). The above description is based on our observations.

J. tanjorensis J.L. Ellis and Saroja

LM: Pollen ± spheroidal, radially symmetrical, 61.6 μm in diam., inaperturate (omniaperturate), heavily sculptured with clavate/pilate processes aligned reticulately (penta or hexagonally) to form a crotonoid pattern, the pattern dense. Processes fairly large 3.8–5.4 μm long, heads triangular to polygonal 3.8 μm in diam., lumina small, locally with a few small clava/pila. Ectexine much thicker than endexine which is feebly developed (Fig. 4.1d).

SEM: Pollen ± spheroidal, inaperturate (omniaperturate) heavily sculptured with clavate processes, aligned reticulately in a crotonoid pattern. Processes seem to be larger than heads than in other species, apparently confined to angles of faint brochi; crotonoid pattern moderately dense. Lumina irregular of various sizes, muri faint, not seen distinctly; heads of clava rounded, clava prominently striate, striae narrow 16–24 (average 18) per clava/pila; radiating from a central smooth area on clava heads, lumina usually with 1–3 much smaller free clava/pila lumina/clava also striate. Pollen of this species has been studied by Bahadur et al. (2000). The above description is based on our observations (Fig. 4.5e, f).

J. glandulifera Roxb.

LM: Pollen ± spheroidal, radially symmetrical 60.0 μm in diam., inaperturate (omniaperturate). Heavily sculptured with pilate-clavate processes aligned reticulately (pentagonally or hexagonally) to form crotonoid pattern. Processes 3.0 μm long, heads of processes 2.3 μm in diam., ± triangular, rather loosely spaced. Lumina with 1–3 inconspicuous free pila/clava. Ectexine much thicker than endexine, feebly developed (Fig. 4.1c).

SEM: Pollen ± spheroidal, inaperturate (omniaperturate) heavily sculptured with clavate/pilate processes, aligned reticulately in a crotonoid pattern; processes apparently confined to angles of faint brochi; muri delimiting lumina indistinct, heads of clava/pila ± rounded to subtriangular, processes faintly stri-

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ate, striae 13 (average 10), radiating on clava/pila heads; lumina large, irregular, smooth devoid of free processes. Pollen of *J. glandulifera* has been earlier studied by Bahadur et al. (2000). The above description is based on our observations (Fig. 4.6a, b).

J. panduraefolia Andr.

LM: Pollen ± spheroidal, radially symmetrical 66.0 μm in diam., inaperturate (omniaperturate) heavily sculptured with pilate/clavate processes aligned reticulately (penta or hexagonally) forming a crotonoid pattern; crotonoid pattern lax, processes 1.8–3.0 μm long heads more or less triangular 1.5–2.3 μm diam., lumina fairly large, 3.8–5.2 μm in diameter, psilate, ectexine much thicker than endexine is feebly developed (Fig. 4.1g).

SEM: Pollen ± spheroidal, inaperturate (omniaperturate), heavily sculptured with clavate/pilate processes aligned reticulately in a crotonoid pattern moderately dense, brochi penta or hexagonal, muri distinct, locally narrow, psilate lumina irregular, fairly large, mostly smooth occasionally with 1–3 much smaller free clava/pila; clava/pila heads more or less rounded to angular smooth (Fig. 4.6c, d).

Pollen of *J. panduraefolia* has been studied earlier by Chaturvedi and Jehan (1982) and Bahadur et al. (2000). Although the species is considered as synonymous to *J. integerrima,* the microcharacters of pollen and leaf and some other characters are different and needs further taxonomic assessment.

Pollen of *J. hastata* considered synonymous to *J. integerrima* has also been scanned and studied; it shows lax crotonoid pattern with deep lumen with few luminal bacules, and psilate clava. Images are not provided.

J. integerrima Jacq.

LM: Pollen ± oblate to prolate spheroidal, radially symmetrical 58.5 μm in diam., circular in outline inaperturate (omniaperturate) heavily sculptured with pilate/clavate processes aligned reticulately penta to hexagonal crotonoid pattern; moderately dense processes 3.8–5.0 μm long, heads ± rounded 2.3 μm in diam., closely spaced, lumina small. Ectexine much thicker, 6 μm thick, endexine 1.9 μm, feebly developed (Fig. 4.1f).

SEM: Pollen ± spheroidal, inaperturate (omniaperturate) heavily sculptured with clavate/pilate processes aligned reticulately in a crotonoid pattern. Processes confined to angles of faint brochi, crotonoid pattern moderately dense, brochi penta or hexagonal, muri faint, Heads of clava/pila ± round to sub- triangular apparently with vertical striations, can be seen with the shadow effect. Lumina small to medium of different sizes smooth, locally with 2–6 small luminal bacules (Fig. 4.6e, f).

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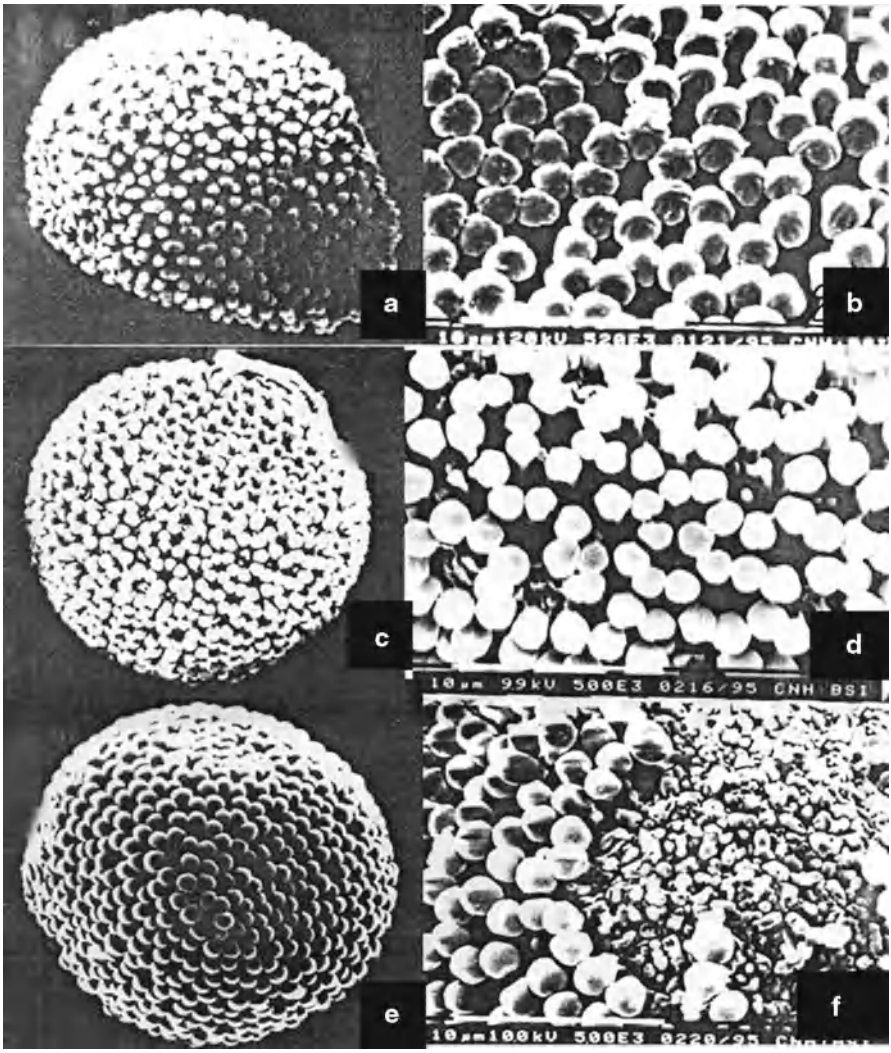

Fig. 4.6 Pollen morphology in (**a**) *J. glandulifera:* entire pollen grain showing lax crotonoid pattern (× 500), (**b**) poly-hexagonal lax crotonoid pattern of round shape clavae with vertical striae (× 2550), (**c**) *J. panduraefolia:* entire pollen grain with lax crotonoid pattern (× 550), (**d**) exine showing poly-hexagonal pattern, psilate clavae (× 2600), (**e**) *J. integerrima:* entire pollen grain showing dense and compact clava arrangement (× 550), (**f**) exine showing crotonoid pattern of clava distribution. Loss of clavae during acetolysis can be seen on the right side. Clavae round and striated (× 2500) (Source: Bahadur et al. 2000)

Pollen of *J. integerrima* has been studied by Chaturvedi and Jehan (1982), Dehgan and Webster (1979) and Bahadur et al. (2000). The above description is based on our observations.

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J. multifida L.

LM: Pollen ± spheroidal, radially symmetrical 51.0 μm in diam., inaperturate (omniaperturate) heavily sculptured with clavate processes aligned reticulately (penta or hexagonal) forming crotonoid pattern; crotonoid pattern lax, muri smooth, locally seen clearly; pila/clava at angles of brochi, processes 3.8 μm long, heads triangular, 1.8 μm in diam., lumina 3.8–4.5 μm in diam, psilate. Ectexine much thicker than endexine which is feebly developed (Fig. 4.1i).

SEM: Pollen ± spheroidal, inaperturate (omniaperturate) heavily sculptured with clavate/pilate processes aligned reticulately in a crotonoid pattern. Brochi penta to hexagonal, processes confined to angles of brochi, crotonoid pattern moderately dense lax due to distinct psilate, muri delimiting lumina. Clava/pila smooth heads ± rounded to sub-circular, lumina appears smooth, careful examination shows 4–6 linear striations. Lumen with 1–3 luminal bacules (Fig. 4.7a, b).

Pollen of *J. multifida* has been studied earlier by Dehgan and Webster (1979) and Bahadur et al. (2000). The above description is based on our observations.

J. podagrica Hook.

LM: Pollen ± spheroidal, radially symmetrical 61.6 μm in diam., inaperturate (omniaperturate) heavily sculptured with pilate/clavate processes (penta to hexagonal) forming a crotonoid pattern. Crotonoid pattern moderately dense, processes 2.3–3.0 μm long, heads angular (polygonal), 1.5–2.3 μm in diam., lumina 2.3–3 μm in diam., psilate, ectexine much thicker than endexine, feebly developed (Fig. 4.1h).

SEM: Pollen ± spheroidal, inaperturate (omniaperturate) densely studded with clavate processes, aligned reticulately in a crotonoid pattern. Crotonoid pattern seems lax, heads of clava/pila triangular to more or less rounded. Brochi penta to hexagonal, muri distinct locally narrow smooth irregular, lumen with 1–3 much smaller free clava/pila, pila heads round 4.3–63 μm smooth (Fig. 4.7c, d).

Pollen of *J. podagrica* has been earlier studied by Dehgan and Webster (1979) and Bahadur et al. (2000). The above description is based on our observations.

J. isabelli Muell. Arg.

LM: Pollen ± spheroidal, radially symmetrical 61.0 μm in diam., inaperturate (omniaperturate) heavily sculptured with pilate/clavate processes (penta or hexagonally) to form a crotonoid pattern; crotonoid pattern moderately dense. Processes 2.3–3.0 μm long, heads angular (polygonal), 1.5–2.3 μm in diam., lumina 2.3–3 μm in diam., psilate. Extexine much thicker than endexine, feebly developed. Image not given.

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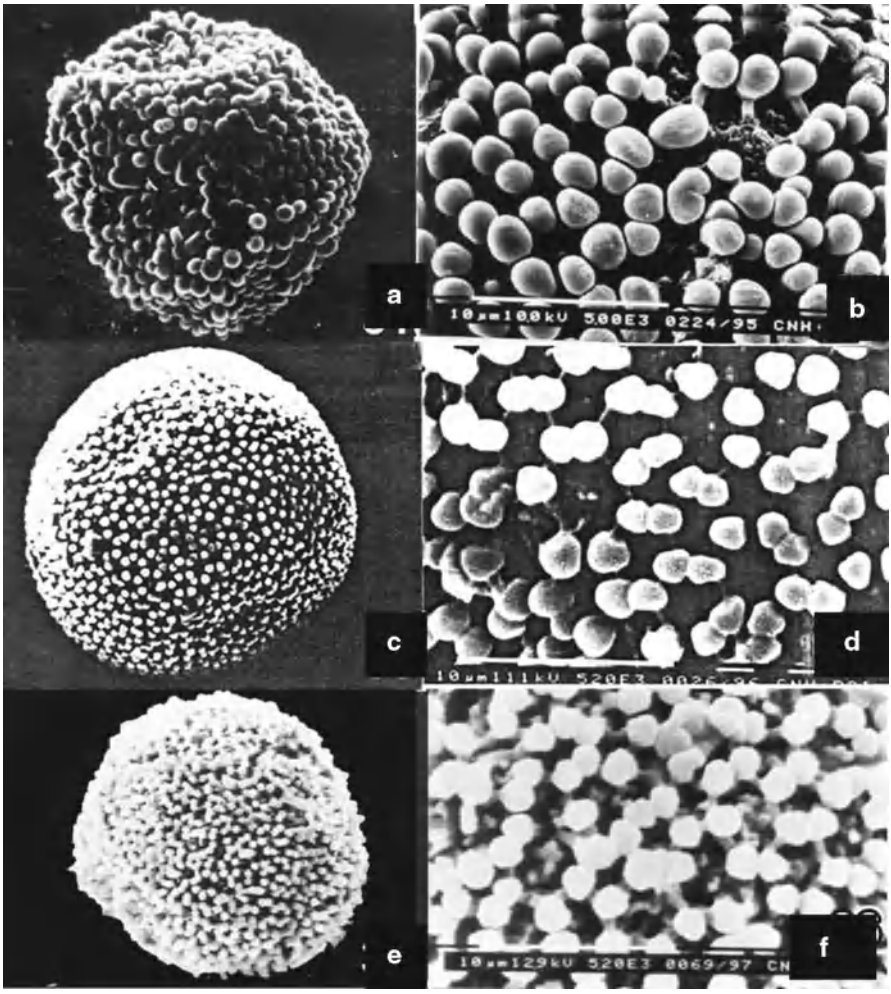

Fig. 4.7 Pollen morphology in (**a**) *J. multifida:* entire pollen grain with moderately dense crotonoid pattern of clava distribution (× 550), (**b**) exine showing psilate clavae of triangular to round section with faint striations. The lumen is buried and enclosed in thin muroid ridges (× 2500), (**c**) *J. podagrica:* entire pollen grain with lax crotonoid pattern (× 550), (**d**) exine of round psilate clavae and large, shallow lumina (× 2500), (**e**) *J. isabelli:* entire pollen grain showing moderately lax crotonoid pattern (× 500), (**f**) exine showing poly-hexagonal crotonoid pattern with deep lumina and small luminal bacules (× 2500) (Source: Bahadur et al. 2000, 2012)

SEM: Pollen ± spheroidal, inaperturate (omniaperturate) densely studded with clavate processes, aligned reticulately in a crotonoid pattern. Crotonoid pattern seems lax, heads of clava/pila triangular to ± rounded. Brochi penta-hexagonal, muri distinct locally narrow smooth irregular with 1–4 much smaller free clava/pila, pila, head round, smooth (Fig. 4.7e, f).

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J. rufescens Brandg.

LM: Pollen ± spheroidal, radially symmetrical, 61.6 μm in diam., inaperturate (omniaperturate) heavily sculptured with pilate/clavate processes (penta or hexagonal) to form a crotonoid pattern; crotonoid pattern moderately dense, processes 2.3–3.0 μm long, heads angular (polygonal), 1.5–2.3 μm in diam., lumina 2.3– μm in diam., psilate. Ectexine much thicker than endexine which is feebly developed. Image not given.

SEM: Pollen ± spheroidal, inaperturate (omniaperturate) densely studded with clavate processes, aligned reticulately in a crotonoid pattern. Crotonoid pattern seems lax, heads of clava/pila triangular to ± rounded. Brochi penta-hexagonal, muri distinct, locally narrow smooth irregular with 1–4 much smaller free clava/pila, pila heads rounded and smooth (Fig. 4.8a, b).

J. lagaranthoides Sond.

LM: Pollen ± spheroidal, radially symmetrical, 61.6 μm in diam., inaperturate (omniaperturate), heavily sculptured with pilate/clavate processes (penta or hexagonal) to form a crotonoid pattern; crotonoid pattern moderately dense, processes 2.3–3.0 μm long, heads angular (polygonal), 1.5–2.3 μm in diam., lumina 2.3–3 μm in diam., psilate. Ectexine much thicker than endexine which is feebly developed. Image not given.

SEM: Pollen ± spheroidal, inaperturate (omniaperturate) densely studded with clavate processes, aligned reticulately in a crotonoid pattern. Crotonoid pattern seems lax, heads of clava/pila triangular to ± rounded; brochi penta-hexagonal, muri distinct, locally narrow irregular with 1–4 much smaller free clava/pila, pila heads round smooth (Fig. 4.8c, d).

J. induta Chodat and Hassl.

LM: Pollen ± spheroidal, radially symmetrical 61.6 μm in diam., inaperturate (omniaperturate) heavily sculptured with pilate/clavate processes (penta or hexagonal) to form a crotonoid pattern; crotonoid pattern moderately dense process 2.3–.0 μm long heads, angular (polygonal), 1.5–.3 μm in diam., lumina 2.3–3 μm in diam. psilate. Ectexine much thicker than endexine which is feebly developed. Image not given.

SEM: Pollen ± spheroidal, inaperturate (omniaperturate) densely studded with clavate processes, aligned reticulately in a crotonoid pattern. Crotonoid pattern seems lax, heads of clava/pila triangular to ± rounded. Brochi penta–hexagonal, muri distinct locally narrow smooth irregular with 1–4 much smaller free clava/pila, pila heads rounded smooth (Fig. 4.8e, f).

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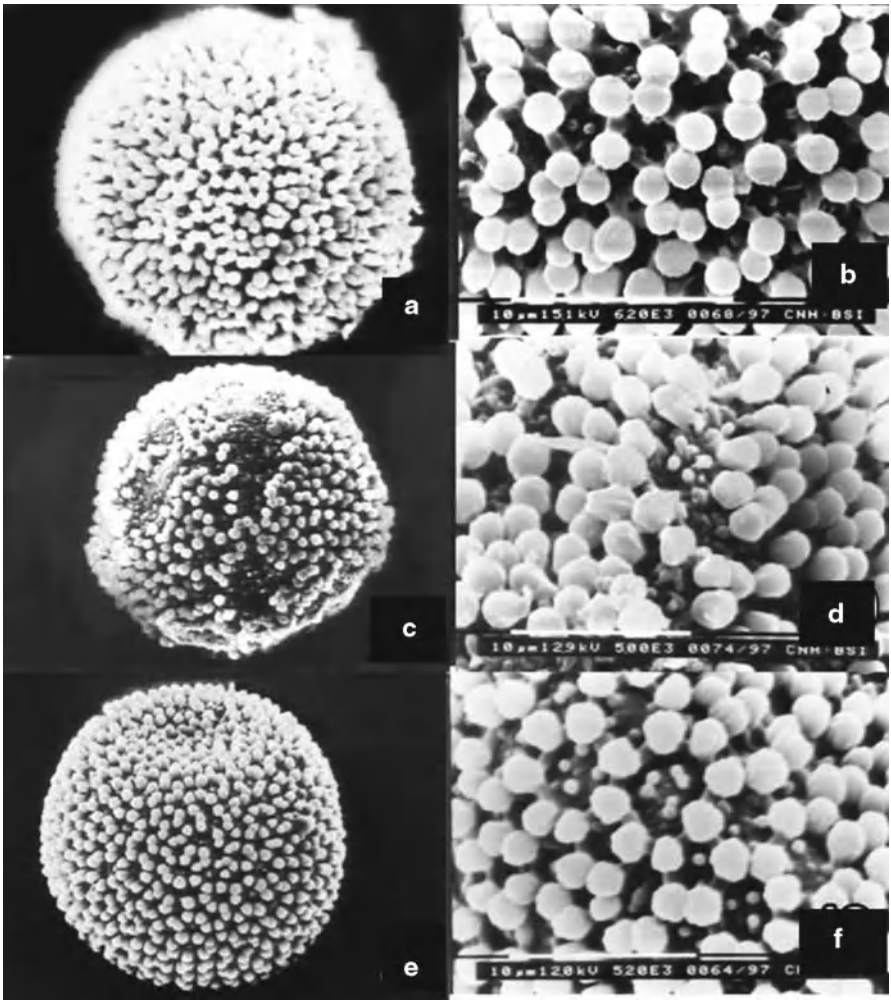

Fig. 4.8 Pollen morphology in (**a**) *J. rufescens:* entire pollen grain showing moderately dense pattern, (**b**) crotonoid pattern of 5–6 clava forming ring, muri thin, lumen large irregular with luminal bacula, (**c**) *J. lagaranthoides:* entire pollen showing loss of clava during acetolysis, crotonoid pattern dense, (**d**) faintly striated clava, lumen large irregular with luminal bacules (× 550), faintly striated clava held with thick muri, lumen deep with luminal bacules, conspicuous at clava base (× 2500), (**e**) *J. induta:* entire pollen showing moderately lax crotonoid pattern (× 500), exine with hexa to polygonal pattern, clava triangular to circular with striations conspicuous at base, (**f**) deep lumen, thick muri with large luminal bacules (× 2500) (Source: Bahadur et al. 2012)

J. mutabilis (Pohl) Baill.

LM: Pollen ± spheroidal, radially symmetrical 61.6 μm in diam., inaperturate (omniaperturate) heavily sculptured with pilate/clavate processes (penta to hexagonal)

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forming a crotonoid pattern; crotonoid pattern moderately dense, processes 2.3–0.0 μm long, heads angular (polygonal), 1.5–2.3 μm in diam, lumina 2–3 μm diam., psilate ectexine much thicker than endexine which is feebly developed. Image not given.

SEM: Pollen ± spheroidal, inaperturate (omniaperturate) densely studded with clavate processes, aligned reticulately in a crotonoid pattern. Crotonoid pattern seems lax, heads of clava/pila triangular to ± rounded 4.2 μm. Brochi penta-hexagonal, muri distinctly local narrow, smooth irregular with 5–7 much smaller free clava/pila, pila heads rounded psilate (Fig. 4.9a, b).

J. mollissima (Pohl) Baill.

LM: Pollen ± spheroidal to oval radially symmetrical, 50.6 μm in diam., inaperturate (omniaperturate) heavily sculptured with pilate/clavate processes (penta or hexagonal) to form a crotonoid pattern; crotonoid pattern lax, clava processes psilate, 2.3–3.0 μm long, heads triangular-angular (polygonal), 2.2–3.8 μm. Lumina 2.3–5 μm in diam., deep with 1–3 luminal bacules psilate, ectexine much thicker than endexine, feebly developed. Image not given.

SEM: Pollen ± spheroidal, inaperturate (omniaperturate) densely studded with clavate processes, aligned reticulately in a crotonoid pattern. Crotonoid pattern seems lax, heads of clava/pila triangular to ± rounded. Brochi, penta–hexagonal, muri distinct, locally narrow smooth irregular with 1–3 much smaller free clava/pila, pila heads round and smooth (Fig. 4.9c, d).

J. berlandieri Torr.

LM and SEM: Pollen ± spheroidal, radially symmetrical, 66.6 μm in diam., inaperturate (omniaperturate) heavily sculptured with pilate/clavate processes (penta or hexagonal rarely more) to form a crotonoid pattern; crotonoid pattern moderately dense, muri indistinct, processes 2.3–3.0 μm long, heads round angular, 1.6–2.3 μm in diam., coalesced forming distinct rings, semi circular structures or chain of 2–3 clava, lumina 2.3–3 μm in diam. deep, clava with few faint striations. Ectexine much thicker than endexine, feebly seen (Fig. 4.9e, f).

J. maheshwarii Subram and M.P. Nayar.

LM: Pollen grains ± oblate spheroidal, 80 μm across, inaperturate (omniaperturate) circular, aligned reticulately in a crotonoid pattern, pattern moderately dense. Exine 6.4 μm thick, ectexine thick, sculpturing clavate, clavae 4.8 μm long, 3.5–4.5 μm in

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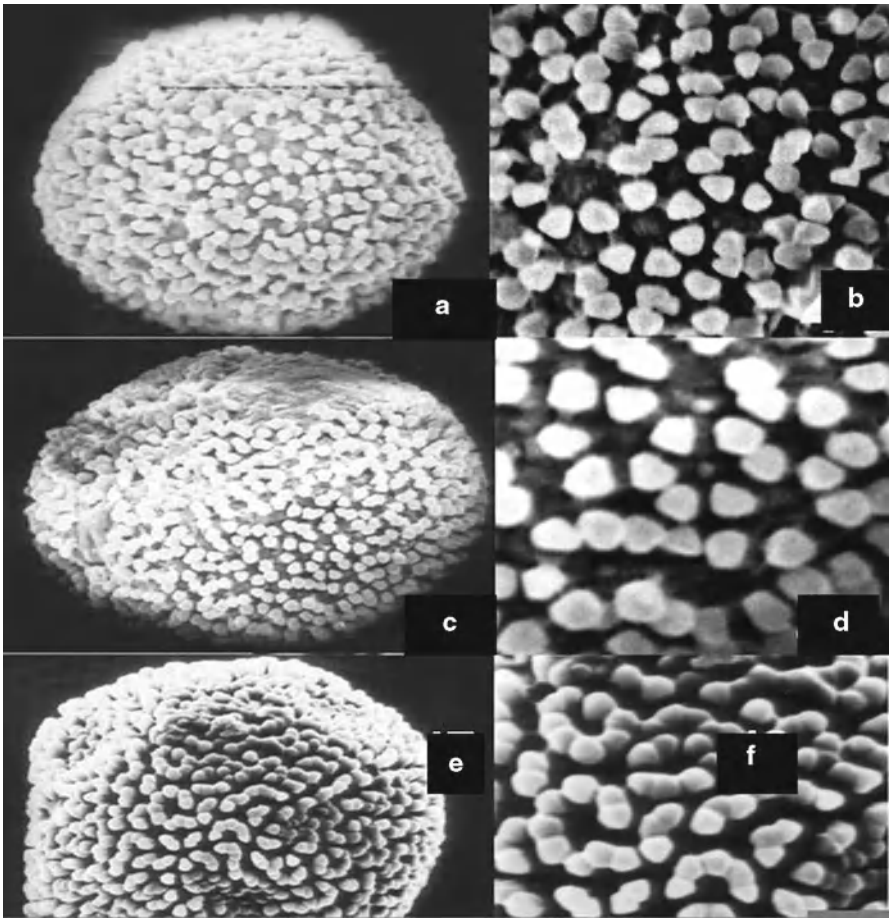

Fig. 4.9 Pollen morphology in (**a**) *J. mutabilis:* entire pollen grain showing moderately lax crotonoid pattern (× 500), (**b**) exine poly-hexagonal, 5–8 clava forming ring, muroid ridges short and thin, lumen deep and luminal bacules (× 2700), (**c** and **d**) *J. mollissima:* entire pollen grain showing lax crotonoid pattern with 7–9 luminal bacules (× 500), (**e** and **f**) *J. berlandeiri:* entire pollen grain showing crotonoid pattern moderately compact crotonoid pattern of 7–9 clava, laterally fused forming circular/rod like structures, muri indistinct, lumina deep with few luminal bacules (Source: Bahadur et al. 2000)

diameter, clava striated, muri indistinct lumen deep irregular with several luminal bacules (Fig. 4.1k).

SEM: Pollen oblate spheroidal, inaperturate (omniaperturate) aligned reticulately in a dense crotonoid pattern of penta-hexagonal pattern, clava round to circular about 5 μm across the head of clava deeply grooved forming 7–9 distinct thick transverse bands, muri indistinct, lumen small irregular studded with round luminal bacules. This species shows some degree of resemblance with *J. tanjoresnsis* in view of the similarity in clava ornamentations (Fig. 4.10a–c).

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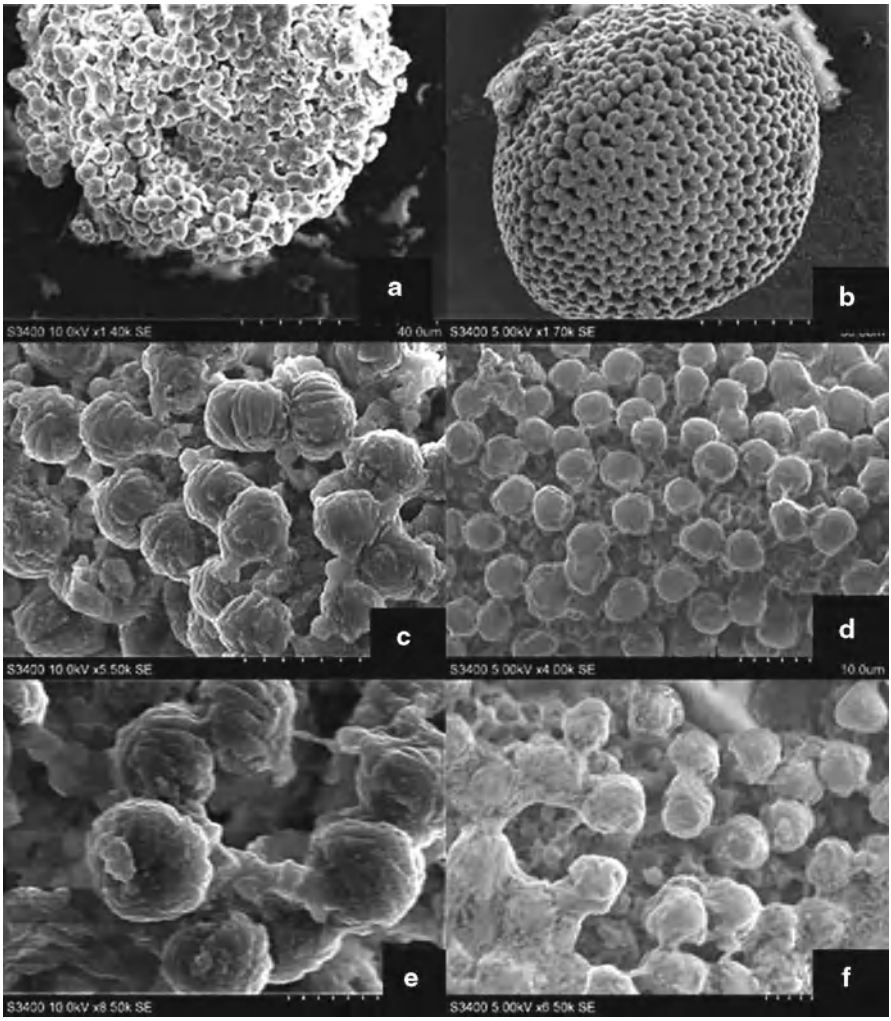

Fig. 4.10 Pollen morphology in (**a**) *J. maheshwarii:* entire pollen grain damaged during acetolysis showing compact crotonoid pattern (× 470), (**b**) exine showing pentagonal crotonoid pattern, clava round to spherical, transversely striated (× 2500), lumina deep with luminal bacules, muri indistinct, (**c**) (× 3000), (**d**) *J. heynei:* entire pollen grain showing moderately dense crotonoid pattern hexagonal clava (× 560), (**e** and **f**) clava with round head with distinct downwardly projecting dentate ring/cap/teeth crotonoid pattern of 6–9 clava, muri feebly developed, with 5–7 luminal bacules (× 2950) (Source: Bahadur et al. 2012)

J. heynei Balakr.

LM: Pollen grains oblate to spheroidal, 86.7 μm across, outline circular, inaperturate (omniaperturate) aligned reticulately in a crotonoid pattern, crotonoid pattern moderately dense. Exine thick (4.8–6.4 μm, ectexine 1.6 μm thick), sculpturing

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clavate, clava 4.8 μm long, 33.5–4.5 μm in diam., clava with cap like structure on the clava head, muri indistinct, lumen deep with luminal bacules (Fig. 4.1j).

SEM: Pollen oblate spheroidal, inaperturate (omniaperturate) in crotonoid pattern of 5–6 clava, clava head with distinct cap with dentate/serrations facing downwards the rim of identical serrations, giving the appearance of soda bottle lid cap (Fig. 4.10d–f).

J. pelargoniifolia Courb. *(=J. glandulosa* Vahl*).*

LM: Pollen spheroidal, radially symmetrical, 55.6 μm in diam., inaperturate (omniaperurate) heavily sculptured with pilate/clavate processes (penta to hexagonal) forming crotonoid pattern, pattern moderately dense, processes 2.3–3.0 μm long, heads round 1.23–2.3 μm in diam., lumina 2.3–3 μm in diam., with 5–6 vertical striations, ectexine much thicker than endexine, feebly developed. Image not given.

SEM: Pollen spheroidal, inaperturate (omniaperturate) densely studded with round clavae, processes aligned reticulately in a crotonoid pattern, crotonoid pattern moderately dense, heads of clava/pila triangular to ± rounded. Brochi penta-hexagonal, muri distinct, locally narrow irregular with 1–3 much smaller free clava/pila (Fig. 4.11a, b). This study is based on the images given by Dehgan and Webster (1979).

J. villosa Wight *(= J. wightiana* Muell. Arg*).*

LM and SEM: Pollen grains radially symmetrical, 55.3 μm across, aperturate, (omniaperturate), heavily sculptured, pattern moderately dense, penta-hexagonal; loss of clava during acetolysis can be seen, clava round psilate, muri faint or indistinct, lumen deep without luminal bacules (Figs. 4.1h and 4.11c).

Cnidoscolus quercifolius Phol *(= J. phyllacantha* Muell. Arg*).*

LM and SEM: Pollen ± spheroidal, radially symmetrical 63.6 μm in diam., inaperturate (omniaperturate) heavily sculptured with pilate/clavate processes (penta or hexagonal) form a crotonoid pattern; crotonoid pattern moderately dense, clava processes club shaped, 2.3–3.0 μm long, heads cylindrical, 2.3–5.0 μm in diam., muri indistinct lumina indistinct, psilate. Ectexine much thicker than endexine, feebly developed (Fig. 4.11d).

J. cinerea (Ortega) Muell. Arg.

SEM: Pollen ± spheroidal, radially symmetrical, 58.0 μm in diam., inaperturate (omniaperturate) heavily sculptured with pilate/clavate processes (penta or hexago-

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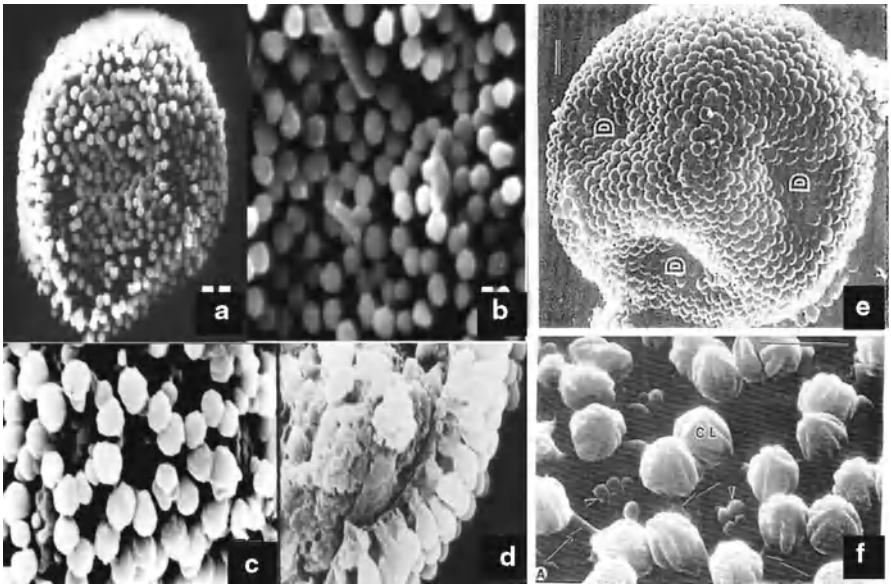

Fig. 4.11 Pollen morphology of (**a**) *J. pelargonifolia:* exine enlarged showing moderately dense crotonoid pattern, (**b**) clava round to spherical with vertical striations and luminal bacules, (**c**) *J. villosa:* exine enlarged showing crotonoid pattern, net work of 8–9 round clava (× 2550), (**d**) *Cnidoscolus quercifolius* Phol (=*J. phyllacantha):* damaged pollen showing clava base, one can visualize this as distinct concave impressions on spongy subexinous spongy layer. Clava densely studded club shaped, psilate (× 2500), (**e** and **f**) *J. glauca:* showing depressions over the grain (c ×700), exine showing crotonoid pattern, muri and clavae with linear striations (×2600) (Source: Bahadur et al. 2012; Sadd and Ghazaly 1988)

nal) to form lax crotonoid pattern; muri thin, heads of clava triangular to polygonal vertically striate; lumen with smaller bacules. Ectexine much thicker than endexine, feebly developed. Images are not given.

J. glauca Vahl.

This Egyptian species was investigated by Saad and Ghazaly (1988) under LM, SEM and TEM to understand the nature of exine at the ultrastrcutural level. The palynodescription given by them is somewhat modified and presented as under. Pollen grains inaperturate, spheroidal 65–83 μm in diameter, often with few to six concave depressions around the exine wall. These sunken areas are of the spine and real and probably function for adaptation of volume changes (may be due to the arid conditions) and represent loss of function of endexine as a protective and harmomegathic layer. Exine semitectate, 0.5 μm thick, intine 2.5 μm thick. Crotonoid pattern lax but conspicuous, clavate, clava ampullo/hemispherical, vertically striated supported by few short stalks located on the foot layer, muri connecting the clavae, with large prominent luminal bacules (Fig. 4.11e, f).

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Discussion

The Euphorbiaceae is one of the largest cosmopolitan family that includes an enormous variety of pollen types and represents a great interest for palynologists. Based on the present knowledge of pollen characters and the classification by Punt (1987), the various sub-families can be briefly summarized palynolgically as follows:

1. **Phyllanthoideae**:

(a) Colporate pollen with a weakly reticulate pattern without much ornamentation and considered primitive.
(b) Coarsely reticulate and oblate pollen.

2. **Oldfieldioideae**: Homogenous and polyaperturate (six or more apertures) or pantoaperturate pollen.
3. **Acalyphoideae**: Heterogenous pollen with large variations of morphology. The pollen types might be linked to phylogenetic relationships.
4. **Crotonoideae**: The crotonoid pattern is found in different pollen types and varies in several features, such as apertures, shape and size of clavae.
5. **Euphorbioideae**: Colporate pollen belonging to 3–6 types, consistent in shape, size and ornamentation.

According to Novicke (1994) the sub-families Oldfieldoideae, Crotonoideae, Euphorbioideae would form a natural group while the Phyllanthaoideae and Acalyphoideae would belong to different groups on the basis of palynological features.

The sub-family of Crotonoideae comprises 173 genera distributed among 13 tribes, with *Croton, Jatropha* and *Manihot* accounting for about 1,000 species on their own. The pollen of Crotonoideae is spheroidal, inaperturate with a sexine that consists of well defined, regularly arranged excrescneces, which may be triangular or rounded and are supported by a baculate, baculoidate or spongy layer. The triangular supratectal elements are attached to a network of muri having short or irregular columellae visible on SEM micrographs where clavae were detached by chemical treatments. The triangular elements can be striate, furrowed and ridged or psilate with long attenuate apices, which may be echinate. The sub-units may be closely spaced or arrayed in a more open configuration pattern (Novicke 1994; Bahadur et al. 2000). The description above fully supports the observations by Punt (1987) that have been made investigations on various species of the family. Table 4.1 provides the palynofeatures of all *Jatropha* species studied to date in a nut shell.

From the foregoing, it is obvious that the palynofeatures of *Jatropha* species are considered taxonomically significant. These features include (1) size, shape and ornamentation of clava heads, (2) presence or absence of free processes in lumina, (3) dense or lax nature of crotonoid pattern, and (4) nature of muri. In possessing striate clavate/pilate heads (under SEM), *J. tanjorensis* and *J. maheshwarii* pollen shows striking similarity with the pollen of *J. gossypiifolia* and grow wild in south India. The density of crotonoid pattern is related to and controlled by the degree or

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Table 4.1 Summary of comparative exine features visible under LM or SEM, which are important for pollen classification in *Jatropha*

S. no.	Species	Length of clava (μm)	Clava processes shape, diameter in μm and nature of clava head	Crotonoid pattern	Luminal free processes	Muri	Figure
1	***J. gossypiifolia* var. *elegans***	2.3–3.0	Triangular 2–3, with crenate margin, prominently striate (12–15 striae)	Dense	Absent	Faint	4.5a, b
2	***J. gossypiifolia* var. *gossypiifolia***	2.3–3.0	Triangular, 1.5, margin crenate, prominently striate (8–15 striate)	Lax	Present, 2–5	Distinct	4.5c, d
3	***J. glandulifera***	3.0	Triangular, 2–3, faintly striate (8–10 striate)	Moderately	Absent	Faint	4.6a, b
4	***J. tanjorensis***	3.6–5.4	Triangular to polygonal, 3.6, prominently striated (20 thick striations)	Moderately dense	Present, 1–3	Faint	4.5e, f
5	***J. curcas***	3.0–4.5	Ellipsoid to rounded, 3.75–5.25, faintly striate, vertical ridges	Lax	Present, 4–7	Thick, distinct	4.2a–c
6	***J. panduraefolia***	1.8–3.0	Triangular to round, 1.5–2.3, smooth	Moderately dense	Present, 5–6	Faint	4.6c, d
7	***J. integerrima***	2.3–3.8	Round, 2.3, faintly striate vertically (5–8 striae)	Moderately dense	Present, 2–6	Distinct	4.6e, f
8	***J. multifida***	3.8	Triangular to round, 1.8, faintly striate	Lax	Present, 2–4	Distinct	4.7a, b
9	***J. podagrica***	2.3–3.0	Triangular, 1–2.3 smooth/psilate	Lax	Present, 2–4	Distinct	4.7c, d
10	***J. heynei***	2.3–3.0	Clava round with a distinct ring at tip	Moderately dense	Present, 5–7	Faint	4.10d–f
11	***J. maheshwarii***	3.7–4.2	Clava round, deeply lobed striations	Moderately dense	Present	Indistinct	4.10a–c
12	***J. villosa***	3.3–4.3	Clava with 5–6 vertical striations	Moderately dense	Present, 1–4	Distinct	4.11c
13	***J. mollissima***	3.4–4.4	Clava triangular to round, psilate	Lax	Present, 1–2	Distinct, thick	4.9c, d
14	***J. induta***	3.3–4.6	Clava round, vertically striated	Moderately dense	Present, 2–6	Distinct	4.8e, f
15	***J. berlandeiri***	3.4–4.5	Chain of 2–6 clavae laterally fused forming ring	Dense	Absent	Indistinct	4.9e, f
16	***J. rufescens***	4.5–5.6	Clava round, psilate	Dense	Present, 1–5	Distinct, thick	4.8a, b

(continued)

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Table 4.1 (continued)

S. no.	Species	Length of clava (μm)	Clava processes shape, diameter in μm and nature of clava head	Crotonoid pattern	Luminal free processes	Muri	Figure
17	***J. isabelli***	–	Clava round, psilate	Moderately dense	Present, 5–7	Distinct	4.7e, f
18	***J. lagaranthoides***	2.9–4.0	Clava vertically striated	Lax	Present, 1–2	Distinct	4.8c, d
19	***J. urens***	3.3–4.5	Clava club shaped	Dense	Present 1–2	–	4.4e, f
20	***J. phyllacantha***	5.6–6.7	Clava club shaped, hirsute	Dense	–	Distinct	4.11d
21	***J. cathartica***	3.8–4.9	Clava round, 4.0, vertical striae	Dense	Present, 1–2	Distinct	4.2d–f
22	***J. gallabatensis***	5.0–6.5	Clava spherical to ellipsoid, 3.0–3.5, vertically striate	Dense	Present, 1–2	Indistinct	4.3a, b
23	***J. macrorhiza***	3.6–4.7	Round ellipsoid, 3.3–4.2, vertically striated	Lax	Present, 1–2	Distinct	4.3d
24	***J. moranii***	2.5–4.0	Round, 3.6–4.3, vertically striate and echinate	Lax	Present, 2–3	Distinct	4.3e, f
25	***J. canescens***	3.2–3.6	Triangular to umbrella shaped 3.5, vertically striated, furrows deep	Lax	Present, 1–2, much smaller many	Distinct	4.3c
26	***J. dioica***	3.2–4.5	Round, umbrella shaped, 4.0, deep vertical striae	Dense	Present, 1–3	Fairly distinct	4.4a, b
27	***J. capensis***	5.0–6.4	Round to conical, 4.5, vertically striated forming distinct lobes	Lax	Present, 1–2	Distinct, thick	4.4c
28	***J. augustii***	3.3–4.3	Sub-triangular of various shapes, faintly and vertically striated	Moderate dense	Present 1–2, microbacules in 3–4 rows/ lm	Distinct	4.4d
29	***J. mutabilis***	3.1–3.8	Triangular to round clavae, smooth/ psilate	Moderate lax	Present, 5–7	Indistinct, short, thin	4.9a, b
30	***J. pelargonifolia***	2.5–3.7	Round clavae with moderate, vertical striae (5–7)	Dense	Present, 1–3	Indistinct	4.11a, b

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development of the muroid ridges. When the muroid ridges are discrete and well developed delimiting the lumina, the crotonoid pattern appears lax. Conversely, when the muroid ridges are poorly developed, faint and obscure, the crotonoid pattern appears dense. Thanikaimoni et al. (1984) noted similar features in the crotonoid pattern of the pollen of *Croton* and *Domohinea.* Instead of any single character, it is the totality of the finer aspects of sculpture enumerated above that are of utility value for a meaningful characterization of various species of *Jatropha*; not withstanding a high degree of overlapping of various characters as observed among the species of the genus.

Miller and Webster (1962) reported pollen size in 11 *Jatropha* species and correlated it with petiolar traces, nature of clavae (verrucae) and diploid chromosome number. Pollen size in all species belonging to four sections studied by them is reported to range from 54 μm as in *J. divaricata* to 87 μm in *J. podagrica*, but similar comparison was not possible due to scattered data although there were variations in pollen size (see the pollen diagnosis). In this study, pollen grains of *J. curcas, J. heynei, J. glauca* and *J. maheshwarii* were found to be the biggest being in the size range of 80–90 μm and the smallest measuring 40–45 μm in diameter as in *J. pohliana, J. rufescens* and *J. capensis*.

The above authors also studied the exine pattern in *Jatropha* species and noted the clavae to be triangular to round in *Jatropha* species, but triangular in *Cnidoscolus* species. Reduction in pollen grain diameter seems to be directly correlated with the latitude, reduction in reproductive structures, geo-climatic distribution of the taxa and consequently increasing aridity.

The genus *Jatropha* is represented by about 175 species distributed under two sub-genera, *Jatropha* (with six sections) and *Curcas* (with four sections) and as many sub-sections (Dehgan and Webster 1979). Therefore, a comprehensive palynological study of representative species covering all sections is desirable; especially the Somalian endemics of Africa (Heming and Radcliffe-Smith 1987) belonging to the section *Spinosae viz., J. spinosa, J. fissipina, J. crinita, J. rivae, J. ferox,* and *J. afrocurcas* and *J. macrophylla* both belonging to subsection *Curcas* whose representative species have not been studied yet. In addition, the Mexican endemic species *viz., J. hintoni*, *J. pseudocurcas*, *J. mcvaughii*, *J. andrieuxii*, *J. bartlettii* and *J. yucatensis* etc., need to be investigated before suitable conclusions can be drawn on the relevance of palynocharacters at the sub-generic and sectional classification of *Jatropha*, and a comparison must be established with a similar study based on epidermal features of 37 species representing all the ten sections of *Jatropha* (Dehgan 1980). Pollen grains of both the genera *Jatropha* and *Cnidoscolous* are almost similar in size and the verrucae are round to triangular. However, the grains of *Jatropha* are invariably inaperturate, whereas those of *Cnidoscolus* are either 6–10 porate depending on the species (Miller and Webster 1962). They opined that the pollen grains of *Cnidoscolus* are less specialized than that of *Jatropha. Cnidoscolus* perhaps represents a transitional stage between typical tricolporate grains of Crotonoideae such as *Acalypha* and *Euphorbia* and the crotonoid pattern of *Jatropha*.

According to Punt (1987), the pollen in *Phyllanthus orbicularis* and *P. acumianthus,* is inaperturate and the exine is psilate with endoapertures resembling *Jatropha*

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pollen. Palynological and cytological evidence among the four *Jatropha* sections is also striking suggesting that *Jatropha* is closer to *Aleurites* as thought earlier by Miller and Webster (1962) and this supports Pax's (1910) grouping of these genera in the sub-tribe *Jatrophinae*. Erdtman (1952) concluded that imbricate calyx, pollen morphology and other characteristics of Buxaceae are similar to all species Euphorbiaceae and thus denote their ancestry (plesiomorphic characters).

The significance of sculptural features of the exine in *Jatropha* species (Table 4.1) may be summed up in the light of its finer aspects considered taxonomically important (apomorphic characters):

1. There is subtle variation in the crotonoid pattern between dense, moderately dense or lax in the species studied. The network may be polygonal with orders 5–6, but some species show pattern with orders 8–9.
2. The clavae/pila are arranged in a regular penta- or hexagonal pattern around a depression. The clavae are often laterally connected by or project from a ridge, which bound its depression; the size of the ridge depression is variable being shallow or deep, narrow.
3. The size and shape of clavae. Clavae can be spherical to oval, round to conical, club shaped, with circular cap, with a dentate ring, with transverse or vertical striae or both, smooth or psilate on the *hirsute* mode of density.
4. The presence or absence of free processes in lumen (luminal bacules) and their number (1 or 2–4 and 4–6) depending on the species.
5. Details of brochi and muri thick, thin, etc. This is an important primitive feature, which is obvious in the sub-genus *Curcas.*

Further palynological investigations on additional *Jatropha* species belonging to various sections are not investigated as mentioned in the beginning, which hopefully would resolve the taxonomic ambiguities and provide a clear picture on the phylogenetic basis of *Jatropha* and its closely related taxa. Dehgan and Schutzman (1994) made a phenetic and phylogenetic analysis of 77 *Jatropha* species based on 32 vegetative, floral and fruiting characters and concluded to the affinities of *J. hernandifolia, J. divaricata* and *J. gaumeri* for a new sub-section *Hernandifoliae* in the section *Polymorphae.* However, their study is devoid of the application of pollen characters in tracing the phenetic and phylogenetic relationships. They concluded sub-genus *Jatropha,* sections *Jatropha* and *Mozinna* to be monophyletic while sub-genus *Curcas,* sections *Curcas, Loureira, Peltatae* and *Polymorphae* display paraphyly.

Interestingly, reports of the occurrence of fossil *Jatropha* pollen referable to *Crotnidaepollenities euphorbiodis* and *Crotonipollis neyveli* from palynoflora of Neyveli lignite dating back to Tertiary period; establishes its origin to South America indicates long ancient history (Ramanujam and Reddy 1984). During their long journey of migration and evolution from South America to Central America, Africa, Asia and Australia the species from the genus *Jatropha* and in particular *J. curcas* and related species of Section *Curcas* adapted to semi-arid climates as can be deduced from floral and exine evolution. With respect to these characters, *J. curcas* that originated from Mexico is considered to display a primitive stage of evolution.

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This study clearly shows unity and diversity in various palynofeatures. With additional information from the unexplored new and old world species, particularly from Mexico, Somalia (Africa) may shed vital information on the origin and evolutionary history of the genus *Jatropha* (see also Carels 2009).

Apart from the above described studies, we suggest, the need for similar comparative studies on various accessions of toxic and non-toxic varieties as this will provide useful information on the nature of germplasm, its utility in breeding programme as well as in vitro pollen banks. Likewise, knowledge of pollen biology, both in vivo and in vitro is necessary for better understanding of pollen-pistil interactions when one plans for interspecific crosses with desirable characters for jatropha improvement programme to develop hybrids with desirable economic and commercial features.

Li et al. (2010) stressed the need for using pollen as best model system, since each pollen grain represents a genotype, to detect alterations in germination pattern, pollen selection and even for genetic transformation studies of various economically useful jatrophas.

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Chapter 5
Embryology of *Jatropha:* A Review

K.V. Krishnamurthy

Introduction

The Euphorbiaceae is a very large and diverse family, consisting of about 300 genera and 8,000 species (Radcliffe-Smith 2001). *Jatropha* is a very important genus of this family, not only because some of its species have great potential as sources of biodiesel but also because of their toxicity, pest control properties, fodder and fertilizer value and various pharmacological activities (Liu et al. 2007a). However, this genus has not been adequately investigated for its embryology (Davis 1966; Johri et al. 1992). This review summarizes the available information on the embryology of this taxon.

Floral Organization and Organogenesis

Jatropha is monoecious with male and female flowers borne on the same plant. The female flowers are invariably greenish while the male flowers are often red or scarlet. The male flowers possess a dichlamydeous perianth and many botanists are inclined to call them respectively petals and sepals (Liu et al. 2008). The inner perianth lobes (=petals) are almost free or connate, at least up to half way up, depending on the species. The number of stamens is usually 10 but in some species it may be only eight or nine. They are arranged in two whorls (dicyclic stamens), with the outer whorl of stamens lying opposite to the petals (obdiplostemony) and inner whorl opposite to the sepals (Singh 2005; Liu et al. 2008). There is nectariferous disc in the male flower and female flowers outer to the sepals. There is no pistillode,

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right from the beginning of floral ontogeny, in the male flowers. The female flowers do not have perianth, although staminodes could be seen, at least in the earlier stages of ontogeny of female flowers. There are three carpels (tricarpellary), all united (syncarpous) to form a trilocular structure. The placentation is axile. The styles are either absent or very short and three in number, often bilobed.

Floral organogenesis has been studied in three species of *Jatropha*: *J. curcas, J. podagrica* and *J. gossypiifolia* (Singh 2005; Liu et al. 2008). The first sepal primordium is initiated in non-median abaxial position and the second one in median abaxial position. The five sepal primoridia, thus, arise in a 2/5 sequence on the periphery of the floral apex and are initiated anticlockwise or clockwise in different floral buds of the same species. On the contrary, the five petal primordia are initiated simultaneously inner to the sepals. Two types of stamen ontogeny have been recorded in this genus (Liu et al. 2008). In *J. curcas*, belonging to the subgenus *Curcas*, the five stamen primoridia of the outer whorl arise simultaneously at first and then five of the inner whorl arise simultaneously. In *J. gossypiifolia* and *J. podagrica*, belonging to the subgenus *Jatropha,* the eight to nine stamen primordia are initiated simultaneously. In the female flowers all the three carpels are produced simultaneously (Liu et al. 2008).

Microsporogenesis and Microgametogenesis

These aspects have been studied in detail only in one species, *J. curcas* (Liu et al. 2007a, b). The anthers are terasporangiate and bithecous. The young anther has an epidermis, an endothecium, two or three middle layers, one layer of glandular (=secretory) tapetum and a mass of sporogenous tissue. The mature anther has an epidermis, a highly fibrous–thickened endothecial layer and a tapetal layer enclosing the developing pollen grains (Figs. 5.1, 5.2, and 5.3).

The anther primordium, in transverse sections (TS), appears squarish to slightly rectangular containing a mass of cells enclosed by a single surface layer of cells. The archesporium arises at the four corners of this primordium in the hypodermal layer and in TS may contain one to a few cells depending on the species. It undergoes periclinal division to result in a *primary parietal cell*(s) (PPC) on the outside and a primary sporogenous cells on the inside. The PPC divides periclinally to result in two *secondary parietal cells/layers* (SPC). The outer SPC divides again periclinally to produce an endothecial layer and a middle layer, while the inner SPC layer directly functions as the tapetum. The middle layer undergoes one or two periclinal divisions to result in two to three middle layers. Thus, the development of the anther wall belongs to the dicot type (Davis 1966). Wall development gets completed by the time the microsporocytes are formed. They have dense cytoplasm and small vacuoles. During tetrad formation, tapetal cells are tangentially elongated, have a conspicuously folded cell wall and have large vacuoles on their adaxial side. The tapetum on the connective side is formed from connective tissue and hence is dual in origin (Swamy and Krishnamurthy 1980).

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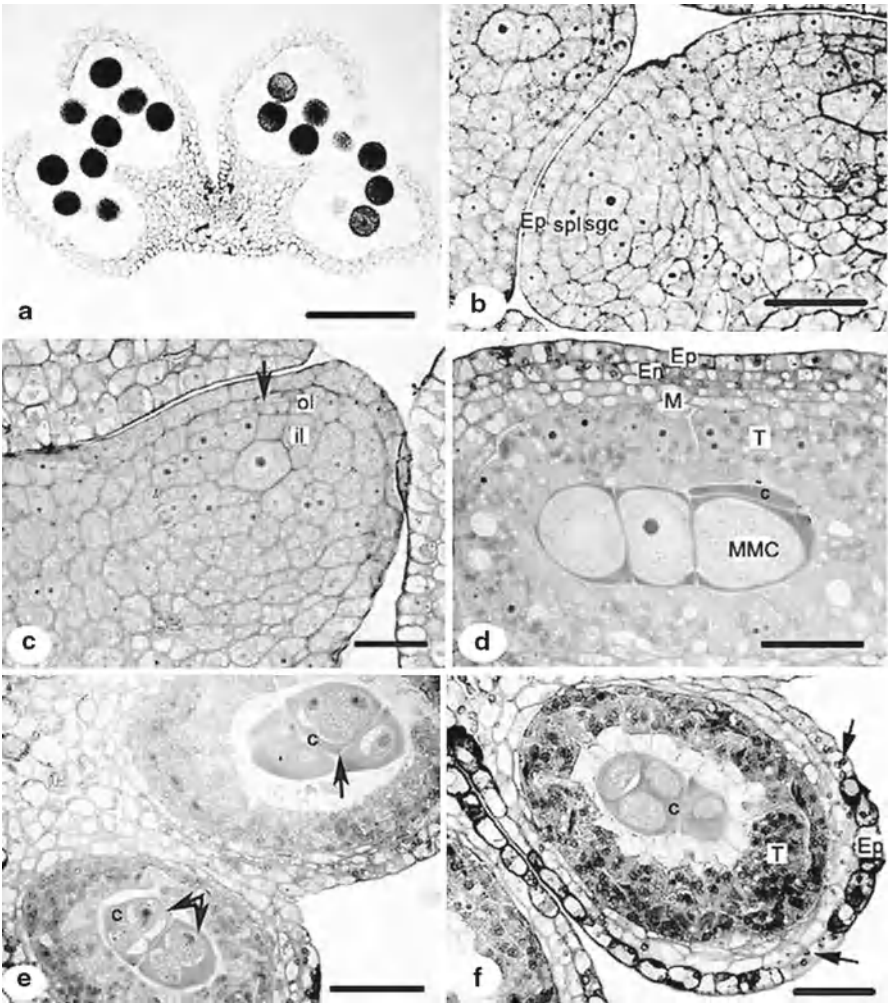

Fig. 5.1 Light micrographs of anther structure and the early stages of microsporogenesis. (**a**) Four microsporangia at the time of dehiscence. (**b**) Sporogenous cells (*sgc*) and secondary parietal layers (*spl*) beneath the protoderm (*Ep*). (**c**) Cell divisions in the outer secondary parietal layer (*ol*, *arrow*) produce the endothecium and middle layer, while the inner layer (*il*) functions directly as the tapetum. (**d**) Microspore mother cells (*MMC*) surrounded by callose (*c*), and an anther wall composed of epidermis (*Ep*), endothecium (*En*), middle layers (*M*), and tapetum (*T*). (**e**) Microspore mother cells in the process of meiosis (*arrows*). (**f**) Locule with a tetrahedral tetrad surrounded by callose (*c*), and tangentially elongated tapetal cells (*T*). The epidermal cells (*Ep*) are rich in starch grains (*arrows*) and fat globules. (**a**) Scale 5 = 200 mm, (**b**, **e**, **f**) scale 5 = 30 mm, (**c**) scale 5 = 20 mm, (**d**) scale 5 = 50 mm (After Liu et al. 2007a; Reproduced with permission from the Journal of Torrey Botanical Society)

The primary sporogenous cells are polygonal, larger than the cells of the secondary parietal layer and have large nuclei. They divide on all planes to result in a group of sporogenous cells (=sporogenous tissue) which function as microscope mother

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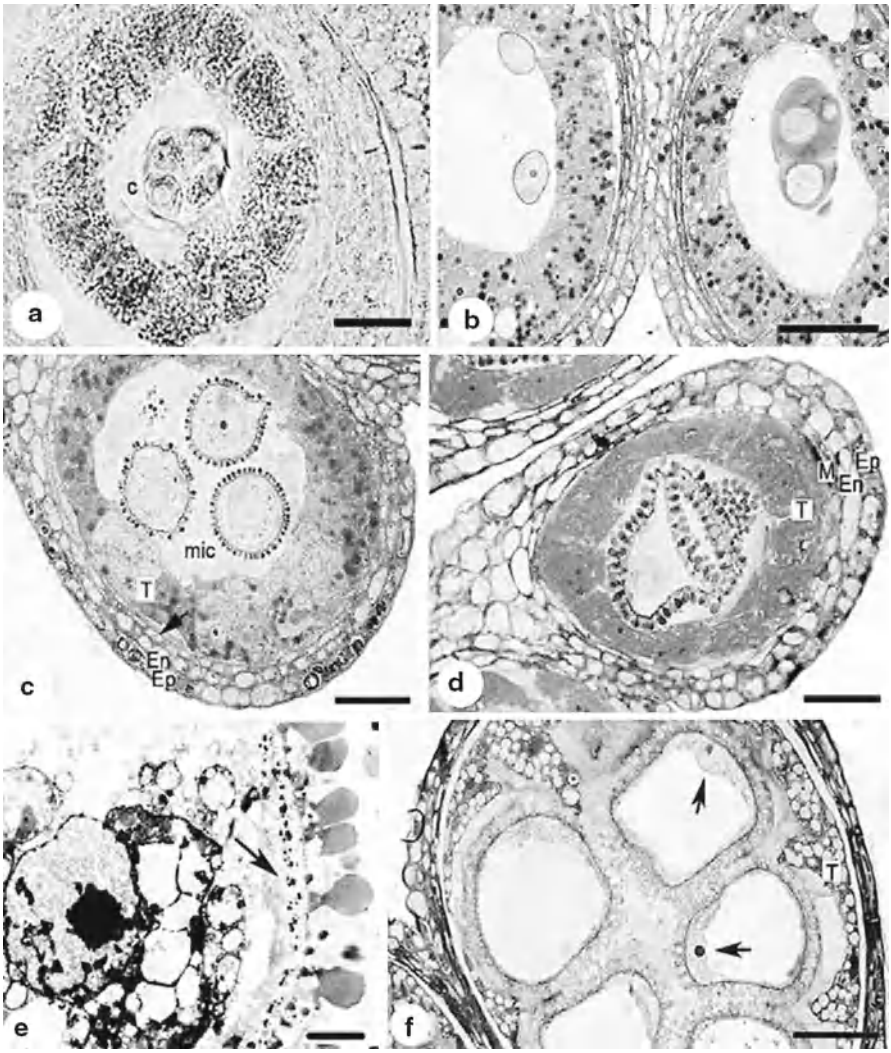

Fig. 5.2 Tetrad to microspore enlargement. All photographs except E (TEM) are light micrographs. (**a**) An irregular tetrahedral tetrad surrounded by callose (*c*). (**b**) Two stages of pollen formation in different microsporangia: free microspores in the left, and a tetrad in the right. (**c**) Free microspores with prominent centrally placed nuclei. The middle layers of the anther wall are flattened (*arrow*). Ep 5 epidermis; En 5 endothecium; T 5 tapetum. (**d**) Enlarged microspores at the stage where the tapetum (*T*) is beginning to degenerate. M 5 middle layer. The radial walls of the tapetal cells have dissolved by this stage. Ep 5 epidermis; En 5 endothecium. (**e**) Intine (*arrow*) present in a free microspore. (**f**) Uninucleate microspore with the nucleus displaced to one side (*arrows*). The tapetal cells (*T*) contain numerous vesicles and have partially degenerated at this stage. (**a**) Scale 5 = 20 mm; (**b**) scale 5 = 40 mm; (**c**, **d**, **f**) scale 5 = 30 mm; (**e**) scale 5 = 2 mm (After Liu et al. 2007a; Reproduced with permission from the Journal of Torrey Botanical Society)

cells (MMC). The MMCs develop callose walls around them and get separated from each other. They then undergo meiosis to result in microspore tetrads which may be tetrahedral or irregularly tetrahedral. Cytokinesis in MMC is simultaneous and

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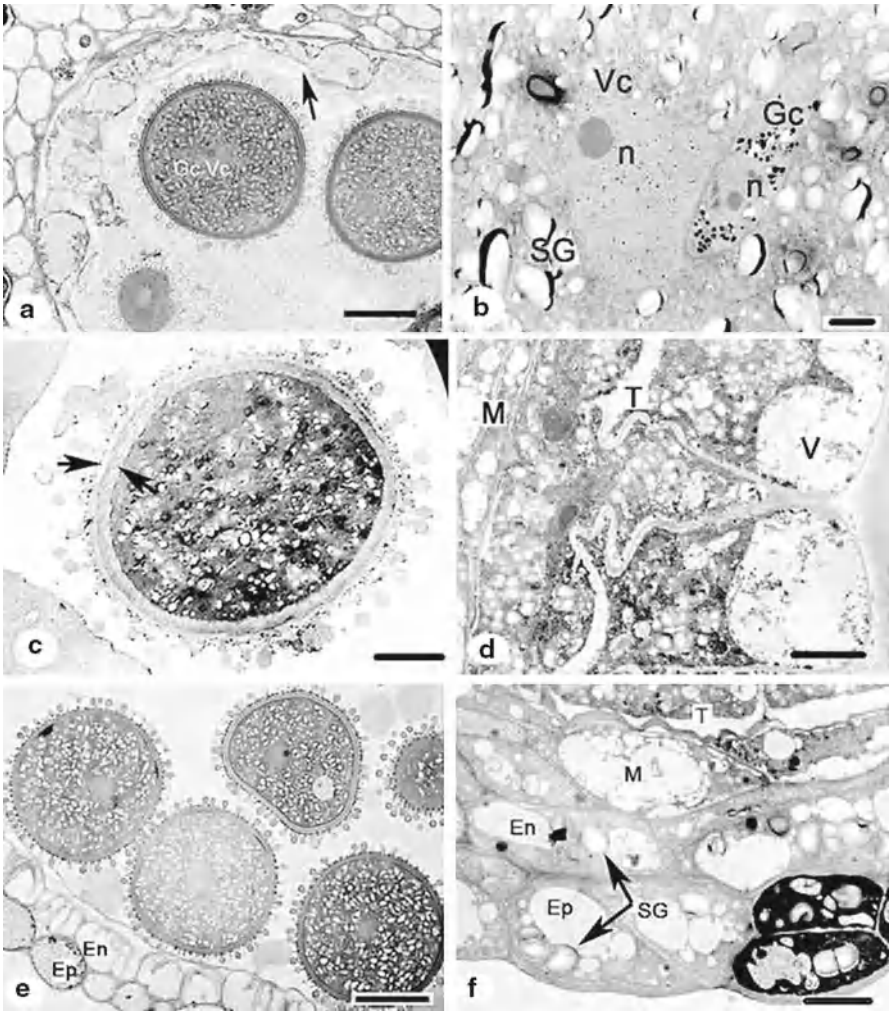

Fig. 5.3 Microgametogenesis and dissolution of the tapetum. (**a**, **e**) are light micrographs; (**b**, **c**, **d**, **f**) are TEM. (**a**) Two-celled pollen grains. All the tapetal cells (*arrow*) degenerate in their original positions. Gc 5 generative cell; Vc 5 vegetative cell. (**b**) Two-celled pollen grain with many starch grains (*SG*). Gc 5 generative cell; n 5 nucleus; Vc 5 vegetative cell. (**c**) A pollen grain with exine thicker than the intine (*arrows*) (SG 5 starch grains). (**d**) Tangentially elongated tapetal cells (*T*) with folded walls and large vacuoles (*V*) adjacent to the anther locule. M 5 middle layers. (**e**) Cross section of mature anther wall, showing epidermis (*Ep*), highly fibrous thickened endothecium (*En*). (**f**) Cross section of an anther wall at the stage of tetrad formation, with the innermost of the middle layers (*M*) degenerating (*arrow*), and the endothecium (*En*) tangentially elongated. Ep 5 epidermis; SG 5 starch grains; T 5 tapetum. (**a**, **e**) Scale 5 = 30 mm; (**b**) scale 5 = 2 mm; (**c**) scale 5 = 10 mm; (**d**, **f**) scale 5 = 4 mm (After Liu et al. 2007a; Reproduced with permission from the Journal of Torrey Botanical Society)

through furrows. The development of MMCs is synchronous within each microsporangium. The dissolution of callose walls results in the release of free microspores into the microsporangial chamber.

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The just formed microspores have an intine and are spherical, haploid, and with dense cytoplasm and a prominent and centrally located nucleus. Several endoplasmic reticulum, mitochondria, dictyosomes and plastids are present in the microsporocyte at the time of meiosis and tetrad stage. Mitochondria and endoplasmic reticulum continue to be abundant during early and late microspore stages (Liu et al. 2007b). The appearance of a central vacuole in each microspore pushes the nucleus to one side of the MMC where mitotic division ensues. This division is asymmetric resulting in a small spindle shaped generative cell and a large vegetative cell. The vegetative cell is densely packed with starch grains, while the generative cell is rich in lipids (Liu et al. 2007b). Simultaneously there is exine development and thus, a pollen grain is formed.

Once the microspores are released into the microsporangial chamber, degeneration of anther wall starts except in the endothecium and epidermis. The inner periclinal and radial walls of tapetal cells dissolve by the microspore stage and by the pollen maturation stage, the tapetal cells are completely degenerated. The degeneration of the innermost of middle layers begins at the tetrad stage followed by the other middle layer(s). All the middle layers get flattened by the free microspore stage and are fully degenerated at the mature pollen stage. The cells of the endothecium elongate tangentially during the microspore tetrad stage. They become vacuolated and contain a few starch grains. The cells enlarge at the free microspore stage and develop fibrous thickenings.

Some degree of pollen abortion is noticed in *J. curcas* (Liu et al. 2007b) and that too only in one or two microsporangia of an anther. Occasional irregularities are reported to occur in meiosis, during tetrad formation and free microspore stage. A few abnormally shaped microspores are formed. These irregularities appear to be related to abnormal tapetal behaviour.

Pollen Grains

Some aspects of pollen structure and morphology of *Jatropha* have been studied by Kajale and Rao (1943), Miller and Webster (1962), Bahadur et al. (1997, 2000) and Liu et al. (2007a, b). The pollen grains are fairly large (50–87 μm) Miller and Webster (1962), while those of species studied by Bahadur et al. (2000) ranged between 51 and 66 μm (Fig. 5.4). Bahadur et al. (2012) have recently studied additional 14 species under LM and SEM and recorded wide variation in various pollen exine features. They are two-celled at the time of release and inaperturate (Kajale and Rao 1943; Liu et al. 2007a, b). They have a thin intine and a thick exine divisible into an ectexine and an endexine (the former thicker than the latter). They are more or less spheroidal, radially symmetrical and heavily sculptured i.e. the exine shows crotonoid pattern of ornamentation (Erdtman 1952) which is essentially due to triangular to rounded (in cross section) excrescences (clava/pila) aligned in a regular polygonal or circular reticulate pattern around depressions delimited by muroid ridges of the exine.

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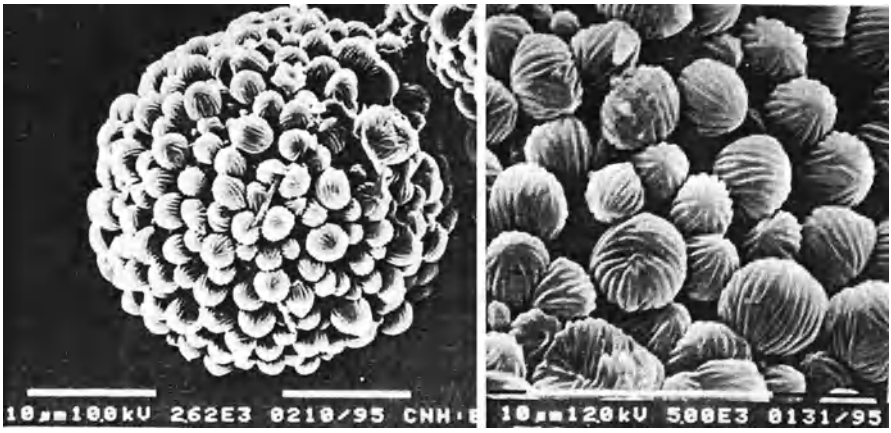

Fig. 5.4 Pollen of *J. tanjorensis* under SEM and a portion enlarged, respectively (*Photographs courtesy:* Prof. Bahadur)

Megasporogenesis and Embryo Sac Development

The ovule is anatropous, suspended, solitary per locule, bitegmic, crassinucellate, and with an undeveloped or extremely short funicle. An obturator is present. The outer integument is 3–8 cells thick while the inner integument is 3–25 cells thick depending on the species. The micropyle of *J. curcas* is formed by the outer integument, but it is often occluded by the nucellar projection reaching to the obturator. In other words, the nucellus protrudes through the micropyle. In *J. gossypiifolia* the nucellus is less protruding (Singh 1970). It is likely that in most species of *Jatropha* the nucellus protrudes to various degrees. There are 16–18 procambial strands in *J. curcas* more or less anastomosing from the chalaza to the periphery of the inner integument (Fig. 5.5).

The female archesporium is hypodermal in origin and cuts off an outer parietal cell and an inner sporogenous cell. The parietal cell repeatedly divides periclinally and to some extent anticlinally to form an extensive amount of nucellar tissue in the micropylar region, so that it protrudes to various extents beyond the micropylar orifice. This also pushes the sporogenous cell fairly deep into the nucellus (Kajale and Rao 1943). A hypostase is usually differentiated at the chalazal end of the nucellus.

The megaspore mother cell undergoes meiosis to form a linear tetrad of four megaspores, of which the lowermost (chalazal) alone develops into the female gametophyte or embryo sac. Thus, the development of the embryo sac is monosporic. The mature embryo sac is eight-celled with an egg and two lateral synergids (one on either side of the egg) at its micropylar end, three antipodals at the chalazal end and a central cell with two polar nuclei. Hence, the embryosac development is of the polygonum type. In *J. gossypiifolia,* a small degree of secondary multiplication of antipodal cells occurs and four or five cells are formed (Singh 1970).

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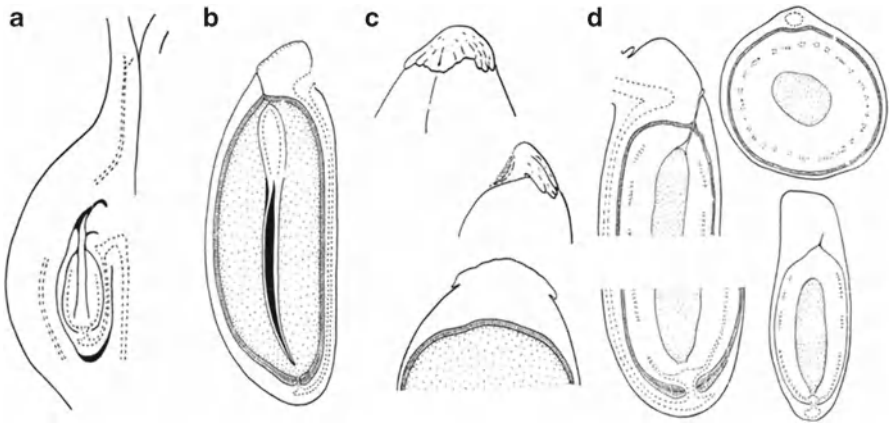

Fig. 5.5 *J. curcas*; (**a**) LS of ovary showing an ovule; (b) LS of mature seed; (**c**) Apex of seed in micropyle and side-view and in transmedian to show the slight aril; (**d**) Immature seed in LS and TS and in transmedian to show the tegmic and the nucellar remains at the micropyle (Reproduced from Corner 1976 with the kind permission of Cambridge University Press)

Fertilization is porogamous with the pollen tube produced after germination of pollen on the wet type stigma, enters almost straight into the micropyle of the ovule through the nucellar beak. The obturator guides the pollen tube into the micropyle. Syngamy and triple fusion apparently take place as per the condition seen in many of the plants to respectively, result in the diploid zygote and the triploid primary endosperm nucleus.

The endosperm is *ab initio* nuclear but soon becomes cellular; the nuclei are separated by fairly thick walls. The unpublished observation of the author of this paper indicates the presence of a vermiform endosperm haustorium in the chalazal end in some species of *Jatropha*, but this needs to be verified. Endosperm cells are rich in oil and the quantity depends on the species. Thus, the seed is distinctly albuminous.

Embryo development usually follows the Onagrad type. The embryo is relatively large, straight or slightly curved with a short and cylindrical radicle and thin and flat cotyledons (Samant 1973).

Seed Development and Structure

Some aspects of seed development and structure have been studied in the past by Mandl (1926), Netolitzky (1926), Wiehr (1930), Corner (1976), (Roth 1977), Singh (1970) and Pal and Khan (1978). Dehgan and Webster (1979) in their study on infrageneric relationships in the genus *Jatropha* noted various types of fruits and commented on caruncle characters. Bahadur and Goverdhan (1998) investigated the seed and caruncle characters in several *Jatropha*

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species under LM and SEM and noted differences in seed and caruncle features while Bahadur et al. (1996) made a comparative study of pericarp in several *Jatropha* species. Seed development is pachychalazal (Swamy and Krishnamurthy 1980). The seed is 6–21 mm long and up to 80 mm wide depending on the species. It is albuminous and with a caruncle (sometimes called aril). It is subtrigonous in TS in *J. curcas.* Some details on seed structure are available for *J. curcas, J. glandulifera, J. gossypiifolia* and *J. hastata*. The testa is around 6–20 cells thick. In *J. curcas* it is 10–12 cells thick in the micropylar region, while around 20 cells thick near the chalaza. In *J. glandulifera* it is eight cells thick near the micropylar region. The cells of testa are generally thin walled and unlignified. Testa is pulpy to dry depending on the species. In some it is initially pulpy but later dries up into a pellicle. It is multiplicative, i.e. the number of cell layers increase as the ovule develops into a seed. In *J. curcas* the outer epidermis is made of narrow columnar cells with slightly thickened and pitted radial walls and dark brown contents. It is not lignified but has small patches of thin-walled, unspecialized cells (drying into fine pits on the surface of the testa); it has no stomata. The inner epidermis generally is palisadal with short, thin-walled, columnar cells, not or slightly lignified or with deposits in the lumen or on the walls. The mesophyll is aerenchymatous with latex tubes; the cell walls are scarcely thickened (Fig. 5.6). In *J. glandulifera* and *J. hastata,* the inner epidermis shows little or no calcification. In *J. gossypiifolia* the structure of testa is similar to that of *J. curcas* but it is without patches of thin-walled cells.

The tegmen's outer epidermal layer is a mechanical layer i.e. as a palisade of malpighian cells, each 0.2–3 mm long. The epidermal cells are with straight or curved walls on the sides of the seed with the inner end towards the micropyle. The epidermis is uniform or with groups of shorter cells giving a pitted appearance in surface view. The tegmen is 4–80 cells thick (26–30 cells in *J. curcas*), generally much thicker than the testa, but eventually all layers are crushed except the palisade. Before degeneration, the tegmen has a width of 115–400 μm (150–180 μm near micropyle, 250–400 μm on the sides and 500–600 μm around the chalaza in *J. curcas*). The tegmen's mesophyll is aerenchymatous and large-celled. The inner epidermis is unspecialized or with slightly thickened and subligneous, radial walls with slight ridge–like thickenings. The tegmic palisade of *J. gossypiifolia* is 130 μm thick (Wiehr 1930), while that of *J. hastata is* 115 μm thick (Corner 1976).

The caruncle or aril is small, hard, brownish white, minutely lobed and found round the micropyle but not on the funicle or antiraphe side in *J. curcas.* It is made up of firm but unspecialized cells. The caruncle shows variation in structure from species to species and taxonomically useful Bahadur and Goverdhan 1998).

The vascular supply to the seed is often complicated with testal and tegmic bundles. The tegmen attached to the short chalaza is supplied by vascular bundles from the heterophyte (original sporophyte) to form a peripheral and longitudinal network (Corner 1976).

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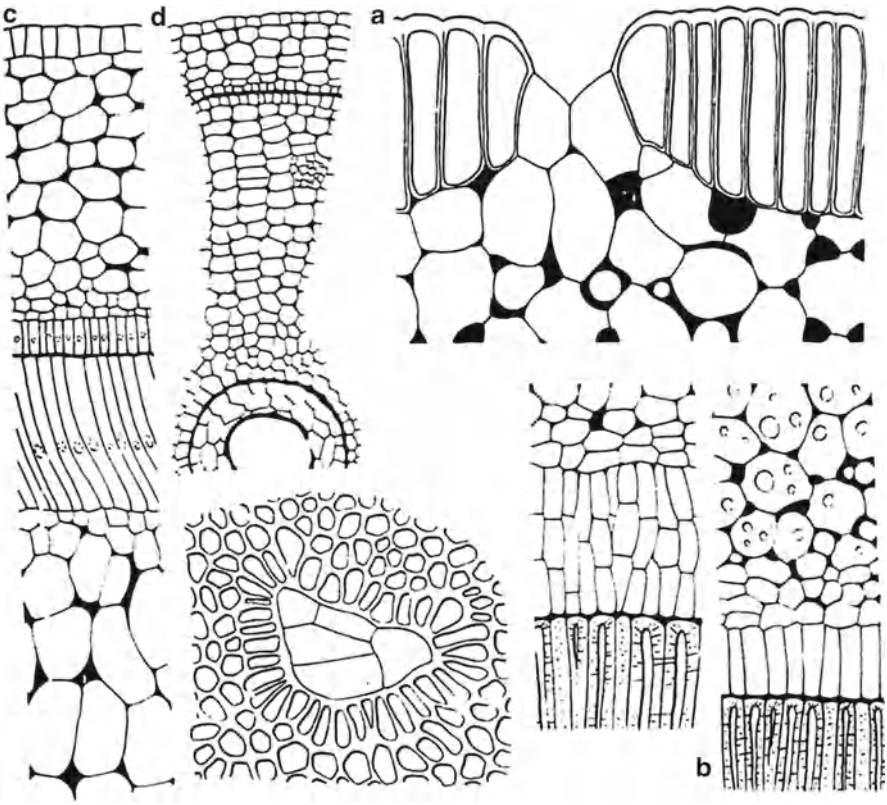

Fig. 5.6 *J. curcas*; Ovule-wall and seed-coats in TS: (**a**) mature seed with outer part of the testa (with a colourless thin-walled patch in the exotesta) and (**b**) inner part of the testa at the junction with the exotegmic palisade (with multiple endotesta in the chalazal region); (**c**) immature seed; (**d**) surface-view of a thin-walled colourless patch in the exotesta (Reproduced from Corner 1976 with the kind permission of Cambridge University Press)

Conclusions

It is to be mentioned here that inspite of the potential importance of the various species of *Jatropha* to human kind, not much attention has been paid to the study of the embryology of this taxon. Of the 165–175 species of this genus there is only one detailed work on microsporogenesis in one of its species, *J. curcas* (Liu et al. 2007a, b). Euphorbiaceae shows basic, monocot and dicot types of anther wall development (Davis 1966), and *J. curcas* has dicot type of wall development. As in most Euphorbiaceae, microspore mother cell division is of the simultaneous type to result in a tetrahedral tetrad of microspores. Mature pollen is inaperturate and two-celled at the time of release in *J. curcas* (Liu et al. 2007a) and *J. gossypiifolia* (Kajale and Rao 1943). Nowicke (1994) found inaperturate pollen in Crotonoideae which includes the genus *Jatropha*. According to Nowicke (1994) several early branching lineages of the subfamily Crotonoideae share inaperturate pollen, an unusual feature among the angiosperms and a strong synapomorphy for most of the sub-family.

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Like other members of Crotonoideae, the species of the sub-family *Jatropha* studied have uniovulate condition, a feature also shared by the sub-family Acalyphoideae and Euphorbioideae (Webster 1975, 1994). Dehgan and Webster (1979) noted that the main distinguishing characters for establishing the phylogenetic relationships in the two sub-genera i.e, *Jatropha* and *Curcas* are the shape of seed and morphology of the caruncle. The presence of vascular bundles in the inner integument appears more conservative and characteristic of Crotonoideae to which *Jatropha* also belongs.

However, embryological work on the species of *Jatropha* is so scanty that no valid taxonomic conclusions can be drawn from them at present.

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Chapter 6
Structure and Development of Fruit and Seed of *Jatropha gossypiifolia* L.

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Introduction

The genus *Jatropha* L. belongs to the tribe Joannesieae of subfamily Crotonoideae of the Euphorbiaceae (APG II 2003) and contains about 175 species. Dehgan and Webster (1979) revised the subdivision of *Jatropha* made by Pax (1910) and now it is distinguished into two subgenera, *viz., Jatropha* and *Curcas*, with 10 sections and 10 subsections to accommodate the old and new world species.

Jatropha gossypiifolia L. is a bushy shrub of about 1.8 m in height and it is very common in wastelands, plains along roadways and sandy soils. The flowers were scarlet red with greenish seed in capsule (Morton 1981; Oudhia 2001). The leaves of *J. gossypiifolia* have been used for intermittent fevers, carbuncles, eczema, itches, sores on tongues of babies, swollen mammae, stomach-ache and veneral diseases (Balee 1994; Chetty et al. 2008). Ogbobe and Akano (1993) studied the physico-chemical properties of its seed oil with reference to human foods.

Though there were reports on seeds of *Jatropha* (Singh 1970; Corner 1976; Pal and Khan 1978; Dehgan and Webster 1979; Anez et al. 2005), as such there is no detailed study on its seed and fruit development. In view of this, the present investigation has been taken up to reveal the structure and development stages of fruit and seed of *J. gossypiifolia*.

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The fruits of *J. gossypiifolia* were collected from the surrounding environs of Acharya Nagarjuna University at their sequential developmental stages right from the ovary of unopened flower bud and ovary from opened flower to ripening ovary and, the mature and dry stages. Usual procedures of fixation, dehydration and embedding were followed (Berlyn and Miksche 1976; Khasim 2002). Microtome and freehand sections were stained with safranin O and fast green FCF. Photomicrographs were taken with Leica microscope.

J. gossypiifolia is a gregarious shrub with three-lobed palmate leaves possessing hairs (Fig. 6.1a). Flowers are unisexual in axillary paniculate cymes. The ovary is trilocular with three ovules and pondulous with three styles. Capsule is three-lobed (Fig. 6.1b) and seeds are oblong.

Ovary and Fruit Wall

The capsular fruit of *J. gossypiifolia* develops from a trilocular, syncarpous and superior ovary. The ovary wall is formed by uniseriate outer and inner epidermis. The Mesoderm is parenchymatous with 8–10 cell layers (Fig. 6.1c).

After anthesis, mesoderm is differentiated into outer, middle and inner mesoderm. The mesodermal cells just beneath the outer epidermis may divide anticlinally and periclinally and adding to the middle mesoderm, whereas inner mesoderm exclusively undergoes anticlinal divisions.

Structure and Development of Fruit

The development of fruit begins with the fertilization and shedding of withered floral organs enclosing it. The fruit wall, i.e., the pericarp is differentiated into epicarp, mesocarp and endocarp (Fig. 6.1d, e).

Epicarp: The outer epidermis of ovary wall forms a single layered epicarp. The squarish or isodiametric cells with dense cytoplasm constitute outer epidermis of the pericarp; these cells undergo anticlinal divisions only. The outer tangential walls of the epidermal cells are slightly thickened and covered with cuticle. This uniseriate layer represents the main protective layer in fruits.

Mesocarp: It is 25–30 layers thick and developed from the mesoderm of ovary wall. It is subsequently differentiated into outer, middle and inner mesocarp. Just beneath the epicarp, the 7–10 layered outer mesocarp consists of slightly collenchymatous and also isodiametric cells with abundant laticiferous vessels (Fig. 6.1f). The middle mesocarp with 7–12 layers of spherical or polygonal parenchymatous cells is interspersed with fibres oriented longitudinally or

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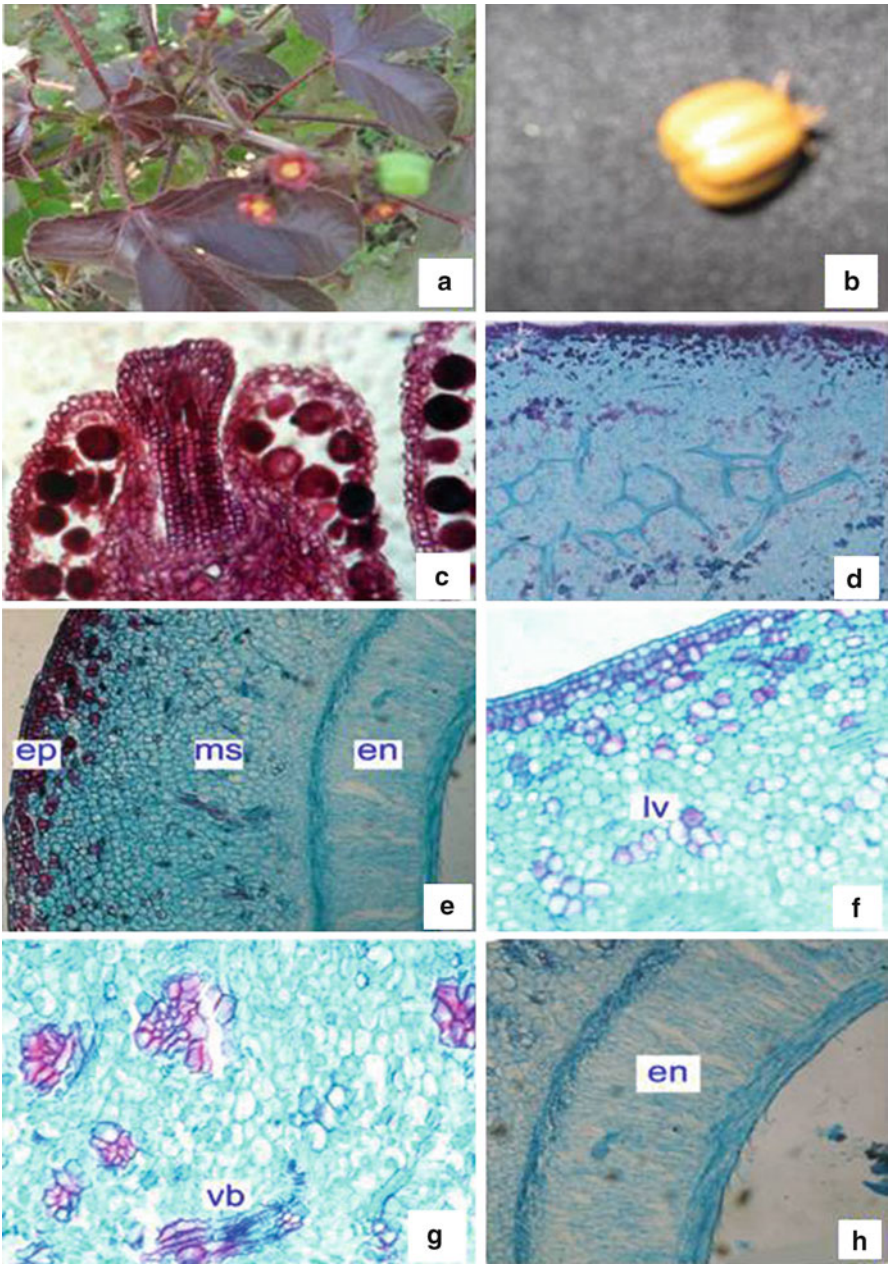

Fig. 6.1 (**a**–**h**) (*ep* epicarp, *en* endocarp, *lv* laticiferous vessels, *ms* mesocarp, *vb* vascular bundle) (**a**) *J. gossypiifolia* plant showing flowers and fruit. (**b**) Capsular fruit. (**c**) LS of unopened flower showing ovary wall and ovule, also pollen grains. (**d**) Developing pericarp showing scattered fibres in mesodermal region. (**e**) Gross anatomy of pericarp showing epicarp, mesocarp and endocarp. (**f**) Outer mesocarp possessing laticiferous vessels. (**g**) Middle mesocarp showing vascular bundle. (**h**) Endocarp with fibrous cells. (**b** × 3, **c** × 200, **d** × 100, **e** × 100, **f** × 100, **g** × 100, **h** × 200)

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obliquely to the longitudinal axis of the fruit (Fig. 6.1d, g). Undifferentiated vascular bundles are also found in this region (Fig. 6.1g). The inner mesocarp consists of lightly stained small oval or isodiametric cells. The cells of this zone undergo less periclinal divisions and they appear very compactly arranged. During development of fruit, formation of large intercellular spaces occurs among the middle mesocarpic cells (Fig. 6.1g).

Endocarp: The inner epidermis of the ovary wall constitutes a single-layered endocarp. The endocarpic cells of developing fruit undergo structural changes and transform into thick walled fibres (Fig. 6.1h) in mature fruit.

Carpophore and septum: The septum originates from the cohesion of carpel walls. The middle portion of the septum consists of abscission tissue (Fig. 6.2b) which is extended from carpophore. Abscission zone is represented by parenchymatous, spongy tissue and accompanied by fibrous cells (Fig. 6.2b).
Carpophore is the central portion of the ovary. It is the region where three carpels join together possessing thick-walled fibrous cells. Central vascular bundle is observed in the carpophore (Fig. 6.2a). The carpophore is separated from the septum by its abscission zone.

Dehiscence zone: The fruit represents two dehiscence regions, the dorsal dehiscence in the pericarp region and other along the carpel sutures. In the dorsal dehiscence, the epicarp and, outer and middle mesocarp with vascular bundles accompanied by fibrous endocarp are involved. There is a groove on the pericarp surface through which dehiscence takes place. Whereas in ventral suture dehiscence, abscission tissue in the carpophore, spongy tissue in the central portion of septum along with neighbouring mechanical layer (fibrous cells) are involved.

Seed Structure and Development

The seed originates from anatropous, bitegmic and crassinucellate ovule. In the developing seed both integuments are multiseriate but the tegmen has larger number of cell layers (Fig. 6.2c–f). The exotesta is uniseriate and thin-walled parenchymatous. The mesotesta is multiseriate and also parenchymatous. Laticiferous vessels are abundant in the very beginning in the mesotesta (Fig. 6.2d) and during its development, these are sparsely distributed (Fig. 6.2e, f). The endotesta is multiseriate and deeply stained, compactly arranged cells zone (Fig. 6.2e), later these cells become sclerenchymatous (Fig. 6.2f). Tegmen is 26–30 cells thick. The exotegmen consists of uniseriate columnar cells with lignified walls (Fig. 6.2f). Mesotegmen is with large-celled aerenchyma; the outer portion of mesotegmen is lightly stained whereas inner portion is prominently stained (Fig. 6.2e). However, during seed development the outermost layer of mesotegmen becomes sclerenchymatous (Fig. 6.2f). The inner cells of mesotegmen become radially elongated before being crushed. The endotegmen represents small tabular cells, they are eventually crushed during development of seed.

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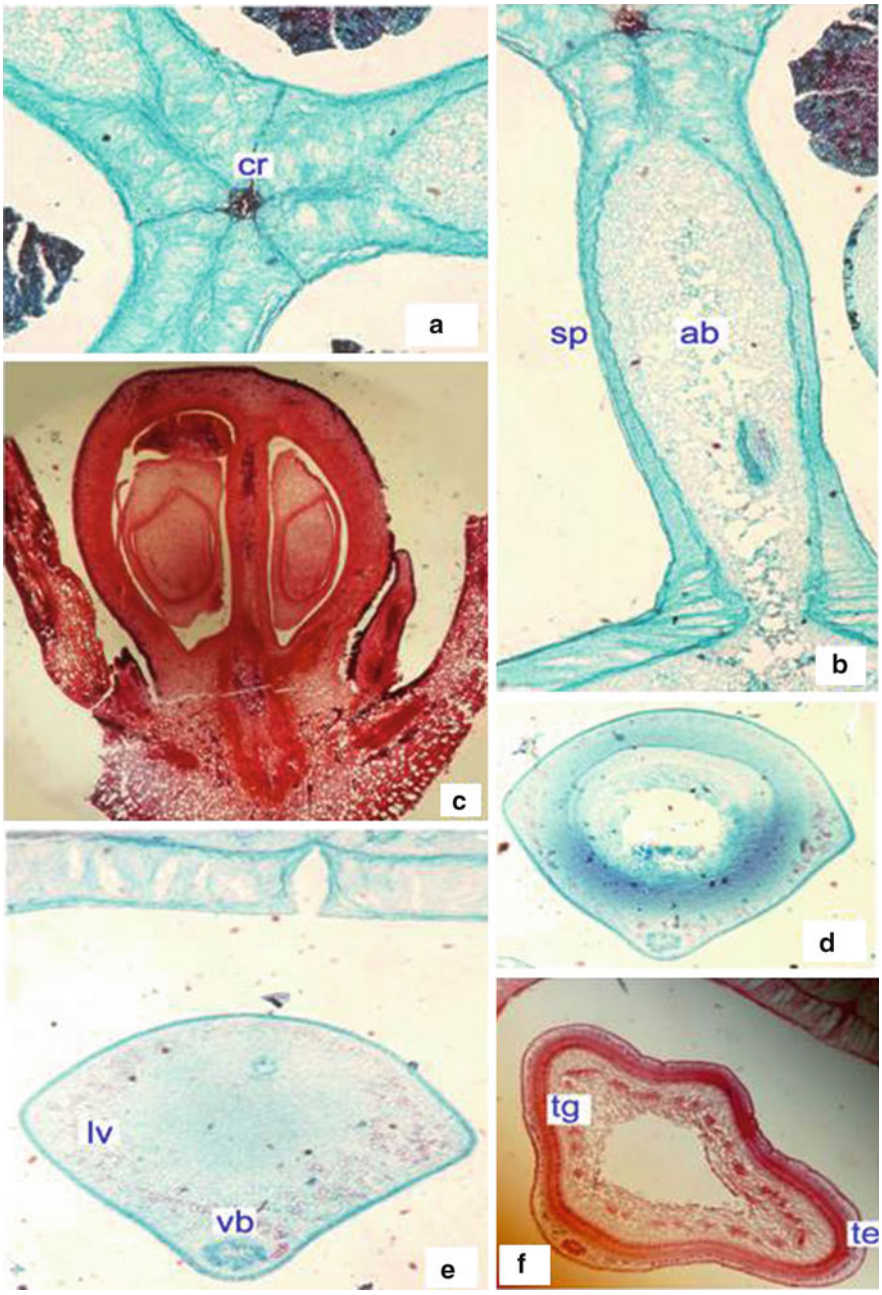

Fig. 6.2 (**a**–**h**) (*ab* abscission tissue, *cr* carpophore, *lv* laticiferous vessels, *sp* septum, *te* testa, *tg* tegmen) (**a**) Carpophore. (**b**) Septum showing abscission zone. (**c**) LS of ovary (initial stage of fruit) in completely opened flower. (**d**) Developing seed showing laticiferous vessels and vascular bundles. (**e**) Immature seed showing different tissues in testa and tegmen. (**f**) Mature seed showing testa and tegmen

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The mature seed is bitegmic with well-developed testa and tegmen. The collateral vascular bundles and latex vessels occur in the mesotesta. Laticiferous vessels are also reappeared abundantly in the mesotegmen region (Fig. 6.2f).

Comparison of Structural Details with Other Euphorbiaceae Members

The wall of the fertilized ovary transforms during development into the pericarp of the mature fruit (Roth 1977). The pericarp may be arbitrarily divided into layers, referred to as epicarp, mesocarp and endocarp (Eames and MacDaniels 1947; Spjut 1994). In ontogenetic study of *Manihot utilissima*, Toledo (1963a, b) considered that the endocarp originates from the subepidermal parenchyma whereas in *J. gossypiifolia* from the inner epidermis of the ovary. The inner epidermal origin of endocarp was also observed in coffee bean in which these cells have been transformed into endocarp fibres (Roth and Lindorf 1971). The endocarp in the present study was uniseriate with fibrous cells whereas in *Manihot caerulescens* and *M. tripartita* possessed either collenchyma or fibre-sclereids (Oliveira and Oliveira 2009). In contrast to the two sclerenchymatous regions in the mesocarp in *M. utilissima* (Toledo 1963a, b) and *Delechampia stipulacea* (Silva and Souza 2009), the pericarp of *J. gossypiifolia* had parenchyma tissue only with different denominations and cell compositions.

During dehiscence of fruits in *J. gossypiifolia,* both spongy tissue in septum and other abscission tissue in the carpophore accompanied by sclerenchyma or fibrous tissue were involved. This investigation supports the view of Roth (1977) that pericarp of dehiscent fruits often consists of parenchyma and sclerenchymatous tissue in the form of endocarp. Hence the pericarp fulfills a double function, serving as protective tissue of the seeds, as well as a dynamostatic tissue during dehiscence (Roth 1977; Fahn and Zohary 1955; Souza 2006). In many capsules of Euphorbiaceae, each mericarp separates from the adjacent mericarps as well as from the central column, when the fruit dries out, dehiscing abruptly along the dorsal suture, so that the seeds are thrown far away upto 14 m in *Hura crepitans* (Roth 1977). Crossing elements of the inner hard layer produce dehiscence which results suddenly, because the separation zone consists not of thin-walled elements, but of fibres oriented parallel to the dehiscence line which resist the opening (Guttenberg 1971).

In *J. curcas* there were patches of thin-walled cells in exotesta (Corner 1976); whereas in the present investigation it was uniseriate parenchymatous. Laticiferous vessels were observed in mesotesta in *J. gossypiifolia*. The same observation was made by Corner (1976) in *J. curcas*. However, laticiferous vessels were also found in the mesotegmen in the present investigation.

The collateral vascular bundles were recorded in the testa in *J. gossypiifolia*. According to Tokuok and Tobe (2003) these are called post-chalazal vascular branches that were observed in 11 genera of Acalyphoideae including *Dalechampia* studied by Silva and Souza (2009). Contrary to the present observation, Corner

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(1976) recorded the post-chalazal vascular bundles in tegmen in *J. curcas*. However, Werker (1997) reported the vascularization in both integuments in *Dalechampia*.

J. gossypiifolia differs from *J. curcas* in lacking the patches of thin-walled cells in the exotesta and presence of post-chalazal vascular bundles. These observations support the Dehgan and Webster's (1979) division of genus *Jatropha* into two subgenera, *viz., Jatropha* and *Curcas*.

According to Corner (1976) there has been an uniformity in the exogenic palisade of Crotonoideae which allies them with Bombacaceae, Malvaceae, Sterculiaceae and Tiliaceae. The present investigation also supports the Corner's view of classifying Euphorbiaceae-Crotonoideae in the Malvalean alliance because of exotegenic palisade.

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Chapter 7
Fruit, Seed and Seedling Characters in *Jatropha* L.

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Introduction

Jatropha L. is a large genus with about 175 species distributed mostly in America, Africa (ca. 50 species) while a few species occur in Arabia and a dozen species in India(Dehgan and Webster 1979; Ratakrishnana and Parmathma 2009). Various aspects of *Jatropha* have been extensively studied (Dehgan and Craig 1978; Dehgan and Webster 1979; Dehgan 1980; Olowokudejo 1993). But for a cursory account and illustrations of fruits, seed and seedlings characters by Dehgan and Webster (1979), the subject continues to be in neglect. Considerable work on the anatomy of fruits and related aspects in many angiosperms has been carried out (Corner 1976; Roth 1977; Kigel and Galili 1995). With the advent of SEM in botanical research, microcharacters of fruit, seed, pollen and leaf surfaces have been studied and shown to be taxonomically reliable. Bahadur et al. (1983b; Bahadur et al. 1989) investigated the seed surface microcharacters under SEM in several species of *Nigella* (Ranunculaceae) and nine species of *Petunia* (Solanaceae) and showed remarkable diversity in testa (spermoderm) microcharacters and showed their taxonomic utility.

Structure of ovules and seeds in *Jatropha* and related genera of Crotonoideae has been studied and their systematic implications discussed by Tokuoka (1998). Ehler (1976) studied seed macrocharacters of large number of species representing various sections of *Euphorbia* and has drawn interesting conclusions of taxonomic value. *Jatropha* pericarp, seeds, testa and caruncle and seedling characters

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have been studied earlier by Bahadur et al. (1996a, b, 1998). The structure and development of seed in few *Jatropha* species *viz., J. multifida* and *J. podagrica* has been studied (Singh 1970; Pal and Khan 1978). Recently, Khasim et al. (2012) have investigated for the first time the structure and development of fruit and seed in *Jatropha gossypiifolia* and made interesting observation regarding the tissues involved in the dehiscence of fruit. In this paper we present a review of fruit, pericarp, seed, caruncle and seedling characters in various *Jatropha* species studied to date.

Fruit Characters

Dehgan and Webster (1979) first noted variation in fruit characters in several *Jatropha* species *viz., J. gossypiifolia, J. brockmanii, J. integerrima, J. multifida, J. macrorhiza, J. hernandifolia, J. dioica, J. cardiophylla, J. platyphylla, J. cinerea, J. canescens* and *J. podagrica* and provided illustrations. The morphology of fruit characters varies considerably not only in shape but its size as well. *Jatropha* is generally characterized by a trilocular capsule with one seeded cocci. The capsule may be explosive or tardily dehiscent with a single seed/locule. It is common in most *Jatropha* species but in the Mexican species the fruit has undergone reduction in conjunction with reduction in the stigma lobes/size in section *Loueria* and is characterized by two locules while in Section *Mozzina,* tardily dehiscent unilocular condition exists as a result of abortion of ovules as in *J. giffordiana* and *J. dioica.* Fruits in sub-genus *Jatropha* are consistently trilocular. The fruit is smallest with thin pericarp and dehisce violently. Fruits in Section *Adenophoreae* are pubescent and in Section *Jatropha* and *Collenucia* are glabrous. Gradual reduction in thickness of the mesocarp is observed in others with the exception of *J. multifida.*

The following are the details of the capsule features of *Jatropha* species studied:

***J. nana*:** Capsule oblong-obovoid, flattened at top, slightly six lobed, 9–10 mm long and 4–6 mm across, capsule pericarp thin, somewhat smooth, dehiscing longitudinally ballistically.

***J. tanjorensis*:** Capsule loculicidal, oblong-obovoid with only one ovule/seed, generally capsules do not develop due to high degree of pollen sterility, seeds are very rarely seen. Pericarp is smooth in young condition becoming rough as it matures; reticulate forming irregular pattern, pericarp stomatiferous, stomata paracytic, epidermal cells polygonal, walls straight and thick.

J. gossypiifolia* var. *gossypiifolia: Capsule oblong, ovoid, trilobed three cocci, 10–12 mm long, reticulate, verruculose reddish green, sparingly hirsutulous. Pericarp reticulate, stomatiferous, stomata paracytic, stomatal density high, stomatal complex sunken, epidermal cells slightly convex with nipple like projections, anticlinal walls thick, with waxy ornamentation, hairs unicellular and uniseriate.

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***J. gossypiifolia* var. *elegans*:** Capsule oblong-ovoid, densely hirsutulous. Pericarp rough, reticulate forming an irregular pattern, surface stomatiferous, stomata paracytic, rarely nisocytic, sunken, stomatal density high, pore elliptic, subsidiary cells waxy, epidermal cells polygonal, striated, anticlinal walls thick, epidermal hairs unicellular, sphaerocrystals present.

J. curcas: Capsule sub-globose, rugose, ellipsoid, trilobed 1.5–3.5 cm long, glaucous, pale yellow, black when dry. Capsule tardily dehiscent, pericarp rough, woody endocarp reticulate, stomatiferous when young, stomata paracytic, pore elliptic, epidermal cells polygonal, with cuticular deposits, anticlinal walls straight to curved, sphaerocrystals density high.

J. podagrica: Capsule oblong, truncate/obtuse, trilobed, 1.5 cm long 12 cm across, glabrous greenish yellow. Pericarp uneven, forming an irregular pattern, stomatiferous, stomata paracytic, large, sunken, pore elliptic to obovoid, epidermal cells polygonal, sphaerocrystals present.

J. multifida: Capsule (sub-drupaceous) globose to pyriform, laterally oblong, two to three lobed with prominent longitudinal ribs, 3 cm across, indehiscent, yellow when ripe, often two seeded. Pericarp smooth, stomatiferous, stomata paracytic and small, pore elliptic, epidermal cells small, quadrangular to polygonal, anticlinal walls straight, sphaerocrystals present. *J. multifida* is the only species with large indehiscent fruits.

J. integerrima: Capsule shallow at apex, three-lobed, 1 cm long, green. Pericarp thin, rough dehiscing ballistically. Pericarp with vertical rows of stomata, stomata paracytic and big with elliptic pore, nodulose striations around stomata present, epidermal cells polygonal, anticlinal walls straight, sphaerocrystals present.

J. panduraefolia: Capsule oblong about 1 cm long, three-lobed. Pericarp thin dehiscing ballistically, slightly bigger than *J. integerrima,* stomatiferous, stomata paracytic, pore elliptic, epidermal cells quadrangular to polygonal, sphaerocrystals large and their density high.

***J. villosa*:** Capsule oblong-globose, 1.5–2.2 cm long, 1.2–1.5 cm across, with deep ridges, three lobed, nodulose thinly pubescent when young. Pericarp thin, capsule dehiscing ballistically into three cocci, cocci one seeded.

J. heynei: Capsule ovoid, 2 cm long, about 1.5 cm across, distinctly deeply lobed pustulate, capsule dehiscing ballistically into three cocci; each with one seed.

***J. maheshwarii*:** Capsule oblong, globose to sub-globose 1.4–1.6 cm long, 1.2–1.4 cm across, rough brownish, surface rough, glabrous with three cocci, pericarp thin, capsule dehiscing ballistically.

***J. glandulifera*:** Capsule oblong-ovoid, sub-globose, distinctly three-lobed, 1–1.5 mm long and 8 mm across, glabrous rugulose, with three one seeded cocci. Pericarp thin, capsule dehiscing ballistically.

J. sotoi: This species from Madagascar is described by Casas and Salas (2008). The capsule and seed show much similarity with species of section *Adenoporium.*

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Pericarp Characters (SEM)

Comparative light and scanning electron microscopic characters of nine Indian *Jatropha* species have been investigated earlier by Bahadur and Goverdhan (1996a) small caruncle. Bahadur and Goverdhan (1996a) have studied seed and caruncle been described by Bahadur and Goverdhan (1996a) and is reviewed as under investigated (Kamilya and Paria 1994; Bahadur and Goverdhan 1996b; Anez et al.

J. gossypiifolia* var. *gossypiifolia: Pericarp reticulate with cuticular ornamentation, stomatiferous, stomata paracytic, stomatal density high, stomatal complex slightly sunken, epidermal cells concave with nipple like projections, anticlinal walls thick. Small unicellular and uniseriate hairs present (Fig. 7.2a, b).

J. gossypiifolia* var. *elegans.: Pericarp surface rough reticulate, stomatiferous, stomata paracytic (rarely anomocytic), sunken, stomatal density high, pore elliptic, surrounding cells polygonal, walls wavy, epidermis hairy, anticlinal walls thick, cells with few sphaerocrystals, hairs unicellular (Fig. 7.2c, d).

J. glandulifera: Pericarp rough with distinct ridges and furrows forming an irregular pattern, stomatiferous, stomata paracytic, sunken, pore elliptic, epidermal cells polygonal, anticlinal walls straight and curved, cells with sphaerocrystals density high, hairs long (Fig. 7.1a–c).

***J. tanjorensis*:** Pericarp rough, reticulate with irregular pattern, stomatiferous, stomata paracytic, epidermal cells polygonal, anticlinal walls thick and straight (Fig. 7.1d–f).

J. integerrima: Pericarp rough stomatiferous with vertical rows of stomata, stomata paracytic, with elliptic pore, nodulose striations around stomata, epidermal cells polygonal, cells with sphaerocrystals, anticlinal walls straight.

J. panduraefolia: Pericarp green, dehiscing ballistically, stomatiferous, stomata paracytic with elliptic pore, epidermal cells quadrangular to polygonal, sphaerocrystals large, abundant.

J. podagrica: Pericarp surface uneven, stomatiferous, stomata sunken, paracytic and large with elliptic pore, epidermal cells polygonal with sphaerocrystals, anticlinal walls straight.

J. multifida: Pericarp stomatiferous, stomata paracytic with small, elliptic pore, epidermal cells, quadrangular with sphaerocrystals, anticlinal walls straight (Fig. 7.2e, f).

J. curcas: Pericarp rough with cracks, reticulate with cuticular deposits, stomatiferous, stomata paracytic with elliptic pore, epidermal cells polygonal, cells with sphaerocrystals in large numbers, anticlinal walls straight to curved (Fig. 7.1g, h).

The salient features of pericarp of *Jatropha* species are:

1. Presence of paracytic stomata with elliptic pore in all *Jatropha* species studied except *J. podagrica* where the pore is elliptic to obovoid.
2. The epidermal cells in most species studied are polygonal, while quadrangular to polygonal cells occur in *J. multifida*, cells are slightly convex with minute nipple like projections in *J. gossypiifolia* var. *gossypiifolia* and with several tubercles in *J. tanjorensis.*

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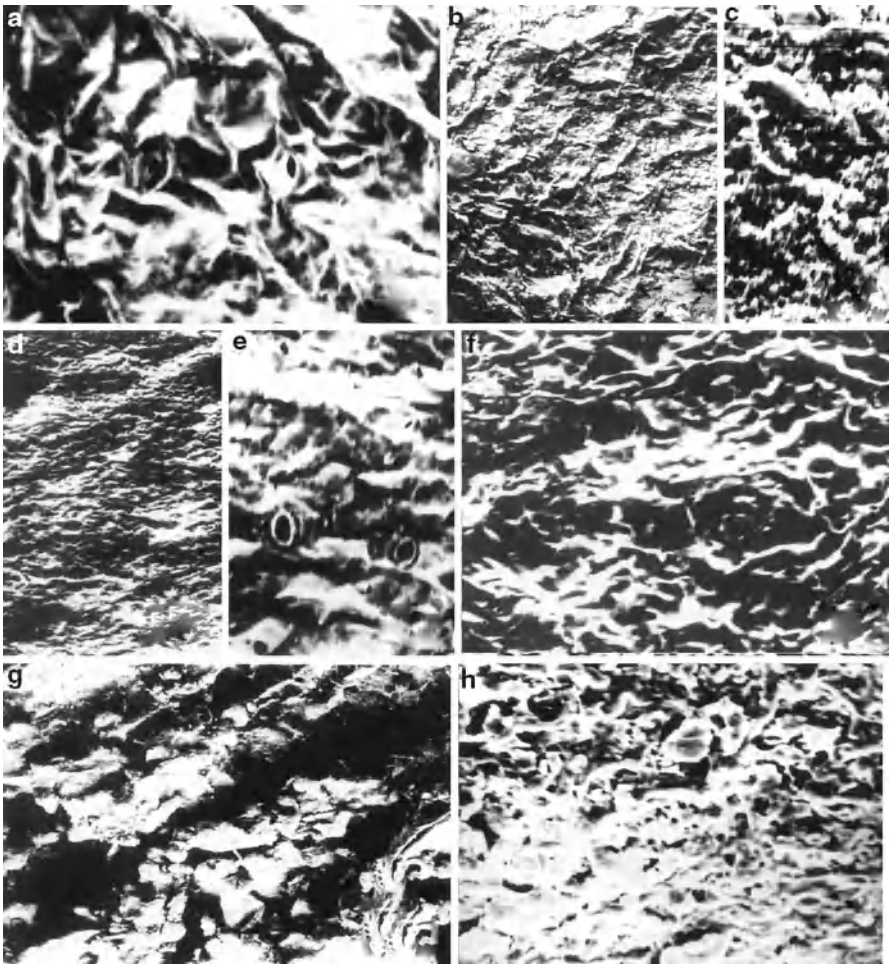

Fig. 7.1 SEM of pericarp. (**a**) *J. glandulifera:* SEM of pericarp (× 90), (**b**, **c**) portion enlarged showing stomata (× 960), (**d**) *J. tanjorensis:* pericarp showing reticulate structure (× 360), (**e**, **f**) portion enlarged showing reticulations, thick wall, lumen, several pin head like tubercles (× 900), (**g**, **h**) *J. curcas* pericarp showing cracks in testa (dark area), portion enlarged showing reticulate spermoderm (× 180, × 3,000)

3. The anticlinal walls are often straight or rarely curved.
4. The cells vary in size both within and between the *Jatropha* species.
5. The sphaerocrystals are dense in some and sparse in others and absent in few species.

These observations are very limited and need to be supplemented with similar data on other species belonging to other sub-genera and sections to verify the utility and validity of microcharacters as has been done effectively by Dehgan and Craig (1978) and Dehgan (1980, 1982) for leaf epidermal features, petiole anatomy and sphaerocrystals.

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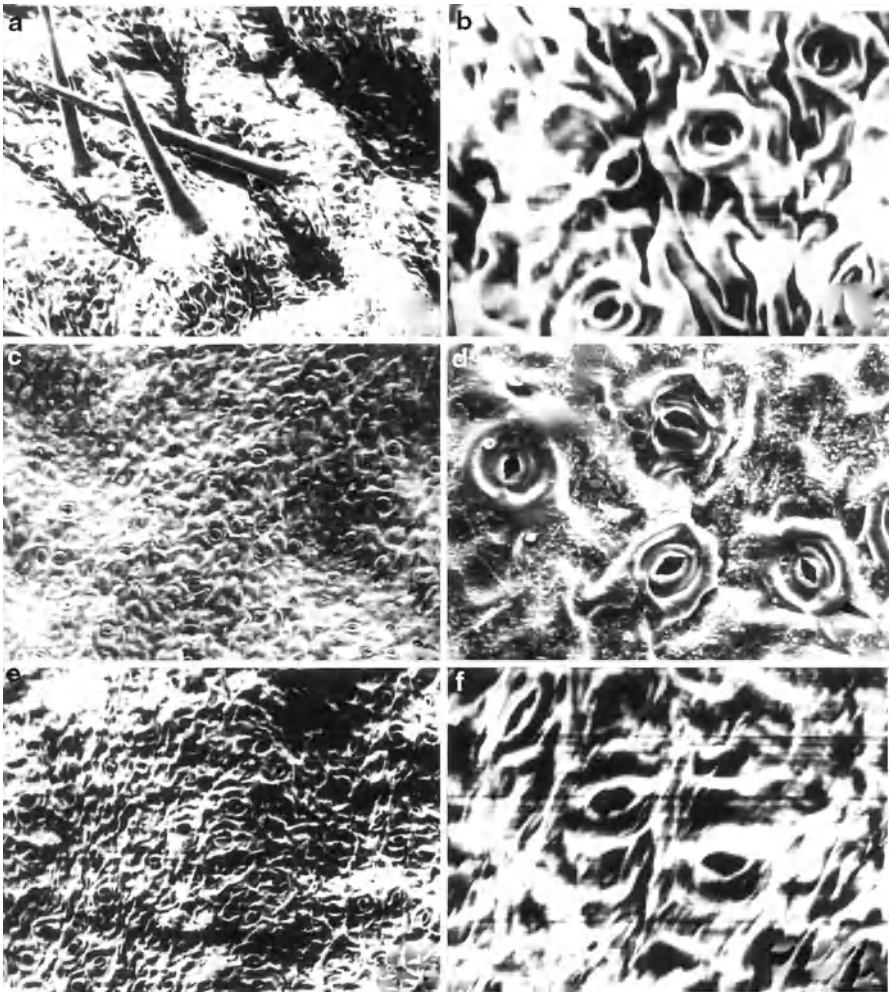

Fig. 7.2 SEM of pericarp. (**a**, **b**) *J. gossypiifolia* var. *gossypiifolia* showing reticulate pericarp with hairs and portion enlarged showing stomata (× 180, × 2,000), (**c**, **d**) *J. gossypiifolia* var. *elegans* showing reticulate pericarp with portion enlarged showing stomata (× 180, × 2,000), (**e**, **f**) *J. multifida* showing reticulate uneven pericarp, portion enlarged showing stomata (× 180, × 2,000)

Seed and Caruncle Characters (LM and SEM)

Of the various species illustrated by Dehgan and Webster (1979), *J. gossypiifolia, J. integerrima, J. multifida* and *J. curcas* have been studied earlier by Bahadur et al. (1998). The other species illustrated by them have not been described for their seed and caruncle characters; therefore, a brief account of the various species is also given to make this review complete as far as possible. The following is a brief

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account of the fruit characters of the various species studied by Dehgan and Webster (1979). Small, round to spherical seeds characterize *J. dioica, J. platyphylla, J. capensis, J. macrorhiza*, *J. hiernoymii,* and *J. cardiophylla,* while in other species the seeds are ovoid/oblong as in *J. canescens, J. unicostata, J. hernandifolia* and *J. brockmanii.* Variegated seeds are found in *J. macrorhiza, J. brockmanii,* and *J. capensis* while mottled seeds are characteristic of *J. unicostata, J. cinerea,* and *J. platyphylla* and such seeds have median ventral ridge/raphae. Large lobed caruncle is characterized in *J. capensis, J. macrorhiza, J. brockmanii,* and *J. hieronmyii* while small caruncle is found in *J. canescens, J. cinerea, J. platyphylla* and *J. cardiophylla.*

Structure and development of seeds and caruncle in Euphorbiaceae has been studied by a number of workers (Corner 1976) and comparative light and scanning electron microscopic data on microcharacters proved reliable for identification and therefore, taxonomically useful (Dey and Roy 1995). Dehgan and Webster (1979) described the seed characters in several *Jatropha* species and commented on the African geophytic species to possess small seeds and small caruncle where as the Central American species are large shrubs/trees characterized with large seeds with small caruncle. Bahadur and Goverdhan (1996) have studied seed and caruncle characters of various *Jatropha* species. Casas and Salas (2008) recently described *J. sotoi* as a new species from Madagascar and provided good description and photographs of seed and caruncle. Comparative light and scanning electron microscopic characteristics of testa and caruncle characters of nine Indian *Jatropha* species has been described by Bahadur and Goverdhan (1996) and is reviewed as under.

J. glandulifera

LM: Seeds oblong, 9.0 mm long × 5.0–6.0 mm wide. Testa shiny dark brown to black with dark brown to black streaks, on both dorsal and ventral sides with a conspicuous median line on the ventral side which is somewhat triangular. Hilum circular between the junction of caruncle and median line, cream yellow 2.0 × 4.0 mm wide, massive, palmately lobed; lobes 14 present bilaterally along the median long (Fig. 7.3a).

SEM: Spermoderm reticulate composed of small polygonal cells, cells convex with pointed tip forming reticulate pattern. Caruncle made up of several elliptic lobes, with a large distinct elliptic pore. Caruncle cellular made up of irregular reticulate convex cells (Fig. 7.7c–e).

J. gossypiifolia var. *gossypiifolia*

LM: Seeds triangular oblong, 7.5–9.5 mm long × 4.5 mm wide, testa smooth yellowish, light brown to dark brown, with two stripes present along the median line on

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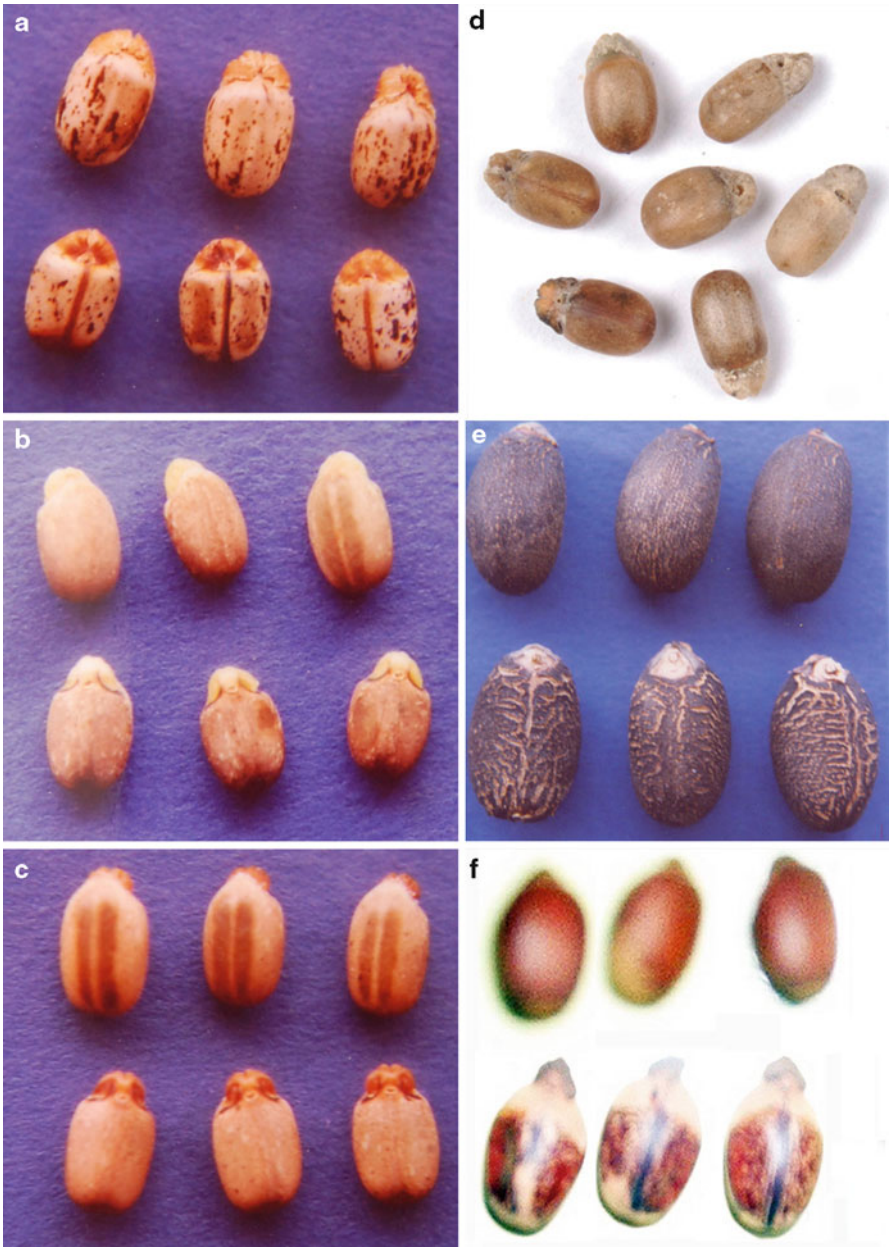

Fig. 7.3 Seeds of *Jatropha* species showing testa and caruncle characters in dorsal and ventral views, ventral view shows a linear streak extending from hilum downwards, testa colour, ornamentation, caruncle size and colour variation can also be seen: (**a**) *J. glandulifera* (× 5), (**b**) *J. gossypiifolia* var. *elegans* (× 6), (**c**) *J. gossypiifolia* var. *gossypiifolia* (× 6), (**d**) *J. nana* (× 7), (**e**) *J. curcas* (× 1.5), (**f**) *J. heynei* (× 4)

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abaxial convex side, testa finely mottled and adaxial side flattened. Caruncle 2.0 mm, ventral surface longitudinally furrowed along at higher magnification 2.0 mm wide, sulphur yellow apparently, two lobed but actually 6–15 lobed with elliptic reticulate and deep lumen. Hilum circular, conspicuous, situated at the junction of caruncle and seed (Figs. 7.3c and 7.7a, b).

SEM: Spermoderm reticulate, composed of polygonal cells, middle lamella conspicuous, pattern irregular, micro reticulate cells with striations, each cell ornamented (Fig. 7.6g).

J. gossypiifolia var. *elegans*

LM: Seeds oblong, 7.0 mm long, 5.0 mm wide, testa smooth light brown with two linear with 8–10 dark light brown stripes. Caruncle dark brown, 2.0 mm long, 3.0 mm wide sulphur yellow branched lobes 15, each lobe with elliptic oval pore. Hilum circular, near junction of caruncle and seed, on the median ridge conspicuous, surface smooth median ridge less conspicuous (Figs. 7.3b and 7.8d).

SEM: Spermoderm reticulate, ribbed, interspace composed of moniliform cells in linear rows, middle lamella inconspicuous, monomorphic pattern irregular, secondary ornamentation in cell surface with hexagonal elevated tubercles. Tertiary ornamentation of granulate surface with tubercles, caruncle massive with deep wide elliptic pore in each lobe, each lobe reticulate (Fig. 7.6f).

J. nana

LM: Seeds oblong 0.9 long × 0.46 cm wide with ventral ridge testa brown, crustaceous with linear stripes. Caruncle yellowish, lobes light brown/somewhat conical about ¼ the size of the seed (Fig. 7.3d).

J. curcas

LM: Seeds large triangular to ellipsoid, 1.6–2.5 cm long × 1.2 × 1.5 cm wide, testa rough, dark brown to black woody encrushed, striate, rather rough, cracking at maturity. Hilum circular, conspicuous situated at junction of median rib and caruncle white, 2.0 mm long × 3.0 mm wide, not distinctly lobed, hilum small basal (Figs. 7.3e and 7.7f).

SEM: Spermoderm coarsely reticulate of polygonal cells, cells polymorphic, anticlinal walls straight to wavy, thick with conspicuous middle lamella. Ridges and cell surface traversed by interlocking wavy striations.

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J. heynei

LM: Seeds ovoid, 0.75 cm long × 0.5 cm wide, testa brown crustaceous. Caruncle small, light Brown (Fig. 7.3f).

J. villosa

LM: Seeds oblong, 1.0 cm long × 0.6 cm wide, with ventral ridge, testa brown glossy, refractive, smooth. Caruncle curved, white at edges (Fig. 7.4a).

J. maheshwarii

LM: Seeds oblong, 1.3 cm long × 0.8 cm wide with ventral ridge testa brown, crustaceous, brownish, shining. Caruncle massive prominent $^{1}/_{4}$th size of seed (3.4 mm) apparently four lobed with white edges, brown in the middle, comprised of six to eight smaller lobes (Fig. 7.4b).

J. inequalis

Endemic to Mozambique, the seeds are oval to somewhat quadrangular mottled with violet-crimson spots of various sizes more on the dorsal side. Ventral side with raphe terminally ending with hilum and caruncle. Caruncle large about one-third of the seed size forming a collar like structures with distinct lobes (Fig. 7.4c).

J. scaposa

Endemic to Mozambique, seeds small, oval to somewhat qudarngular, mottled with a conspicuous median ventral ridge ending with hilum. Caruncle large, disproportionately larger than seed with distinct horny lobes (Fig. 7.4d).

J. mahafalensis

Seeds rather large, ovoid to spherical with a beak and conspicuous ribs. Colour light olive, devoid of any pigmentation or streaks. Caruncle inconspiquous. Hilum at the tip of the beak (Fig. 7.4e).

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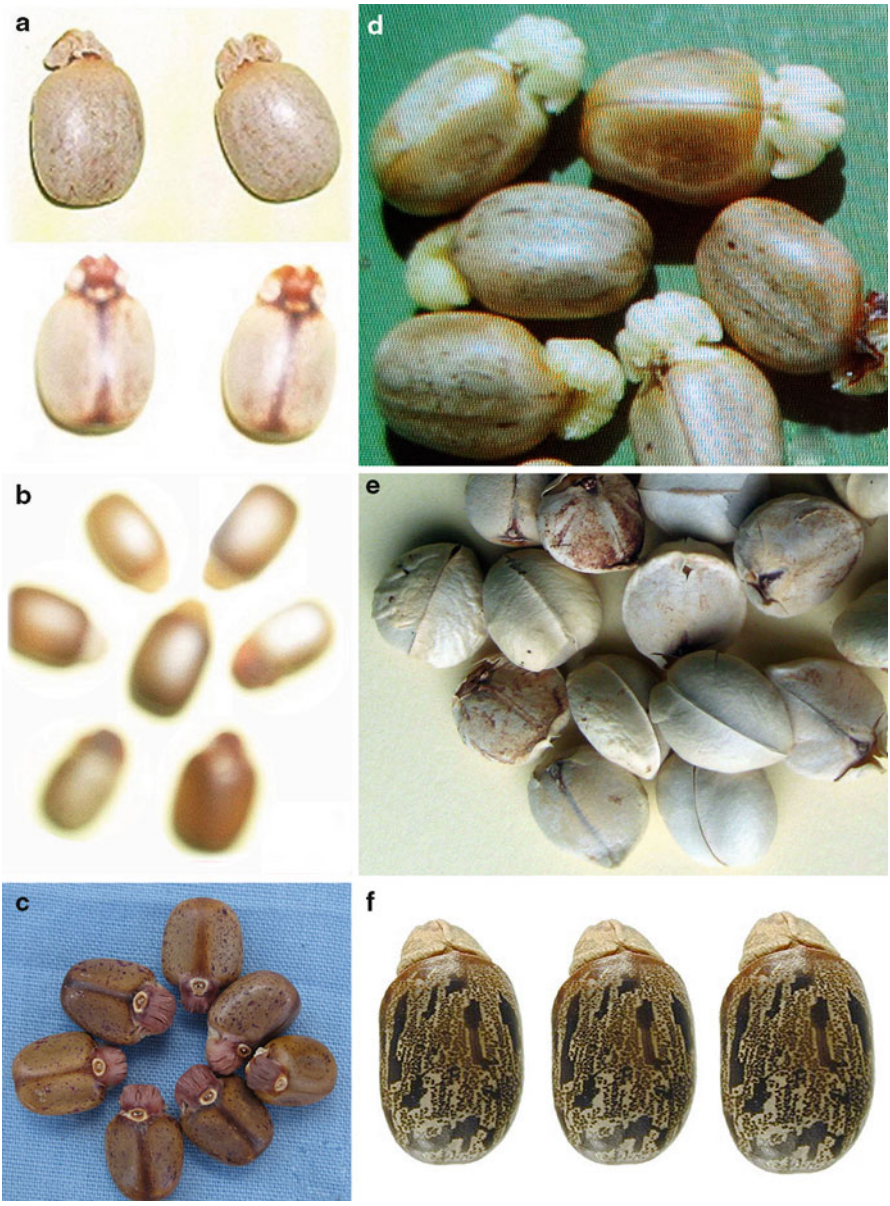

Fig. 7.4 Seeds of *Jatropha* species showing testa and caruncle characters in dorsal and ventral views, ventral view shows a linear streak extending from hilum downwards, testa colour, ornamentation, caruncle size and colour variation can also be seen: (**a**) *J. villosa* (× 5), (**b**) *J. maheshwarii* (× 4), (**c**) *J. inequalis* (× 4), (**d**) *J. scaposa* (× 9), (**e**) *J. mahafalensis* (× 8), (**f**) *J. acanthophylla* (× 6)

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J. acanthophylla

Seeds large measure 1.75 × 1.0 cm. In dorsal view testa is provided with a number of dark brown uneven linear streaks with round spots scattered all over the light brown testa. Caruncle conspicuous 0.7 × 0.34 mm size; cream coloured (Fig. 7.4f).

J. podagrica

LM: Seeds oblong to elliptic, 1.2 cm long × 0.7 cm wide, glossy, testa dark brown, mottled with numerous linear stripes on abaxial surface, ventral surface flat with conspicuous median rib, orange yellow in colour, hilum circular situated at the junction of the median ridge and caruncle. Caruncle small white, conical to triangular (Figs. 7.5a and 7.8a, b). The seed on the top left with greenish yellow colour belongs to the variety found in Africa and is shown here for comparison.

SEM: Spermoderm reticulate of polygonal cells, anticlinal walls raised, middle lamella inconspicuous. Cells concave with numerous tubercles, caruncle cellular, white, lobed (Fig. 7.6a).

J. multifida

LM: Seeds round to spherical, 1.8–1.5 cm long × 1.3 cm wide. Testa crustaceous, uniformly chocolate brown rather rough. Hilum ellipsoidal at the junction of small caruncle (Figs. 7.5b and 7.8c).

SEM: Spermoderm reticulate composed of deeply concave cells with thick anticlinal walls, deeply concave with lumen, anticlinal walls unevenly thick (Fig. 7.6b, c).

J. panduraefolia

LM: Seeds oblong, 8.0 mm long 4.5 mm wide. Testa light-pink, mottled, shiny with median ventral on abaxial side, smaller in size than *J. integerrima.* Caruncle white, forming 'T' shape conspicuous structure on the ridge, ventral side with linear striations 1.0 mm long, 2.0 mm wide (Fig. 7.5c).

SEM: Spermoderm reticulate, polymorphic anticlinal walls, straight to slightly wavy forming raised structures at junctions, middle lamella inconspicuous. Cells convex with irregular interlocking and overlapping reticulate discontinuous striations of uneven sizes and lengths, present between and around the convex dome-like cells (Fig. 7.6h).

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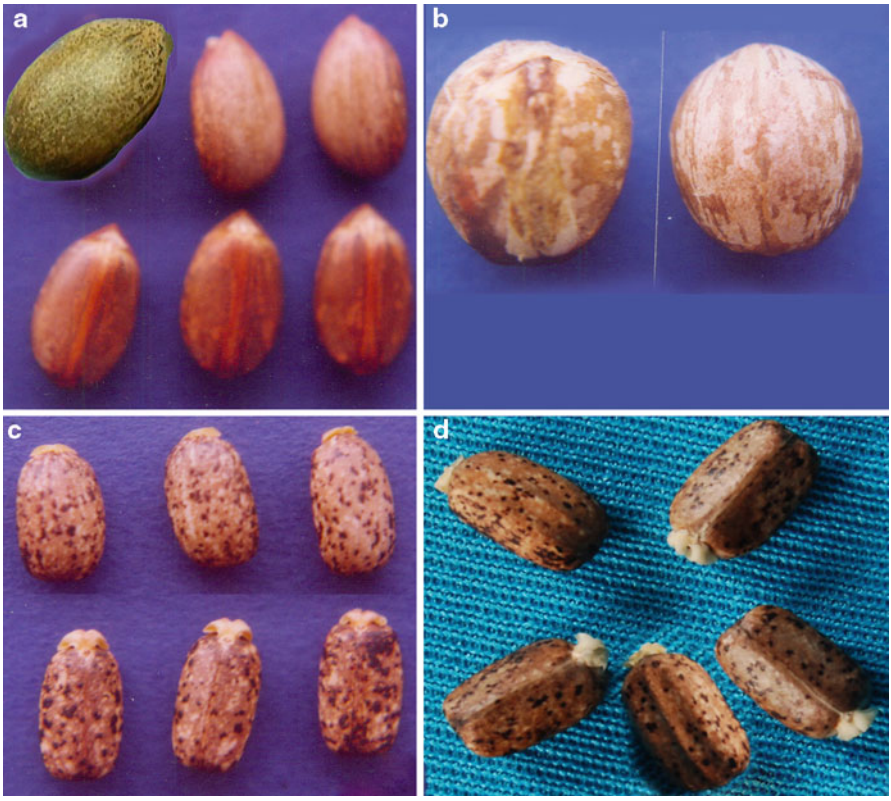

Fig. 7.5 Seeds of *Jatropha* species showing testa and caruncle characters in dorsal and ventral views, ventral view shows a linear streak extending from hilum downwards, testa colour, ornamentation, caruncle size and colour variation can also be seen: (**a**) *J. podagrica* (× 6), (**b**) *J. multifida* (× 6), (**c**) *J. panduraefolia* (× 10), (**d**) *J. integerrima* (× 10)

J. integerrima

LM: Seeds triangular to oblong to rod shaped, 0.9 long and 1.5 cm wide, testa shiny, light to dark brown, mottled with numerous uneven black spots, irregularly distributed on both abaxial and adaxial sides. Median ventral ridge raised, spots purple coloured in undeveloped seeds. Adaxial face with median ridge joining with circular hilum and caruncle. Caruncle reflexed, two lobed lacerate, white to cream, 1.0 mm long × 3.0 mm wide more conspicuous than *J. panduraefolia* (Figs. 7.5d and 7.7g).

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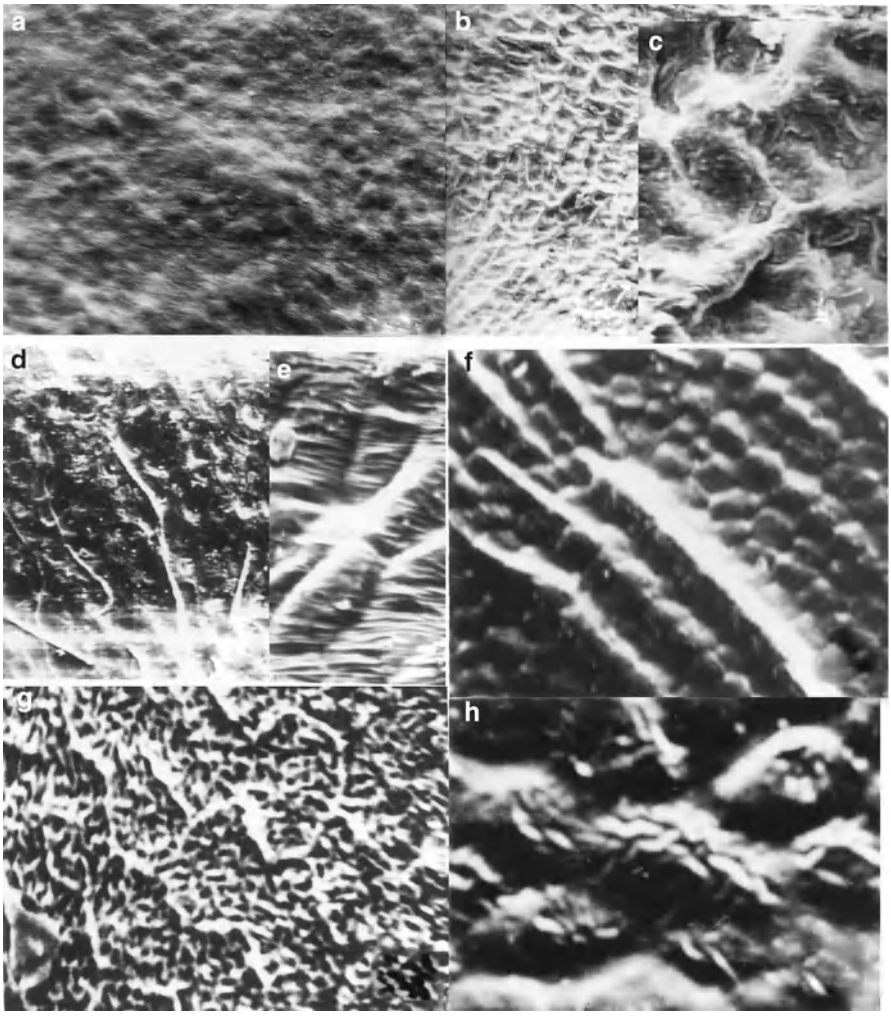

Fig. 7.6 SEM micrographs of spermoderm. (**a**) *J. podagrica* spermoderm showing unevenly scattered tubercles (× 600), (**b**, **c**) *J. multifida* spermoderm with scalariform reticulate cells, portion enlarged showing concave pits and thick wall (×550, ×2,200), (**d**, **e**) *J. tanjorensis* (× 600, × 2200), (**f**) *J. gossypiifolia* var. *elegans* spermoderm uneven, ribbed, ridges with bulbous cells of various sizes in rows (× 350), (**g**) *J. gossypiifolia* var. *gossypiifolia* spermoderm showing reticulate cells with ornamentation (× 350), (**h**) *J. panduraefolia* spermoderm with reticulate cells, convex with conspicuous striations (× 1,500)

SEM: Spermoderm reticulate, compact polymorphic, anticlinal walls thick straight to slightly wavy, middle lamella inconspicuous with clusters of transverse striations, scattered, forming depressions, caruncle distinctly lobed with linear striations and pore deep elliptic.

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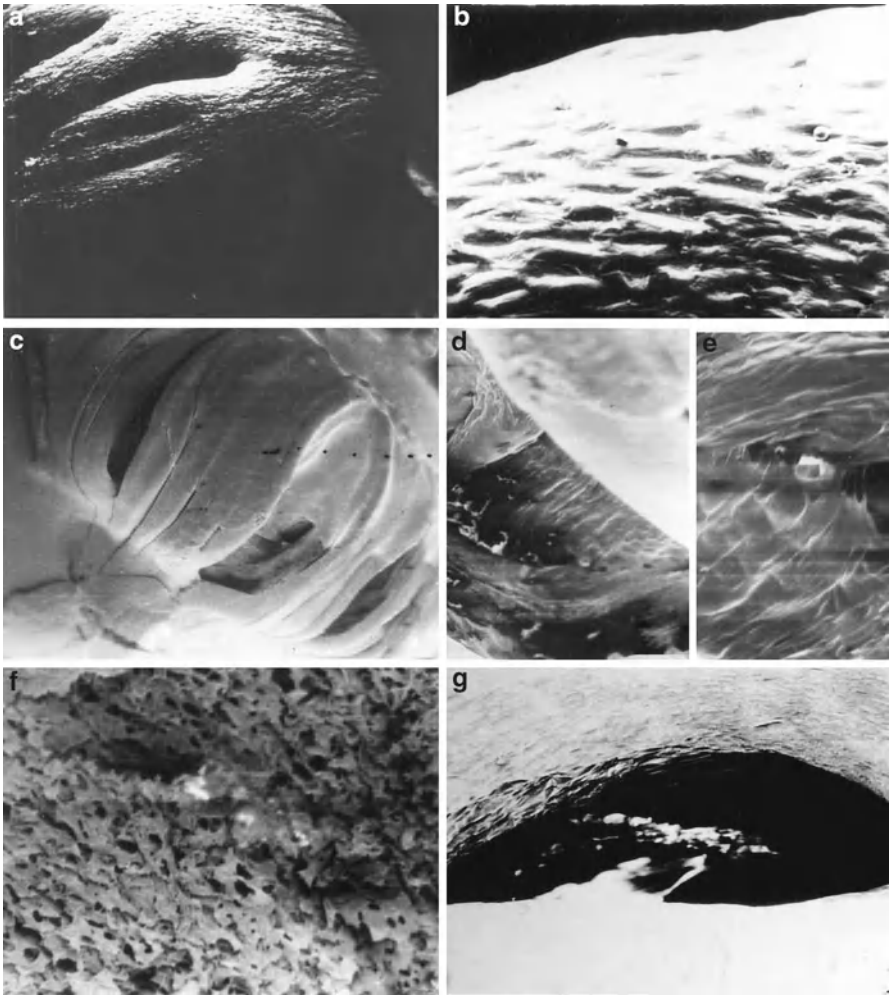

Fig. 7.7 Caruncle of *Jatropha* species. (**a**) *J. gossypiifolia* var. *gossypiifolia* caruncle showing deeply lobed linear (× 150), (**b**) Portion enlarged showing reticulate surface, thick walled, deep elliptic pits (× 1,280), (**c**, **d**, **e**) *J. glandulifera* caruncle showing semi-circular ring with distinct lobes, each with elliptic aperture surrounded by distinct rings with a median furrow, inner concave portion with rim, cellular detail of elliptic are made of two different types of cells – one with round pits, the other reticulate cells (× 140, × 2,000), (**f**) *J. curcas* caruncle showing unorganized mass of reticulate cells with pits (× 250), (**g**) *J. integerrima,* caruncle several lobed, lobes enlarged showing elliptic pore with linear reticulate cells around the margin and surrounding area (× 300)

J. tanjorensis

LM: Seeds ovoid to oblong, 1.2 cm long × 0.7 cm wide, pale green with deep pink linear streaks spots dark brown when dry, hilum circular, conspicuous at the junction of adaxial ridge, caruncle conspicuous striated, white 2.0 mm long × 3.0 mm wide (Fig. 7.8e, f).

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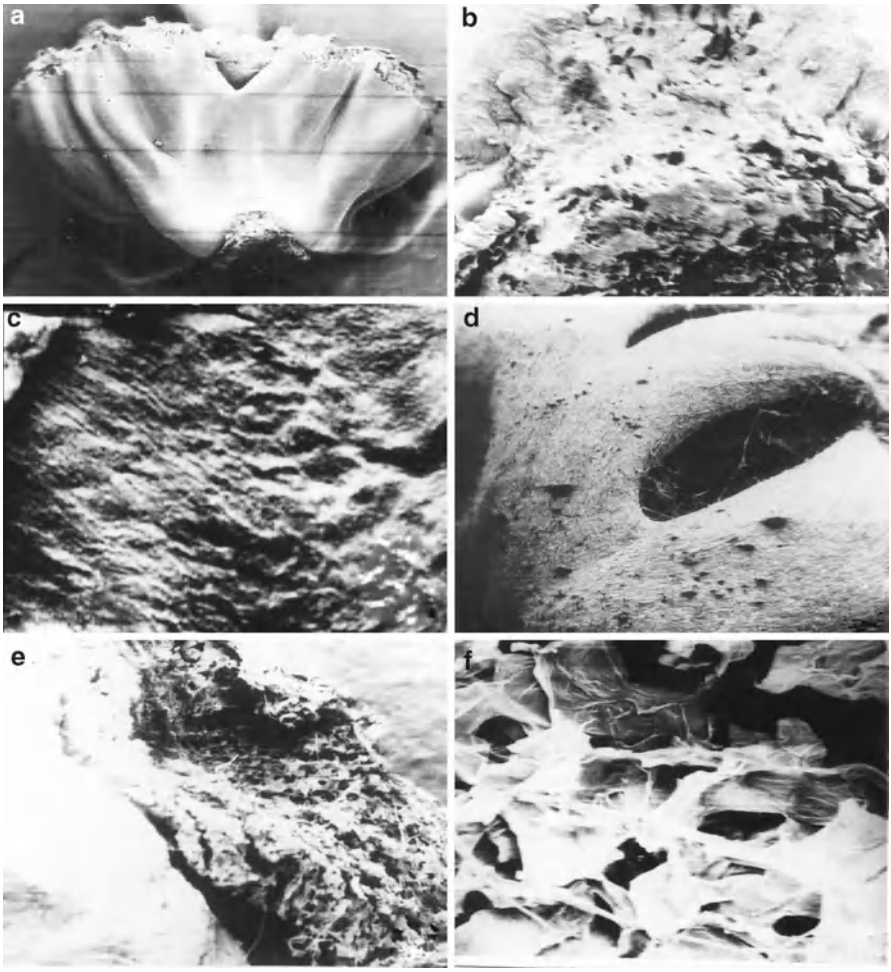

Fig. 7.8 Caruncle of *Jatropha* species. (**a**, **b**) *J. podagrica* caruncle crown like with number of lobes, lobe tip distinctly cellular and undifferentiated mass of cells (× 120, × 700), (**c**) *J. multifida* showing round gemmae/tubercles (× 1,200), (**d**) *J. gossypiifolia* var. *elegans* caruncle made of several oval-elliptic lobes with cottony mass in the deep concave pit, cells around pit reticulate. (× 150), (**e**, **f**) *J. tanjorensis* caruncle showing unorganised reticulate cells, portion enlarged showing cells with pits of various shapes and sizes, inside the pith some ingrowth can be seen (× 100, × 650)

SEM: Spermoderm with concentric layers of tubercles of uneven sizes and scattered, inter tubercular space reticulate with interlocking striations with thick anticlinal walls. Caruncle is made up of uneven spongy cellular tissue, cells unevenly reticulate with pits and striations (Fig. 7.6d, e).

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J. sotoi

Seeds small, ventral side flat. Testa variegated in coffee and brown tones with graininess somewhat obscure, back of the seed convex, gently carinate in all its length, apex obtuse. Hilum between the ventral keel and caruncle is relatively large about 2–2.5 mm, borne above the hilum and extended back covering the apex, fringed chestnut coloured with white extremes of the fringes.

Because of the taxonomic stability and reliability of various fruit and seed characters, both macro and microcharacters have been explored to find out the relationship between various *Jatropha* species. The seeds of *Jatropha* studied are macroscopic and vary in size 1.5 × 1.3 cm to 7.0 × 4.0 mm. Smaller seeds are found in *J. gossypiifolia* var. *gossypiifolia* and large seeds in *J. multifida*. Seed colour (testa) being mostly light brown to dark brown, black seeds are seen only in *J. curcas* and black brown in *J. tanjorensis*. Seed shape is variable, being triangular, oblong (*J. glandulifera, J. nana, J. gossypiifolia* var. *elegans, J. gossypiifolia* var. *gossypiifolia, J. tanjorensis, J. maheshwarii, J. heynei, J. curcas, J. integerrima* and *J. panduraefolia,* oblong to elliptic in *J. podagrica* and round to spheroidal in *J. multifida*. Seeds are carunculate of various shapes and sizes and are a prominent feature in most *Jatropha* species. The seed size character appears to be related to the shattering nature of the capsules for effective dispersal and hence gene flow, particularly in the wild species which are generally provided with thin pericap. The spermoderm of *Jatropha* in various species is primarily reticulate but ribbed spermoderm with moniliform cells is seen in *J. gossypiifolia* var. *elegans* and polygonal cells in *J. panduraefolia, J. curcas, J. gossypiifolia* var. *gossypiifolia, J. podagrica* and *J. glandulifera*. The cell surface is ornamented with secondary and tertiary structures which are species specific. Elevated tubercles with granulate surface *(J. gossypiifolia* var. *elegans)*, interspaces of tubercles are traversed within curved structures (*J. podagrica*), ridges forming elevated tips at junctions traversed by interlocking wavy structures (*J. curcas, J. glandulifera* and *J. tanjorensis*), striations on wall ornamentation within each cell (*J. gossypiifolia* var. *gossypiifolia*) were observed.

Testa ornamentation (spermoderm) and caruncle features show a great degree of diversity and hence are of taxonomic value. Accordingly, the Indian *Jatropha* species can be categorized into four groups.

1. ***Spermoderm reticulate, cells reticulate*** (*J. tanjorensis, J. multifida*)
2. ***Spermoderm reticulate, cells polygonal*** (*J. gossypiifolia* var. *gossypiifolia, J. curcas, J. podagrica, J. glandulifera, J. panduraefolia*)
3. **Spermoderm reticulate, anticlinal walls straight to wavy** (J. integerrima)
4. ***Spermoderm ribbed, moniliform cells*** (*J. gossypiifolia* var. *elegans*)

The spermoderm characters in other *Jatropha* species need to be studied on similar lines as has been done by Ehler (1976). In his study of 123 *Euphorbia* (Euphorbiaceae) he noted spermoderm pattern to be not only species specific but section specific.

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Table 7.1 Seed weight and oil content of *Jatropha* species

Sl. No.	Species	Weight in g (25 seeds)	Oil content (%)[a]
1	*Jatropha gossypiifolia* var. *gossypiifolia*	3.33	27.1
2	*J. gossypiifolia* var. *elegans*	3.15	–
3	*J. glandulifera*	3.23	28.3
4	*J. tanjorensis*	4.56	–
5	*J. curcas*	18.05	30–42
6	*J. podagrica*	6.55	Up to 54
7	*J. multifida*	22.40	32–40
8	*J. integerrima*	3.58	Not reported
9	*J. panduraefolia*	3.20	Not reported
10	*J. villosa*	8.57	Not reported
11	*J. maheshwarii*	9.83	Not reported
12	*J. nana*	3.40	40
13	*J. heynei*	4.20	Not reported

[a] *Source*: Rata Krishnan and Paramathma 2009; Bhagat and Kulkarni 2006; author's unpublished data

The present study is though very limited, suggest such a correlation to be possible in *Jatropha* and therefore additional species belonging to various sections including the endemics of Africa and Americas need to be studied.

Comparison of mean seed size, weight and oil content of various Indian *Jatropha* species (Table 7.1) reveal that *J. multifida* seeds are comparatively heavier (22.1 g) followed by *J. curcas* (18.05 g), *J. maheshwarii, J. villosa, J. heynei, J. panduraefolia* and *J. integerrima*. Smaller seeds are found in *J. nana, J. gossypiifolia, J. glandulifera* and *J. scaposa.* The bigger the seed size, the higher the oil content with *J. nana* as an exception.

The seed dispersal mechanism appears to be directly related to seed and caruncle size and the nature of pericarp whether thin or thick. Dehgan and Schutzman (1994) in their monographic study of *Jatropha* noted that capsule dehiscence as a feature, and recognized (1) violently dehiscent, (2) tardily dehiscent, (3) fruit may explode or remain intact for some time on plant but the differences between these categories are sharp. Most of the *Jatropha* species exhibit violent dehiscent and fall under category one stated above; although the demarcation is difficult to make in some. *J. multifida* is the only species with large fleshy indehiscent fruits and largest seeds. Anez et al. (2005) have studied fruits and seeds of *J. elliptica* and noted explosive fruits with oval seeds, smooth testa with marmoreal colour with distinct longitudinal raphae and caruncle on the ventral side with visible hilum. Unlike *J. curcas* with large black seeds, inconspicuous, elaiosome the seeds of *J. hieronymii* and *J. excelsa* show explosive dispersal of seeds with the former being eight times larger than the latter and reaches 18 m in distance because of their aerodynamic shape and explosive fruit. Further, the removal of seeds by ants has been correlated with the presence of elaiosome and this was greater in *J. excelsa* by 83.6% while *J. hieronymii*

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has only 31.6%. Thus, seed size was the major factor affecting the removal of seeds by large bodied ants. The advantages of seed dispersal mode in the arid zones of Argentina are also discussed (Rickert and Francchia 2006).

Autochory (ballistic) discharge of seeds by explosively dehiscent capsules is followed by myremechory a common feature of many taxa of Euphorbiaceae (Webster 1994). Despite pantropical distribution of *Jatropha,* a vast majority of its species are endemic and often restricted to small regions other than *J. gossypiifolia,* which is reported to be eaten and disseminated by pigeons (Standley 1923). Long-distance dispersal has been reported and the present distribution of the genus appears to be the result of progressive overland dispersal with the exception of a few taxa that have become spontaneous or sub-spontaneous with the help of humans. Thus, no truly disjunct species have been reported within or between the continents of Africa, America, and the subcontinent of India. Their complete absence from all oceanic islands except *J. divaricata* and *J. hernandifolia* in Jamaica is a further indication of their inability to disperse by birds or ocean currents (Dehgan and Schutzman 1994). It may be of interest to note that *J. tanjorensis* endemic to Tamil Nadu, which rarely sets seeds is naturalized in southern Nigeria. It is possible that this species in view of its antidiabetic potential has been smuggled out from India, whether it sets seeds in Nigeria is not known (Idu et al. 2009).

Several genera in the Euphorbiaceae exhibit ballistic/dehiscent fruits and caruncled seeds, such as *Aleurites* (Asia), *Hevea, Joannesia, Cnidoscolus, Manihot* (American) and *Grossera. Crotonogyne* (African) although widespread within the continent, are not dispersable by interoceanic means. Therefore, it is assumed that disjunct presence of Jatrophas in three widely separated regions is a good indication of its antiquity, and hence its primitiveness (Dehgan and Schutzman 1994).

According to Dehgan and Webster (1979), the main distinguishing characters for establishing the phylogenetic relationship in the two sub-genera are the shape of the seed, size and morphology of caruncle. *J. curcas* has a relatively small caruncle appressed to the beak and is distinctly lobed. More or less spherical to ellipsoidal seeds with a small caruncle are common in other species of the sub-genus *Curcas.* Seeds of *J. fremontioides* resemble with those of *J. hieronymii.* Caruncle measures 8–10 mm with distinct lobes. In general, the seeds of sub-sections *Jatropha* are grayish brown mottled with a massive caruncle in proportion to the seeds. Smallest seeds are found in African species of the section *Collenucia. J. multifida* has the largest seed in the genus. It is interesting to note that McVaugh (1945) was the first to suggest the importance of seed and caruncle characters in the various sections and sub-sections of *Jatropha* in his study of American species. The salient features of his study are as under:

Section *Adenorhopium*: Seeds are small with a small scar and large lobes fimbriate caruncle (*J. gossypiifolia*).
Section *Macranthae:* Seeds are small with a small scar and large fimbriate caruncle (*J. macrantha*).
Section *Polymorphae:* Seeds are oblong with reflexed and lacerate caruncle (*J. integerrima*).

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Section *Mozinna:* Seeds are globose with large hilum, caruncle recurred, gray to dull brown (*J. capensis* and *J. inequalis*).
Section Eucurcas: Seeds are black, encrusted, striated 15–22 mm long, capsule show tardily dehiscent (*J. curcas*) species of sub-section are small, black lustrous, 10–15 mm long (*J. pseudocurcas*) occurring in Mexico while species with black, lustrous, 10–12 mm long and 7–8 mm wide occur in Chile (*J. andriexii*).

Caruncle and Myrmechory

A caruncle is a fleshy to horny outgrowth/appendage rich in dietery lipids and diglycerides situated near the hilum of seed, formed from the integuments or arising from fruit tissue including aril and attracts insects and serves as reward for dispersal and known to occur in 77 families, comprising 334 genera and 11,000 species of angiosperms (Lengyel et al. 2010; Culver and Beattie 1978). The size and shape of the caruncle varies considerably not only in the Indian *Jatropha* species but also in species belonging to various sections of *Jatropha* studied by Dehgan and Webster (1979) mentioned above including the present study.

In Section *Polymorphae* (*J. integerrima*), the caruncle is reflexed and lacerate, Section *Macranthae* (*J. macrantha*) the caruncle is large and fimbriate, Section *Adenoprhopium* (*J. gossypiifolia*) caruncle is large finely lobed, fimbriate, Section *Mozinna* (*J. spathulata)* minute appressed –recurved caruncle while in Sub-section *Eucurcas* (*J. curcas*) the caruncle is minute (Figs. 7.7 and 7.8).

J. scaposa has small seeds and the caruncle is disproportionately large, white and fleshy. In *J. sotoi* the caruncle is large, borne above the hilum and is extended back covering the apex, fringed, chestnut coloured with white extremes of the fringes (Casas and Salas 2008). *J. spinosa* has large seeds with filiform dissected caruncle (Dehgan and Webster 1979).

Myrmechory is a common feature of sclerophyll ecosystem and relevant to seed dispersal mode. It may be of interest to note that the caruncle bearing euphorbs represent the largest group of Caatinga forest of north east Brazil (Leal 2003). Leal et al. (2007) studied true myrmechores seeds of seven euphorbs including some Jatrophas with elaiosomic seeds to quantify the rate of seed removal and influence of both seed size and elaisome presence on seed removal, to identify the seeds dispersed by ants at microsites and the benefit of seed dispersal by ants on seed germination and seedling growth at ant-nest sites, and noted better germination in *J. ribifolia, J. gossypiifolia, J. mollisissima* and two *Cnidoscolus* species in comparison to other elaiosomic seeded species. A similar study on wild *Jatropha* species is required to understand the seed dispersal mechanism under Indian conditions.

The advantage of ant dispersal is the relocation of seed to safe site for safe seed germination thereby reducing predation and increasing germination stimulus with enhanced nutrient supply. In addition, this is the best option and safe method for gene flow characteristic to carunculate species. It is significant to comment that conspicuous caruncle is generally associated with species with small seed and in

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the large seeded species, the caruncle is inconspicuous or may be absent. The small seed size and conspicuous caruncle seems to be an adaptive strategy with myremechorous dispersal, since ants can easily carry and disperse the small light seeded species.

Seedling Characters

Study of seedling characters represent, a juvenile stage in plant germination and their utility in botanical systematics is usually ignored although considerable work during the last 30 years has shown their taxonomic value (Burger 1972; Muller 1978; Bahadur and Rao 1980; Bahadur et al. 1983a; Paria 1996). Smith and Scott (1985) in their study of 93 species of Caesalpinaceae recognized six venation patterns in seedling leaves and discussed their evolutionary significance. A wide range of seedling characters have been found suitable even in genetic studies (Crow 1963); *Carica papaya* seedlings can be easily sexed by merely looking at their leaf morphology. The female plants are characterized by three lobed leaves while the male plants are five lobed and this was confirmed by RAPD analysis (Reddy 2006).

To date there are few studies on seedling morphology by Duke (1969) in *J. hieronymii* and *J. macrorhiz*a followed by Dehgan and Webster (1979) in *J curcas,* and *J. multifida.* Subsequently, seedling characters of various *Jatropha* species have been investigated (Kamilya and Paria 1994; Bahadur and Goverdhan 1996; Anez et al. 2005). They studied freshly raised seedlings (ready in 12–25 days) in various *Jatropha* species and noted seedling length, root characters, cotyledon, leaf colour, thickness, size and number of major veins, petiole length, stomatal size, stomatal index and classified the seedlings into three groups as 3, 5 and 7–9 veined.

Germination in *Jatropha* species is epigeal and phenerocotylar (i.e. the cotyledons are freed from seed coat in the form of a cap) except *J. multifida*. The root system of the seedlings consists of a primary and four to five adventitious roots. The primary root differentiates directly downward from the lower end of the hypocotyl. The cotyledon leaf colour is light to dark green. Large cotyledon leaf is seen in *J. curcas* (5 × 4 cm) while the smallest is seen in *J. multifida* (1.5 × 0.5 cm). The cotyledon leaf of *J. podagrica* is succulent and that of *J. gossypiifolia* var. *elegans* is thin. Most of the *Jatropha* species uniformly show five major veins in cotyledon leaf, except *J. integerrima* which is characterised by three major veins while *J. curcas* has seven to nine veins. Epidermal cells from upper and lower epidermis of the seedling leaf are mostly polygonal with straight to rarely curved anticlinal walls. The cells vary in size both within and between species. The cells on the upper surface are generally bigger than those of the lower surface. Stomata occur on both the surfaces of cotyledon leaves (amphistomatic) in most species except *J. integerrima* where they are hypostomatic. Paracytic stomata are found in *J. curcas* while brachyparacytic condition exists in *J. podagrica* and *J. multifida*. A mixture of paracytic and brachyparacytic types characterise epidermal surfaces of both varieties of *J. gossypiifolia* var. *elegans* and *gossypiifolia* respectively. Bigger stomata were

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found in cotyledons of *J. gossypiifolia* var. *gossypiifolia* while small stomata were seen in *J. podagrica.* Stomatal index was high in species with species amphistomatic with and *J. integerrima* is hypostomatic. Thus, on the basis of the above characters *J. integerrima* can be separated as distinct species as also *J. gossypiifolia* var. *gossypiifolia* and *J. gossypiifolia* var. *elegans.* Deore and Johnson (2008) reported viviparous germination in 2-year old *J. curcas* plants; a feature of mangroves. Vivipary is associated with lack of dormancy; and *J. curcas* seeds loose 50% dormancy in about 15 months. This may be an adaptive strategy to enable seedlings to establish rapidly under favourable humid conditions.

The seedling characters of *Jatropha* species were found to be diagnostic and serve as identification markers in *Jatropha* taxonomy. Apart from the seedling size paracotyledon leaf and its associated characters particularly the venation pattern i.e. three or five veined grouping coupled with stomatal index, stoma size, whether amphi- or hypostomatic help in delimiting the various species. Thus, the application of seedling characters is important in taxonomic consideration of the large genus *Jatropha.* On the basis of the above observations, seedlings of *Jatropha* can be grouped into two categories.

1. Seedling length 9–11 cm (*J. gossypiifolia*, *J. podagrica*, *J. heynei*)
2. Seedling length 19–24 cm (*J. curcas, J. multifida, J. maheshwarii*)

Furthermore, the cotyledon veins in *J. integerrima* are three as against five in *J. panduraefolia*, *J. macrorhiza* and *J. hieronmyii* in addition cotyledon leaves in most of the species are in amphistomatic where as hypostomatic condition is characteristic of *J. integerrima.* Thus, *J. integerrima* differs from *J. panduraefolia*. Similarly, *J. gossypiifolia* var. *gossypiifolia* differs from *J. gossypiifolia* var. *elegans.* In addition, *J. maheshwarii* seedlings are different from the rest of the species studied with their reniform leaves and long petiole, a character also shared by *J. multifida* but differ from *J. maheshwarii* in possessing large palmate leaves. The cotyledon veins are three in section Polymorphae but are five in all other species with three dominant and two weaker laterals arising from the base and the cotyledons are pseudopalmately veined (Dehgan and Webster 1979).

It may be of interest to point out that in any plant improvement programme, seed source, colour, seed weight, seed germination and host of related characters are vital for screening and selection of seed sources for desirable traits in biodiesel plant like *J. curcas* and other species of horticultural importance and considerable work on *J. curcas* has been done which has direct relevance to germplasm conservation.

From the foregoing it is obvious that there is a need for further work to explore and exploit the microcharacters of seeds and caruncle in *Jatropha* taxonomy and their utility in *Jatropha* improvement.

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Chapter 8
Genetic Improvement in *Jatropha curcas* Through Selection and Breeding

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Introduction

Jatropha curcas L. has attracted the attention world over as an alternate fuel (Takeda 1982; Banerji et al. 1985; Martin and Mayeux 1985; Openshaw 2000) and due to this fact commercial plantations have been taken up in a number of countries. But it has led to the downfall of the species due to lack of information related to quality planting material with high oil content despite the fact that the plant is known for several desirable characters like hardiness to grow under adverse conditions including stony rocks as seen in Fig. 8.1, easy propagation either by seeds or cuttings, drought endurance and rapid growth. Reliable yield assessments with conventional agronomic methods on wastelands are not yet available for Jatropha. Therefore, selection and multiplication of superior germplasm for quality planting material is now the prime aim in achieving domestication and improvement in productivity of the species under adverse climatic conditions.

The process of making biodiesel of European standard (EN14214) from *Jatropha* has been developed by CSMCRI, Bhavnagar and patented (US patent no. 7,666,234 dt. 23.02.2010), but much needs to be done in terms of improving the productivity which is erratic and low and also the major bottleneck in making it commercially viable. In spite of the best downstream technologies available, the plant has not been accepted by growers for commercial production on large scale. Moreover, the plant has shown very low genetic variability in the germplasm around the world. Under these circumstances, the future strategies needs be focussed on selection of plants exhibiting desirable traits, improving them further using conventional and molecular breeding techniques and multiply clonally for raising more homogeneous populations until unusually high yielding genotype(s) are identified

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Fig. 8.1 *J. curcas* plants growing on rocks

or developed. During the studies conducted at CSMCRI, Bhavnagar, *J. curcas* germplasm was collected from a number of locations in India and planted on wastelands having poor nutrient content. Variability has been observed in relation to morphological, yield and oil characters as reported by number of workers earlier. Efforts are on to determine the production potential under adverse conditions and also to identify consistently high yielding genotypes for genetic improvement of this species.

Existing Variability for Various Traits

Till date, a number of studies have been conducted for understanding the extent of existing natural genetic variation in the species which is available in wild condition. Initially, Heller (1992) studied a collection of 13 accessions in multiple locations field trials in two countries of Sahel region: Senegal and Cape Verde. In Cape Verde, the climate is semi-arid with a short rainy season (approximately 4 months) and longer dry season (approximately 8 months) with a wide variation in rainfall (200–800 mm). The trials were conducted on the island of Santiago at Sao Jorge and Tarrafal (Chao Bom) and the vegetative development was evaluated at each location. Significant differences were reported in the vegetative development among the various provenances. Ginwal et al. (2005) reported seed source variability in central India, while Kaushik et al. (2007) studied the variation in seed traits and oil content in 24 accessions collected from Haryana state, India.

In one of the experiments, seeds from 23 candidate plus trees of *J. curcas* were collected from different geographical areas of the country and seedlings were planted in progeny trial plot at CSMCRI, Bhavnagar (Fig. 8.2) so as to determine the existing genetic variability and production potential of this species on

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Fig. 8.2 Progeny trial plot at CSMCRI experimental site

wastelands. Observations were recorded on morphological and yield contributing characters for 4 years (2005-06–2008-09) for identifying the most suitable accession with respect to growth and seed production on wastelands.

Floral Biology

The reproductive biology of flowering plants is important for understanding the barriers to seed and fruit set, pollination and breeding systems that regulate the genetic structure of populations. Floral biology has great significance in intra-varietal and intra-specific hybridization which offers possibilities to transfer useful genes from one genotype to another. Information on male to female flower ratio is a prerequisite to select suitable genotypes from the existing populations in Jatropha. Normally, in the inflorescence, a dichasial cyme produces a central female flower surrounded by a group of male flowers which are much more numerous and occupy subordinate positions within the inflorescences. Inflorescences are axillary, formed terminally on the new shoots and are complex, possessing main and co-florescences. We noted that the ratio of male and female flowers in all the 23 accessions in our studies on progeny trial fluctuated tremendously when studied for four consecutive years (Bhuva et al. 2007) and affected the fruit set as well as seed yield. It was concluded that apart from M/F flower ratio, number of female flowers converting into true fruits is more important to select superior genotypes from the existing population (Fig. 8.3). Previously Raju and Ezradanam (2002) studied the pollination ecology and fruiting behaviour in *J. curcas* and found the male to female ratio as 29:1. They also reported that both types of flowers open synchronously and the sexual system facilitates geitonogamy and xenogamy.

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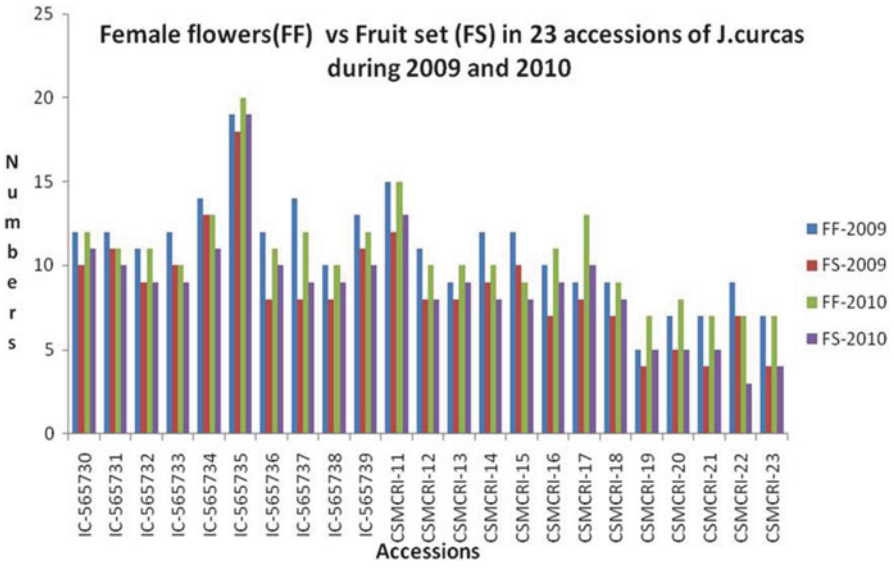

Fig. 8.3 Variation in number of female flowers converting into fruits – data of 2 years (2009 and 2010)

According to Kumar et al. (2008) the number of female flowers per inflorescence showed maximum variation while the difference for the number of male flowers/inflorescence was narrow. This variation is reported to be due to fluctuating environmental conditions.

Pollen Fertility and Fruit Set

In *J. curcas*, the individual flowers are grouped together in cymose pattern, an arrangement which promotes attraction and foraging by the insects. Earlier Dehgan and Webster (1978) reported pollination in Jatropha by moths because of its sweet, heavy perfume at night, greenish white flowers, versatile anthers, protruding sexual organs, copious nectar, and absence of visible nectar glands. Later, Raju and Ezradanam (2002) also reported that flowers of Jatropha are protandrous and pollination is mainly anthophilous affected by insects like bees, flies, ants and thrips, etc. On the contrary, Heller (1992) observed that staminate flowers open later than pistillate flowers in the same inflorescence promoting cross-pollination. Munch (1986) did not observe this chronological order in Cape Verde and Contrary to this that the mechanism is influenced by the environment.

During the experimental trials on 23 accessions conducted by CSMCRI, it was observed that flowers were protandrous in nature and ants and honey bees frequently visited the inflorescences and contributed to a greater extent in pollination. Most of the accessions exhibited more than 80% pollen fertility as shown in Fig. 8.4.

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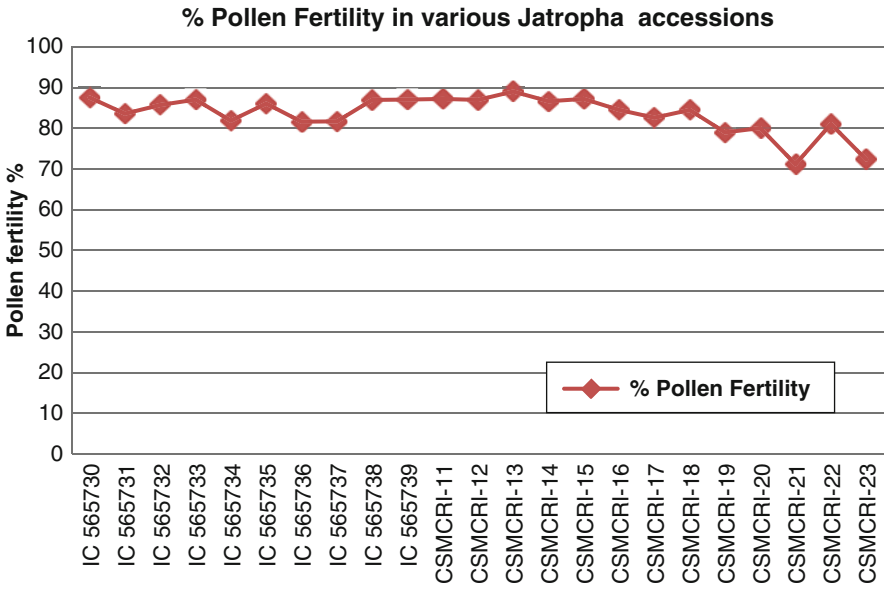

Fig. 8.4 Pollen fertility percentage in 23 accessions of *J. curcas*

Bhattacharya et al. (2005) made comprehensive studies related to floral biology, floral visitors and pollination of *J. curcas* and reported that each male flower produces 1,617 ± 100 pollen with as much as 539:1 high pollen: ovule ratio. The quantity of nectar obtained from female and male flowers was compared and as expected, the female flowers produced higher amount of nectar (4.54 ± 0.82 μL) than the male flowers (1.92 ± 0.44 μL) in 12 h. Apomixis rate was also reported up to 32% by them.

Yield Potential

To initiate breeding programme in any plant species, it is essential to have an idea of the extent of genetic variability available in the species for selection of superior germplasm. In our study we observed considerable variation in flowering and fruiting pattern in all the 23 accessions. The information available in the literature on seed production is from the plants which have not yet reached maturity (<5 years old). Earlier Aker (1997) conducted an exploratory study to detect patterns of variation in flower, fruit and seed production in 1 -year-old plants of *J. curcas* in response to variation in environmental conditions in Nicaragua. According to Kochhar et al (2005) the unreliable and poor flowering and fruiting are important factors responsible for low productivity in the species.

Heller (1996) reported seed yields between 0.1 and 8.0 $t\,ha^{-1}$. The details of the planting material and other experimental details under which this yields were achieved were not clear. Later, Openshaw (2000) reported seed yield between 0.4 and 12 $t\,ha^{-1}$. The realistic figures on seed yield are still not available in the literature but may come in next

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few years, as recently established plantations have begun to reach maturity. Recently, Brittaine and Lutaladio (2010) compiled the seed yields mentioned by various workers in one of the publication in the series of Integrated Crop Management published by Food and Agriculture Organization of the United Nations. It indicates that there is significant variability in seed yield because of locations, soil and environmental conditions.

In our field experiment, maximum variation was recorded for number of capsules, number of seeds and seed yield per plant among different accessions. The seeds were produced in 2–3 flushes and there were significant differences among accessions with regard to seed characters particularly the 100-seed weight. Oil content in seeds showed significant variation among different accessions i.e. from 26.3% to 34.9% and even in different flushes. Cumulative seed yield/plant of all the 23 accessions (date of planting: 2004-05, plant density = 1,667 plants ha^{-1}) for 4 years period (2005-06–2008-09) as shown in Table 8.1 ranged from 594 g (P-23)–1417 g (P-13). It was observed that one of the accessions IC 565735 (1,368 g $plant^{-1}$) was found to be consistently high yielder in all these years in Gujarat as well as in Orissa. During sixth year, 1.9 t ha^{-1} seed yield was obtained in one of the accessions, indicating that seed yield in excess of 2 t ha^{-1} $annum^{-1}$ would be attainable from mature plantations grown on wastelands.

Correlation analysis carried out between growth and yield contributing traits in 2-year-old plants at CSMCRI revealed that maximum and highly significant correlation (0.997**) was observed between number of capsules/plant and number of seeds/plant (Bhuva et al. 2007). Plant height was significantly correlated with plant canopy (0.845**), stem girth (0.898**) and number of secondary branches (0.855**). Seed yield/plant was found to be highly correlated with traits like number of seeds/plant (0.992**), number of secondary branches/plant (0.869**) and tertiary branches/plant (0.703**). The studies indicated that these characters directly influence the productivity in the species. Kumar et al. (2008) conducted studies to assess intra-specific variations and inter-relations among morphological traits in 27 accessions of *J. curcas* and recorded data on various morphological characters. Analysis of variation revealed highly significant differences among the accessions for these characters. A strong correlation between plant height and branch length, number of branches and collar diameter was observed in their study.

Ginwal et al. (2004) observed that genetic differences exist between the seed sources of *J. curcas*. Fair differences between phenotypic and genotypic coefficient of variability were recorded and heritability (broad sense) values were fairly good with regard to leaf area, height and collar diameter in comparison to survival percentage.

Pant et al. (2006) studied seed oil content variation in *J. curcas* in different altitudinal ranges and site conditions in Himachal Pradesh, India and observed that elevation had significant effect on yield attributes and growth parameters. It is due to the excess utilization of photo assimilation for growth over accumulation of oil. Rao et al. (2008) characterized germplasm of 32 high yielding candidate plus trees (CPTs) of *J. curcas* from different locations and found significant trait differences in all the seed characters and oil content in the progeny trial. Broad sense heritability was high in general and exceeded 80% for all the seed traits studied.

Though, the seed yields varied significantly, Basha and Sujatha (2007) and Sun et al. (2008) concluded that the genetic variability in *J. curcas* in Asia is very low

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Table 8.1 Performance of hybrids for yield and oil content parameters

Name of the cross	M/F ratio	No. of female flowers pollinated	No. of capsules set	Success (%)	Single seed weight	Oil (%)
IC 565730 × IC 565731	10.22	64	47	73.44	0.742	29.46
IC 565731 × IC 565730	8.00	63	47	74.60	0.731	26.92
IC 565733 × IC 565734	9.71	55	33	60.00	0.694	36.98
IC 565734 × IC 565733	10.62	51	36	70.59	0.684	34.49
IC 565736 × IC 565737	9.00	68	59v	86.76	0.755	35.93
IC 565737 × IC 565736	8.09	57	41	71.93	0.753	36.10
IC 565735 × IC 565739	**7.46**	**52**	**46**	**88.46**	**0.789**	**40.09**
IC 565739 × IC 565735	10.20	38	33	86.84	0.776	38.92

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and suggested that genetic material from the centre of origin may be useful to identify genetically diverse genotypes for use in breeding programmes. However, it is still doubtful whether yield could be increased through heterosis. It has been possible to increase seed yield of a few experimental plants in our plantation through treatment with paclobutrazol which reduced vegetative growth quite considerably and improved flowering (Ghosh et al. 2007). A further study undertaken at CSMCRI in the same direction, revealed that the vegetative and reproductive behaviour of paclobutrazol treated shrubs has influenced the way biomass was partitioned among plant organs, probably by redistributing assimilates and directing the majority of assimilates toward reproductive growth (Ghosh et al. 2010).

The Indian Council of Agricultural Research has identified the first ever Jatropha variety, SDAUJ-1 (Chatrapati) for commercial cultivation because of high yield and oil content. Though, significant amount of work has been done on selection of superior genotypes of Jatropha, lot of work is yet to be done so that the plant can be grown as captive plantations for biodiesel production on commercial scale. It has been demonstrated that *Jatropha* can also be grown under low saline/alkaline conditions with amendments in soil conditions (Kumar et al. 2010a, b). Hence, large tracts of wastelands with low salinity/alkalinity levels can be exploited for production of biofuels.

Molecular Diversity

It is essential to understand the extent of genetic diversity and identification of molecular markers which in turn can be utilized to breed and improve any plant species using the naturally available plant genetic resources. *J. curcas* has been extensively studied for molecular diversity of the germplasm, genetic transformation, genetic regulation of various biochemical mechanisms in various countries and it is difficult to enlist here. Hence, studies which are only related to improvement of the species are discussed here. The genetic diversity existing in Jatropha has been studied by a number of researchers using RAPD, ISSR SSR and AFLP markers (Basha and Sujatha 2007; Gupta et al. 2008; Ram et al. 2008; Sudheer et al. 2009) and recently summarized by Reddy and Sudheer (2010) and Mukherjee et al. (2011). The molecular studies suggest an immediate need for widening the genetic base of *J. curcas* germplasm due to the existence of very low polymorphism (Basha and Sujatha 2007; Tatikonda et al. 2008; Sudheer et al. 2009, a).

At CSMCRI, genetic diversity was analyzed among the elite germplasm of *J. curcas*, selected on the basis of their performance in the field using *random amplified polymorphic DNA* (RAPD), *amplified fragment length polymorphism* (AFLP) and *simple sequence repeats* (SSR) (Figs. 8.5 and 8.6). The plants were identified on the basis of height, canopy circumference, number of seeds per fruit, weight of 100 seeds, seed yield in grams per plant and oil content. Out of 250 RAPD (with 26 primers), 822 AFLP (with 17 primers) and 19 SSR band classes, 141, 346 and 7, respectively were found to be polymorphic. The percentage polymorphism among the selected germplasm using RAPD, AFLP and SSR was found to be 56.43,

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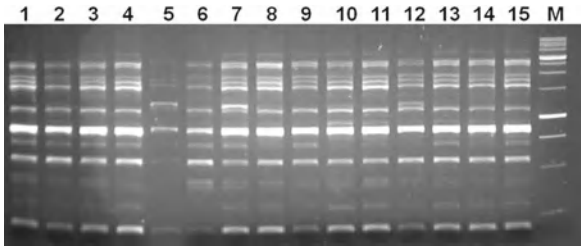

Fig. 8.5 RAPD finger printing profile of selected *J. curcas* germplasm using primer OPQ9; 1–15: JCC1-JCC15 and M: 1 kb marker

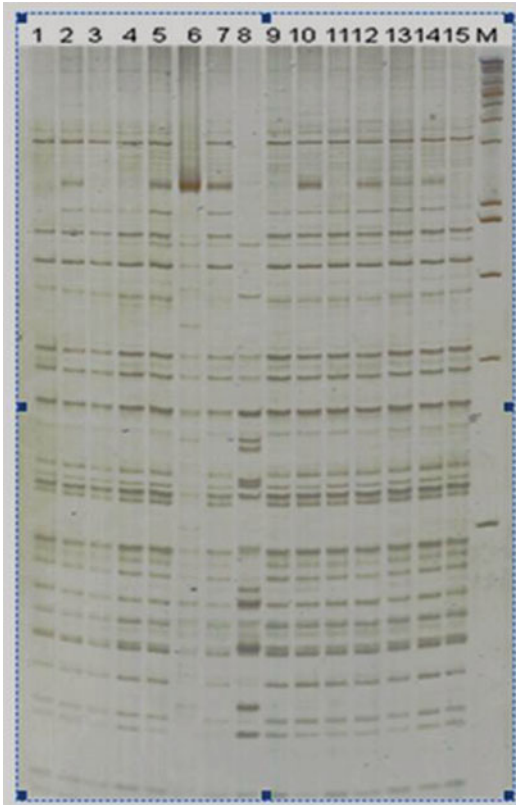

Fig. 8.6 AFLP finger printing profile of elite *J. curcas* germplasm using primer E-ACT/M-CAG; 1–15: JCC1-JCC15 and M: 1 kb marker

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57.9, and 36.84, respectively. The Jaccard's similarity coefficient was found to be 0.91, 0.90 and 0.91 through RAPD, AFLP and SSR marker systems, respectively (Mastan et al. 2012a).

A set of 28 diverse accessions collected from distinct geographical areas in India were studied for genetic relatedness (Sudheer et al. 2010a). The overall percentage of polymorphism (PP) was found to be 50.70 and 60.95 by RAPD and AFLP, respectively. Isolation of novel microsatellites was also undertaken and assessed the genetic equilibrium and diversity that exists among 44 diverse germplasm accessions collected from distinct geographical areas in India using the microsatellites. The overall efficiency of the enrichment of the microsatellites by dual probe in the present study was found to be 54% and among the sequences obtained, the percentage of sequences having suitable flanking regions for the primer designing was found to be 89.58%. The mean co-efficient of genetic similarity (CGS) was found to be 0.97 (Sudheer et al. 2010b). The overall diversity obtained by microsatellites was found to be low in comparison with the diversity reported by multilocus markers systems observed in earlier studies. However, adequate allelic polymorphism was observed in our studies.

Polymorphic markers that were specific to the non-toxic and toxic varieties using RAPD and AFLP techniques were also identified. Totally 371 RAPD, 1,442 AFLP markers were analyzed and 56 (15.1%) RAPD, 238 (16.5%) AFLP markers were found specific to either of the varieties. Genetic similarity between non-toxic and toxic varieties was found to be 0.92 by RAPD and 0.90 by AFLP fingerprinting (Sudheer et al. 2009). Earlier Sujatha et al. (2005) also reported 94.6% of similarity between toxic and non-toxic varieties using RAPD fingerprinting. The RAPD and AFLP markers being identified will help in selective cultivation of specific variety and along with SSRs these markers can be exploited for further improvement of the species through breeding and *Marker Assisted Selection* (MAS). Efforts were made to generate specific SCAR markers for toxic and/or non-toxic *J. curcas* from RAPD markers. Among the markers specific for toxic and non-toxic varieties, four were selected, purified, cloned, sequenced, and primers designed out of which one set of primers NT-JC/SCAR I/OPQ15-F and R could discriminate the non-toxic and toxic *J. curcas* varieties by giving the expected 430 bp size amplicon in the non-toxic variety (Mastan et al. 2012b). Furthermore, novel multiplex PCR was designed using the nrDNA ITS primers to overcome the false negatives. Our work also demonstrates the utility of the conserved regions of nrDNA coding genes in ruling out the artifacts in PCR such as the false negatives that frequently occur in SCAR reactions.

Study of genetic diversity analysis using RAPD, AFLP, and SSRs markers will facilitate the understanding of the population genetic structure, phylogeography, molecular ecological studies, characterization of interspecific hybrids and exploitation of genetic resource management and genetic improvement of the species through marker assisted selection. The characterization of germplasm, with geographically distinct, varied yield characters and genetically diverse accessions can be employed efficiently in breeding programs through marker assisted selection (MAS) and QTL analysis. The specific SCAR markers generated in the present

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investigation will help to distinguish non-toxic from toxic varieties of *J. curcas* or vice versa, and the identified marker along with designed multiplex protocol has applications in quality control for selective cultivation of non-toxic variety and will also assist in breeding and molecular mapping studies.

The low genetic diversity in the germplasm indicates that attempts must be made to create variations in the germplasm. It may lead to identification of some desirable mutants. Therefore, emphasis must be laid on creating genetic variability through mutations using both physical or chemical mutagens. Datta and Pandey (1992) demonstrated the use of induced mutations for improvement in Jatropha. Pandey and Dutta (1995) induced mutations in Jatropha for cotyledonary characters. Sakaguchi et al. (1987) attempted mutation breeding in *J. curcas* for obtaining desirable mutants. Aranez and Guia (1990) studied the effect of gamma rays on development and structural features in Jatropha. Similarly at CSMCRI, attempts were made to induce genetic variation in Jatropha by irradiating the seeds with gamma rays. The RAPD analysis of M1 plants showed increase in the polymorphism. Over all percentage of polymorphism was found to be 72.27 by RAPD and 66.77 by AFLP.

Breeding Strategies

Today a number of strategies are available for the creation of new plant varieties, including conventional breeding, interspecific hybridization, mutation breeding, and genetic engineering. Development of high yielding crop varieties through various plant reproduction systems has significantly increased the agriculture production, especially in the latter half of the twentieth century. In order to domesticate *J. curcas*, there are a number of traits like reduced vegetative biomass with increased number of branches, number of female flowers per inflorescences, low phorbol ester content, etc needs to be targeted. The selection for these traits would ultimately increase the number of seeds produced per plant, leading to an increased seed yield, oil content, and seed toxicity (phorbol ester content) in *J. curcas*.

Sujatha et al. (2008) have pointed out the necessity of biotechnological interventions including breeding for improvement of the species for various characters. Divakara et al. (2009) also indicated that there is a lack of high yielding varieties with high oil content. In our study, all the yield contributing characters varied among the accessions during different years. Inthapanya et al. (2000) also reported genotypic differences in nutrient uptake and utilization for grain yield production of rainfed lowland rice. The development of new cultivars with higher nutrient use efficiency (NUE) coupled with best management practices (BMP's) will contribute to economically viable and environmentally sustainable crop production in Jatropha on vast tracts of wastelands.

In studies conducted at CSMCRI, estimate of oil content indicated that some of the accessions were superior to others for this character. Hence, some of the accessions like IC 565731, IC 565735, IC 56573 and IC 573199 having relatively higher

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yields and high oil content were selected for further multiplication through cuttings/tissue culture for large scale plantation. While other accessions either having higher yield or oil content would be useful for developing the superior germplasm through breeding. Similarly, Sunil et al. (2008) reported that some of the accessions collected zone-wise from Northern as well as Southern Telangana and also from Bastar Plateau Zone of Chattisgarh were found to be high yielding with 35–40% oil content. Recently Ovando- Medina et al. (2011) discussed the geographical origin of *J. curcas* and summarized and contrasted the advances in research on the genetic diversity in the species. They are of the opinion that the collection and characterization of germplasm around the world, including the center of origin, using morphological, chemical and molecular markers and use of genome information from other Euphorbiaceae members is necessary to increase the oil productivity.

The narrow genetic variability in the existing Jatropha genetic resources offers the possibility to create/induce variability through hybridization. To achieve this goal, intra- and interspecific hybridization was undertaken at CSMCRI between identified male and female parents to develop hybrid lines derived from promising combinations. In order to develop Jatropha hybrids through intra-specific hybridization, germplasm was characterized for the traits which can be targeted for improvement including seed yield and oil content. Initially germplasm were assessed for desirable traits and cross-combinations were made between diversified parents**.** Performance of some promising hybrid lines was observed for two successive generations and one of the cross IC565735 × IC565739 proved to be the best with 78.9 g test weight and 40.1% seed oil (Table 8.1). Previously, attempts were also made to generate interspecific hybrids. Sujatha and Prabakaran (2003) reported successful interspecific hybrids between *J. curcas* and *J. integerrima* stating that the interspecific hybrid exhibited intermediate morphological and vegetative characteristics. Further backcrossing of the hybrids resulted in a number of flower colours varying from dark pink through green to white enhancing the ornamental value of the genus.

The amount of genetic variation expressed by different seed characters estimated through phenotypic, genotypic and environmental variances were considerable for undertaking genetic improvement of the species. All the three variations obtained for seed yield per plant showed that productivity can be achieved through a proper expression of G x E interaction. In our study, almost negligible variances recorded for number of seeds per fruit indicated stability for this trait. Higher Heritability values for seed yield/plant and plant canopy indicated the possibility of inheritance of these traits into next generations.

In one of the breeding experiments, all the genotypes were used as parents in various combinations in reciprocal manner and results on seed set from geitonogamy and xenogamy through hand-pollination mechanism revealed that xenogamy exhibited more percentage of fruit set (37–68%) whereas in geitonogamy it ranged from 11% to 38% (unpublished data). More number of fruits obtained in xenogamy suggested that the plant favours successful cross pollination between

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male and female flowers borne on different plants rather than both types of flowers present on the same plant.

In another study, even modification of plant architecture has been found to enhance the yield (Sakamoto and Matsuoka 2004) as increasing the number of branches on *J. curcas* may lead to an increased number of inflorescences and ultimately the number of seeds produced per plant. Recently Zhao (2007a, b) developed the process to regulate the tree shape of *J. curcas* by regulating phosphine and chlormequat.

Though Kant et al. (2011) have indicated the failure of Jatropha as a biofuel crop at the global level, the selection of accessions and their multiplication through vegetative propagation would ultimately lead to increased yields in future populations. The results in our experimental plots obtained so far are quite encouraging. In the absence of sufficient information regarding the suitable germplasm for specific areas, it is necessary to identify the germplasm for different types of wastelands before embarking upon commercial plantations. The plants may survive and look green but may not be productive enough to translate into viable economy. The study also indicated that increased seed yield and oil content can be achieved in *J. curcas* under uniform environment even by single plant selection followed by clonal propagation, however, the process is quite slow. Breeding of such selections would lead to the development of superior hybrids/quality planting material which could be utilized for commercial plantations.

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Part II
Genetic Diversity of Jatropha and Domestication

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Chapter 9
Origin, Domestication, Distribution and Diversity of *Jatropha curcas* L.

Neelam Sunil, Vinod Kumar, and Kodeboyina S. Varaprasad

Introduction

The name *Jatroph*a is derived from Greek word *jatros* meaning Physician or Doctor and *trophe* means nutrition or food (Heller 1996), which is indicative of its potency and widespread use as medicinal herb during ancient times. Besides, evolution of various names in English as well as in regional and local vernacular languages throughout the globe demonstrates its popularity. Commonly known as Physic nut in English and many other synonymous; Purging nut, Barbados nut, Purgeernoot, Big-purgenut, Black vomiting nut (Makkar et al. 1998), Curcas bean, Purgeer boontjie, Purging nut tree (Begg and Gaskin 1994).

Taxonomy

Adanson (1763), a French botanist recognized two subgenera *Jatropha* and *Curcas* based on Linnaeus's *Species Plantarum.* However, McVaugh (1979) discarded the two groups and proposed four homogeneous sections for the American species. Dehgan and Webster (1979), later accepted three of McVaugh's four sections in

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their revised system and recognized them as distinct sections of the sub-genus *Jatropha*. Section *Mozinna* (Ortega) Pax was regarded as more or less equivalent to the sub-genus *Curcas*. Dehgan and Webster's classification appears to be appropriate as it consists of two sub-genera (*Curcas* and *Jatropha*), with ten sections, ten subsections to accommodate the Old and New world species. The sub-genera were distinguished based on growth, habit, calyx aestivation, corolla coronation, monoecious or dioecious nature, stamen number and arrangement, seed shape and presence and absence of caruncle. Based on their classification, *J. curcas* is considered as the most primitive species in the genus. McVaugh (1945) and Wilbur (1954) also regard *J. curcas* as the most primitive species based on anatomical and morphological grounds and compatible with other species in the sections such as *Jatropha integerrima* Jacq. of sect. *Polymorphae* and least compatible with *J. gossypiifolia* of sect. *Jatropha* and *J. dioica* Sesse of sect. *Mozinna.* Species in other sections evolved from *J. curcas* another ancestral form with changes in growth habit and flower structures (Divakara et al. 2010). Thus, *J. curcas* belongs to Division Magnoliophyta; Class-Magnoliopsida; Order-Malpighiales; Family-Euphorbiaceae; Sub family-Crotonoideae; Tribe-Jatropheae; Genus-Jatropha and Species-curcas. The genus *Jatropha* approximately contains 175 known species (Dehgan and Webster 1978). For various synonyms of *J. curcas* the reader may go the website for more information *(*http://www.cabicompendium.org/Namelists/CPC/Full/IAT_CUhtm*)*.

Botany

Jatropha is diploid with $2n = 22$ chromosomes. It is a large shrub or tree that grows up to 3–4 m high. Leaves are 3–5 lobed, cordiform, stipulate, and deciduous. Inflorescence is complex, monoecious with protandry. First branching is racemose and subsequent branches are cymes. Cymes are upto 12 cm in length, flowers greenish white and unisexual (Ratha Krishnan and Paramathma 2009). The inflorescence is axillary, paniculate polychasial cymes formed terminally on branches and are complex, possessing main and co-florescences with paracladia (Divakara et al. 2010). In the middle of the inflorescence, there is a single female flower with tricarpellary gynoecium. In the axil of each bract there are number of male flowers in dichasial cymes. Calyx segments 5, nearly equal, elliptic or obviate. Corolla is campanulate, lobes 5, connate, hairy inside, exceeding the calyx, each lobe bear inside a gland at the base (Divakara et al. 2010). The oldest flower is close to the centre and thus the maturation is centrifugal. Normally the male to female ratio varies from 16 to 27 (male flowers): 1 (female flowers) to 108 (male flowers): 1 (female flowers). Generally, first flowering between September and January and second flowering in June is reported. The inflorescence once it begins flowering, flowers daily, and flowering lasts for 11 days. Cross pollinating is by insects and encouraged by hermaphrodite flowers. Staminate, slightly fused petal-based, stamens 5 + 5, 5 outer filaments only basally united, inner 5 completely united; pistillate petals are free or basally slightly united. Fruits are ellipsoid, mostly trilobed, dehiscing loculicidally;

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seeds compressed ovoid–ellipsoid by 1 cm, caruncle minute and seed weighs about 0.41 – 0.57 g. Propagation is by seeds or cuttings (Ratha Krishnan and Paramathma 2009). *Jatropha*, with a life span of 50 years, can yield 2–4 t of seed/ha with 30–42% of oil content after 3 years of planting (Raina and Gaikwad 1987). Based on the variation in the plant traits, a descriptor has been developed based on the germplasm collected from South India involving vegetative, reproductive, fruit, seed and oil content traits (34 traits). More diversity was observed for leaf characters such as leaf pigmentation, leaf size, colour and lobes. Whereas, least diversity was observed for latex color which was cream and red. Good variability was noticed for oil content which ranged from 16.0% to 41.3% (Sunil et al. 2010).

Origin

The origin of *J. curcas* was long debated. However, it is agreed that it is native to Mexico and Continental America (Achten et al. 2010) where it is found in the forests of coastal regions. The Portuguese, in the sixteenth century, learned about *Jatropha's* medicinal properties, and later established commercial plantations for soap and lamp oil production on the Cape Verdian Islands and Guinea Bissau (Heller 1996). Later, *Jatropha* genotypes adopted in Western Africa were spread across other Portuguese colonies in Africa (Mozambique, Angola) and into Asia (India, China and Indonesia). *Jatropha* is now reported to grow pantropically, from Brazil to the tropical islands of Fiji (www.worldagroforestrycentre.org/sea/products/afdbases/af/asp/speciesinfoasp?spid=1013# ecology).

Jatropha distribution is believed to occur naturally under an annual precipitation ranging between 944 and 3121 mm and a growing season (the number of months in which the mean precipitation is higher than half of the potential evapotranspiration) of 5–11 months (Maes et al. 2009). In its natural area of distribution, the species is reported to be most abundant in tropical savanna and monsoon climates (Am and Aw climate types according to Köppen classification, Koppen 1923) and in temperate climates without a dry season and with a hot summer (Koppen 1923) while it is uncommon in semiarid climates and totally absent in arid climates (Maes et al. 2009). The current distribution of *J. curcas* shows that introduction has been most successful in drier regions of the tropics. It is very tolerant and thrives under a wide range of climatic and edaphic conditions. It is particularly hardy at medium altitude and in humid zones. It is not sensitive to day length. It is drought tolerant and can withstand slight frost. The Cape Verdian Islands, where *Jatropha* was first successfully introduced and grown for commercial use, have a typically Sahelian (desert-like conditions during periods of low rainfall climate) with a strong oceanic influence (Pasiecznik et al. 1996). Mean annual rainfall ranging from 100 mm on the coastal plains to 900 mm at the highest elevations, with strong support of oceanic humidity and mild annual and daily temperature changes. In its distribution of herbarium specimen locations recorded in Eastern Africa, where it was probably introduced using genotypes from Cape Verde, *Jatropha* is established in regions with annual

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precipitation ranging between 650 and 2,500 mm, and length of growing season between 4 and 12 months. These latter results were derived by replicating the published methodology for *Jatropha* specimen locations in Eastern Africa (Maes et al. 2009).

Though *J. curcas* originated in Mexico, the plants introduced in India by the Portuguese during sixteenth century were adopted to West Africa's climatic conditions. First, Portuguese landed on the Malabar Coast of South Western India. Most probably from here it might have spread to other regions wherever Portuguese established their trading settlements. However, its large scale commercial utilization has not been reported rather its utilization by the rural and tribal folklore has been reported from time to time in research reports (Nath and Dutta 1988; Kulkarni et al. 2005; Kaushik et al. 2007; Basha et al. 2009).

Domestication

Until recently, *Jatropha* was mainly a fence crop with local soap production from seed oil, Ever since the awareness of *Jatropha* as a cash crop for biofuel is known, farmers look up to *Jatropha* for alternatives to generate income. This is only possible with quality planting material. However, farmers often end up planting the inferior local planting material. As reported domestication can occur at any point along the continuum from the wild to the genetically transformed state (Simon and Leakey 2004). Most tropical tree species have not been domesticated intensively, with the exception of a number of fruit tree species (cacao and mango), some 'beverage' tree species (coffee and tea) and some timber tree species (*Eucalyptus* spp.). Domesticating tree species, like *Jatropha* requires an intensive human intervention to bring species into wider cultivation by means of a farmer-centric and market-oriented process. A wide genetic base will mitigate the potential risk of inbreeding depression problems *viz.,* embryo abortion, limited fruit set, reduced overall seed yield and lower germination rate in such domestication process. Therefore, intraspecific genetic resource management plays an important role in domestication and also in determining the ecological stability of farming systems. Also, since seed oil is the economic part of the plant, besides focusing domestication strategies on increasing oil yield, future research should aim at producing compatible phenotypes in line with the requirement of the farmer's *viz.,* for use as hedge rows or boundary planting (Achten et al. 2010).

It is important to understand the breeding pattern (panmictic/non panmictic) as it is central for design of domestication strategies. Breeding, large-scale mass propagation and distribution across landscapes will obviously be much easier if the species is reproducing by natural selfing without inbreeding depression or by apomixis (Poehlman and Sleper 1995). Species with naturally high levels of inbreeding ('selfers') are expected to show less inbreeding depression but, at present, very little is known about *Jatropha*'s breeding system. Both the small population sizes during introduction history and the erosion of introduced genotypes due to imbalanced

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clonal propagation by farmers may have led to elimination of recessive, deleterious alleles. This could have counteracted inbreeding depression (Glemin 2003). It is possible that the *Jatropha* landraces have reduced growth, due to imbedded inbreeding depression. Crosses with germplasm with wide geographical isolation and diverse regions of diversity for example germplasm from Asia and introductions from Americas should release any inbreeding depression and thereby increase vigour and enhanced fruit production if genetic diversity of American landraces is effectively larger. Farmer domestication and seed collection often causes a major reduction in genetic diversity by following the ways as reported by Lengkeek (2003), Kindt (1997), Brodie et al. (1997). Some of the domestication efforts leading to narrowing of genetic base are collection of germplasm from a relatively small number of mother trees or individual trees, use of germplasm for subsequent planting either from the same or similar planting material. Also, similar to other insect-pollinated plant species, it is more likely that *Jatropha* trees are pollinated by their neighbouring plants than distant ones.

Domestication of *Jatropha* for agroforestry systems, considering its initial gestation period, is a more important step for realization of its economic potential in a cropping system. The two important methods generally followed within domestication strategy in agroforestry system are, farmer based improvement through *on-farm* domestication and improvement through science in research stations (Leakey and Akinnifesi 2008). In recent years, scientific approaches are being integrated with on-farm domestication through participatory approaches. Participatory domestication has its distinct advantages, as it builds on tradition and culture, local experience, indigenous technical knowledge, and promotes rapid adoption by users. Interestingly, *Jatropha* is suitable for quick and efficient domestication compared with other woody species, due to its propagation, establishment and adaptation to various conditions. The short generation turnover and recurrent selection can be performed in a short period. Low-input breeding approaches based on farmland seed sources as an option centralized germplasm procurement systems can be considered for hastening the domestication and utlization. In this, farmers' involvement in testing, procurement and deployment of improved planting stock will be important, to ensure large-scale access to quality planting material. Hence, a number of steps are required for an efficient and effective germplasm domestication programs in *Jatropha*. Important steps for low-input local breeding, as well as for more intensive breeding initiatives for *Jatropha* have been suggested as collection of genetic resources of global landraces compared with the Central American gene pool, analysis of the breeding system of *Jatropha* by use of molecular markers, study the breeding behaviour and to know the extent of inbreeding and outbreeding, improving traits useful for mechanized harvesting, disease resistance and drought tolerance of toxic and non-toxic *Jatropha,* and development of eco-geographic specific breeding and seed-transfer guidelines apart from high oil production traits (Achten et al. 2010). Hence, access to good performing and well-adapted *Jatropha* accessions with a wide genetic base would be the most logical domestication strategy for small-scale farmer systems as it increases the sustainability of production. Other requirements for implementation of domestication strategies have been outlined (Lengkeek

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2007; Akinnifesi et al. 2008; Leakey and Akinnifesi 2008; Akinnifesi et al. 2006; Leakey et al. 2003). A well-designed domestication program provides a route towards independence of all stakeholders after the project implementation period; training in seed-collection practices, in order to prevent narrowing of the genetic base of subsequent *Jatropha* generations, and postharvest handling to ensure viability are such training methods in the domestication strategy. Without such basic practices, selection for favorable traits, such as production, oil content and/or seed size, will not yield benefits; selection may, depending on the heritability of the selected traits, give an initial positive response due to selection of superior genotypes, but this positive effect could be lost in subsequent generations, due to a narrowing of the genetic base (Lengkeek 2007). Apart from selection of promising or elite lines from the germplasm collection, intra and inter-specific breeding approaches would further aid in utilization and domestication of *J. curcas*.

Without market access, farmers should not embark on joint domestication programs on *Jatropha.* The next step will be to establish domestication and breeding programs with farmers, researchers, extension workers and, preferably, private enterprises aiming at small-scale farming and participatory on-farm involvement, as carried out for fruit trees in west and southern Africa.

Geographical Distribution

J. curcas is best adapted to arid and semi-arid conditions in view of its somewhat succulent nature. Most, *Jatropha* spp. occur in the seasonally dry areas (Shweta et al. 2008) grassland-savannah and thorn forest scrub but, are completely lacking from the moist Amazon region. The current distribution of *J. curcas* shows that introduction has been most successful in drier regions of the tropics.

Though, *J. curcas* is native to Mexico, Nicaragua, Panama Belize, Costa Rica, El Salvador, Guatemala, Honduras, its popularity and wide spread adaptability distribution has been evidenced by its diverse names evolved in various languages and countries which is presented in Table 9.1. However, its widespread geographical distribution has been observed and well naturalized beyond its centre of origin in countries such as, Angola, Antigua and Barbuda, Argentina, Bahamas, Barbados, Benin, Bolivia, Brazil, Burkina Faso, Cambodia, Cameroon, Cape Verde, Central African Republic, Chad, China, Colombia, Cote 'Ivoire, Cuba, Democratic Republic of Congo, Dominica, Dominican Republic, Ecuador, Egypt, Eritrea, Ethiopia, French Guiana, Gabon, Gambia, Ghana, Grenada, Guadeloupe, Guinea, Guinea-Bissau, Haiti, India, Indonesia, Jamaica, Japan, Kenya, Laos, Liberia, Madagascar, Malawi, Malaysia, Mali, Martinique, Mauritania, Montserrat, Mozambique, Myanmar, Namibia, Nepal, Netherlands Antilles, Nigeria, Peru, Philippines, Portugal, Puerto Rico, Sao Tome et Principe, Senegal, Sierra Leone, Somalia, South Africa, Sri Lanka, St Kitts and Nevis, St Lucia, St Vincent and the Grenadines, Tanzania, Thailand, Togo, Trinidad and Tobago, Uganda, United States of America, Venezuela, Vietnam, Virgin Islands (US), Zanzibar, Zimbabwe (http://www.worlda-

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Table 9.1 Vernacular and language specific names of *Jatropha curcas*

Country/language	Common names
Arabic	Dandebarri
Assamese	Bongali-botora, Bongali era, Salika kund
Bengali	Bagbherenda
Kannada	Bettadaharalu, Dodda haralu, Kananaeramda
Sanskrit	Dravanti, Kanana eranda, Musikaparni, Parvataeranda, Vyaghraeranda
Gujarathi	Jamalgoto, Parsi erenda, Ratanjyot
Hindi	Ratanjyot, Bagrendi, Jangli arandi
Punjabi	Jamalgota, Kalaerenda
Telugu	Adavi amudamu, Kond amudamu, Nela jidi, Nepalamu
Tamil	Atalai, Kattamanakku
Malayalam	Katalavanakku, Kattamank
Marathi	Mogali eranda
Mizo	Kangdamdawi, Thingthau
Oriya	Dhalajahaji, Jahazigaba
Fijian	Banidaki, fiki, manggele, mbanindakai, ndrala
French	Pignon d'Inde, Purghère, Grand medicinier, Grand ignon d'Inde, Haricot du perou
Portuguese	Pinahao, Grao malucco, Grao muluco, Pinhao de purga
Hawaiian	kuikui Pākē, kuku'ihi
Samoan	Pāfiki, pāfiti
Spanish	Arbol de los pinones de Indias, Pinoncille, Tartago, Arbol santo, Tempate
Chinese	Ma fong chou
Indonesia	Ba dau me, Ba dau nam, Cc dau, Dau me
Caribbean	Herbe du bon dieu, Herbe du diable mancenillier benit, Medicinier benit
Brazil	Figo do inferno Mandubiguasu, Munduyguasu, Ppinhao do paaguay, Pihao de purga
Cameroon	Botije, Botuje, Botuje-ubo, Lobotuje
Cuba	Pinon botija
Cape Verde	Pulguirea
Germany	Purgiernussbaum
Egypt	Habbel-meluk
Ethiopia	Ehanduejot, Erundi
Gabon	Ogombo
French Guiana	Medeicinier
Guam	Tabatuba
Iran	Dandebarri dandenahri
Italy	Giatrofa catarcita
Cambodia	Lohong khvangsu
Sri Lanka	Kaddamanakku
Mali	Baga-ni
Myanmar	Thinbankyekku
Mauritius	Pignon d'Inde
Mexico	Avellanes purgantes
Mozambique	Sassi
Netherland	Purgeernoot
Nepal	Kadam
Philippines	Bolongcauit, Casta, Kator

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groforestrycentre.org/Sea/Products/AFDbases/AF/asp/peciesInfo.asp?SpID=1013). A non-toxic variety is reported to exist in Mexico and Central America. A species said to be endemic to Madagascar, *J. mahafalensis* is said to have equal energetic promise (Sujatha et al. 2005).

In India, the distribution of *J. curcas* and other species such as *J. gossypiifolia, J. glandulifera, J. multifida, J. nana, J. panduraefolia J. podagrica* has been reported (Anonymous 1959). *J. curcas* has widely spread in wild and semi-wild status as hedge plant, as fence on field boundary, road side and forest eco-systems in various states. Sunil et al. (2008) reported wide distribution of highly variant lines of *J. curcas* in the districts of Bastar, Dantewada of Chhattisgarh, and Adilabad, Nizamabad, Prakasam and Ranga Reddy districts in Andhra Pradesh. Kaushik et al. (2007) reported distribution of *J. curcas* with good variability for seed and oil content in Haryana and adjacent areas. Kumar et al. (2008) reported distribution of *J. curcas* in the districts of Ratlam, Jhabua of Madhya Pradesh; Banswara district of Rajasthan; Ambikapur district of Chhattisgarh; Dahod, Panchmahal and Dantiwara districts of Gujarat; districts of Rahuri and Lalitpur in Maharashtra and Uttar Pradesh, respectively. Rajneesh et al. (2009) reported the distribution of *J. curcas* in diverse elevational and environmental conditions of Barrage, Laxman Jhula, Shivpuri, Selakui, Singthali, Chamoli and Saknidar regions of Uttarakhand. Apart from these states, it is also distributed in the states of Karnataka, Rajasthan, Haryana and Assam. Ganesh Ram et al. (2007) reported the availability and distribution of *J. curcas* from Uppupulam, Palapatti, Vedarcolony, Thayanoor and Vachinampalyam regions in Tamil Nadu.

Diversity and Crop Improvement

A sound breeding programme depends on availability of genetic variability for desired traits. Collection of diverse genetic resources through global germplasm exploration, introduction, characterization, and evaluation will provide strong base for development of elite varieties by various improvement methods. Comprehensive work on collection, characterization and evaluation of germplasm for growth, morphology, seed characteristics and yield traits is still in its infancy. *Jatropha* grows on a wide range of climatic conditions, from semi-arid to humid regions (annual rainfall ranging from 300 to 3,000 mm), with high tolerance to high temperatures and little frost and a preference for deep, well drained soil (Antonio et al. 2010). Such wide range of edaphic and ecological conditions suggest existence of considerable amount of genetic variability which could be tapped (Divakara et al. 2010) for exploiting the full potential as a biodiesel plant. Whatever the provincial variability is recorded it is due to genotype and environmental interaction. Priority should be given to assess intra- and inter-accessional variability in the available germplasm, selection of pure lines and then their multiplication. Existence of natural hybrid complexes between *J. curcas-canescens* in Mexico (Dehgan and Webster 1978),

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J. integerrima–hastata complex in Cuba and West Indian islands (Pax 1910) and *J. curcas–gossypiifolia* (*J. tanjorensis*) in India (Prabakaran and Sujatha 1999) is reported. Sudheer et al. (2008) reported highest interspecific genetic divergence (0.419) between *J. glandulifera* and *J. multifida,* the least interspecific genetic divergence (0.085) between *J. gossypiifolia* and *J. tanjorensis*.

Preliminary studies indicated very low variation in simple sequence repeat (SSR) markers within populations of *Jatropha* even in its natural distribution (Mexico) as reported by Sun et al. (2008). Studies based on genetic markers uncovered only modest levels of diversity in India indicating that the gene pool applied at a large scale may rest on a fairly fragile genetic foundation (Basha and Sujatha 2007; Ranade et al. 2008). Tatikonda et al. (2009) studied the diversity of 48 accessions from India based on AFLP markers and found 680 polymorphic fragments, which provided discriminative power for the classification of germplasm accessions into five major clusters. Ganesh Ram et al. (2007) studied five accessions of *Jatropha* and seven *Jatropha* species and found that highest genetic similarity co-efficient (0.85) was measured between TNMC 1 and TNMC 6. Cluster analysis indicated three distinct clusters one comprising all the accessions of *J. curcas*, the second cluster included six species *viz, J. ramanadensis, J. gossypiifolia, J. podagrica, J. tanjorensis, J. villosa* and *J. integerrima.* while *J. gladulifera* formed the third cluster. The latter species has genetic distinctness and wider geographical distribution in India compared to the other seven species studied. Parthiban et al. (2009) attempted crosses between *J. curcas* and other *Jatropha* species and identified 27 distinct hybrid progeny clones. Clones such as FC RI HC 3 (55.26%), FCRI HC 15 (48.50%), FCRI HC 13 (37.01%) exhibited superiority in terms of oil content. Other progenies such as FCRI 22 (357.48 g), FCRI HC 21 (328.07 g), FCRI HC 10 (325.01 g), FCRI HC 18 (305.43 g), FCRI HC 12 (255 g), FCRI HC 20 (252.26 g) and FCRI HC 27 (250 g) recorded maximum seed yield and early flowering at 9 months after planting. These clones can be promoted and utilized effectively as biofuel crop.

Makkar et al. (1997) reported large variations in contents of crude protein, crude fat, neutral detergent fiber and ash on 18 different provenances of *Jatropha* from countries in West and East Africa, the Americas and Asia. Indonesian accessions recorded variation for oil content ranging from 36.06% to 53.08% as reported by Hasman (2007). In China, Liu et al. (2007) studied variation in *J. curcas* in different regions and reported that under hot and humid conditions trees grow high, are less branched with narrow crown and fruit bearing was synchronous. Under semi-humid and semi-arids of the tropics, fruit bearing was once in a year. The maximum 1,000 seed weight (698.9 g) was recorded from Liuku of Lujiang river and minimum seed weight of 500.7 g was recorded from extensive heat regions of Yuanmou basin. However, kernel yield and oil yield of seeds are higher due to short growth period. The oil content of seed ranged from 53.3 (Yuanmou basin) – 64.25% (Taoyuan of Yongsheng country) which is considered as hot region. Kumar et al. (2008) reported maximum variation for female flowers/inflorescence while it was narrow for male flowers/inflorescence. This may be due to intracellular and extracellular activity at different development stages. They also reported strong correlation between plant height and branch length, number

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of branches and collar diameter which help in the selection of superior genotypes. Rao et al. (2008) reported wide variation in 100 seed weight (57–79 g) and oil content (30–37%) for accessions collected from Andhra Pradesh, India. Kaushik et al. (2007), explored the variability in the accessions collected from the state of Haryana, India and found wide variation in 100 seed weight (49–69 g) and oil content (28–39%). Wani et al. (2006) also recorded variation in Indian accessions for oil content (27.8–38.4%) and 100 seed weight (44–77 g).

The phenotypic diversity studies helps in classification and identification of accessions to be used for meeting specific breeding objectives. Sunil et al. (2009) reported phenotypic variation in number of primary branches (1–14), plant spread (14–161), number of inflorescence clusters per plant (1–20) and oil content (21.5–39.8%), from accessions collected from states of Chhattisgarh and Andhra Pradesh. The same author based on DIVA-GIS analysis reported that Prakasam district of Andhra Pradesh had higher CV value for oil content (29–36%). The richness in oil content using rarefaction method of DIVA-GIS showed that Ranga Reddy district to be the potential area for germplasm with high oil content. Sunil et al. (2008) also reported high oil content accessions from Chhattisgarh (42%) and Andhra Pradesh (40.6%). Kumar et al. (2008) studied the intraspecific variation for various morphological traits and found variations for plant height that ranged from 140.95 to 175.30 cm, collar diameter (4.70–6.27 cm), number of branches (4.75–7.93) and branch length (102.06–132.96 cm). Heller (1992) conducted multiplication field trials in 13 provenances from 1987 to 1988 in two countries of the Sahel region: Senegal and Cape Verde. Significant differences in vegetative traits were recorded except leaf shape among the various provenances at all locations. Rao et al. (2008) observed four clusters with phylogeographic patterns of genetic diversity among 32 high yielding candidate plus trees of *J. curcas* for seed traits. Gohil and Pandya (2008) analysed diversity based on phenotypic traits of nine *Jatropha* genotypes and suggested that for varietal improvement, hybridization among the genotypes of divergent clusters (clusters – III, IV and V) may be done in order to obtain better results in terms of variability and diversity. Kaushik et al. (2007) subjected 24 diverse accessions to non-hierarchical Euclidian cluster analysis for seed traits and found that crossing between accessions of clusters IV and VI will yield wide spectrum of variability in subsequent generations. Sunil et al. (2011) studied the possible correlation between the phenotypic and molecular traits (RAPD and ISSR) and concluded that, unique phenotypic traits like, leaf colour (light green), leaf type (leathery), time of flowering, inflorescence type (lax inflorescence), inflorescence number and pedicel length (partially) among the quantitative traits can guide in the identification of diverse accessions. To enhance genetic diversity, inter-specific cross-pollination between *J. curcas* and other *Jatropha* species is necessary to develop new hybrids with higher yield potential and resistance to diseases. Among all the interspecific crosses, the cross between *J. curcas* and *J. integerrima* produced successful hybrids with more seed set, while the other crosses failed to produce seeds due to existence of crossability barriers as reported by Parthiban et al. (2009).

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The National Vegetable Oil Development Board (NOVOD) India identified a total of 1,855 candidate plus plants. In addition, around 5,000 accessions were collected through network of various inter institutional research initiatives with an oil content ranging from 26% to 42.7% (Punia 2007). The production of quality planting material (30 – 40% oil content with 3–5 t seed yield/ha) under micro mission was undertaken by Department of Biotechnology, Government of India. Kaushik et al. (2006) reported that accessions from Uttaranchal recorded high percentage (73%) of high yielding plants. Gujarat Agricultural University released the first variety of *J. curcas* (SDAUJ I, Chatrapati), for commercial cultivation in India. Two superior accessions of *J. curcas* collected by National Bureau of Plant Genetic Resources, Regional Station, Hyderabad from Chilevar village of Ranga Reddy district of Andhra Pradesh (IC537939) and Bastar district of Chhattisgarh (IC541650) registered high oil content of 41.9% and 40.6%, respectively with the Plant Germplasm Registration committee of ICAR.

The breeding strategy for commercial plantations will, in its first steps, have similarities to the farmer-based domestication, with provenance testing in field trials in relevant environmental conditions and in agricultural systems similar to the systems that are expected to be applied in the plantations. The field trial testing of provenances and trees within provenances should be made simultaneously to identify superior oil-producing and climate-robust provenances and trees. The trees can be tested either as clones, (i.e., by ramets [cuttings]) or by progeny (Eriksson et al. 2007). The clonal testing approach will make estimation of genetic values and, thus, selections more precise. This is of course of high relevance if plantations are to be established with clones. In case mass production through seed propagation is desired, clonal testing is still an effective way to increase the certainty in the selections (Dhillon et al. 2009) unless large clonal effects and non additive genetic effects weaken the correlations between estimated genetic values and breeding values. Mass production through seed propagation will require the establishment of seed orchards with the selected trees (Eriksson et al. 2007). The number of clones used in the case of clonal propagation for deployment in the plantations depends on the expected rotation age of the plantations, degree of genotype by environment interaction, risks for new pests, the degree of genetic variation between clones and, finally, the willingness to accept risks to obtain high genetic gains. Thus, short rotation, low genotype by environment interaction, low risk for severe pests and large genetic variation between clones speak in favor of few clones. Additionally, the use of fewer clones will make it easier to obtain homogenous harvest properties (Burdon and Aimers-Halliday 2003).

The breeding strategies for commercial plantations requires a breeding program with crossing between selected genotypes, testing of offspring from the crosses and, finally, deployment of superior offspring through either clonal propagation or seed propagation in seed orchards (Eriksson et al. 2007). To secure long-term genetic gains, the breeding program could be organized using the concept of multiple breeding populations (Namkoong et al. 2004; Eriksson et al. 2007). If the species is mainly regenerating through apomixis, the generation of new variations in a breeding program will be more troublesome, and it may also increase the risk of the genetic variation between clones.

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A clone-based breeding approach could be interesting for *Jatropha*, when the indication of apomixis in *Jatropha* is confirmed. Molecular-assisted breeding and transgenetic approaches are of interest to develop non-toxic genotypes. There is a growing knowledge in support of agroforestry for future ecoagriculture, farm diversification and management of climate change in the tropics. *Jatropha* appears to be ideally suitable for integration in different agroforestry systems.

Conclusions

J. curcas has spread and got well adapted to diverse environmental conditions far beyond the boundaries of its origin. Availability of improved hybrid or varieties for large scale commercial cultivation has not been possible due to locally available inferior planting material for oil and seed yield. The high diversity for 100 seed weight (698.6 g) and oil content (64.25%) has been reported from China (Liu et al. 2007) and moderate diversity has been observed from the germplasm lines collected from various states of India. Exploitation of such variant lines for 100 seed weight and oil content can be utilized for improvement of *Jatropha* by conventional breeding programme such as, selection and modern breeding tools such as interspecific hybridization, gene technology transfer and biotechnology, etc. Further, study is warranted for the creation of trait specific variability by using mutations for more number of pistillate flowers, oil content and fruit yield per plant in order to exploit it by means of heterosis to make it a more profitable venture in terms of its economics and ecofriendly nature.

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Chapter 10
Systematics of Indian Jatrophas

Bir Bahadur, T. Pullaiah, and G.V.S. Murthy

Introduction

The generic name *Jatropha* is derived from Greek word "*jatros"* (doctor/physician) and "*trophe"* (food) to eat, since various *Jatropha* species possess medicinal properties and nutritional qualities.

Jatropha L. belongs to the tribe Jatropheae (Meisn.) Pax, sub-family Crotonoideae Pax, family Euphorbiaceae, characterized by yellow milky latex, palmately veined leaves and simple to stellate hairs. The other related genera in the tribe are *Vaupesia*, *Oligoceras, Deutzianthus*, *Joannesia, Leeuwenbergia* and *Annesijoa* (Webster 1967). *Jatropha* is morphologically diverse genus comprising about 175 species; most of the species are native to the new world and ca. 45–50 species occur in Africa and Arabia. India's share is limited to about dozen species mostly occurring in South India while some species are wild and widely distributed in India (see Map 10.1) and elsewhere around the world.

The family Euphorbiaceae contains a number of genera used by man in a wide variety of ways, and *Jatropha* is one of them. Several species of *Jatropha* are widely grown as ornamentals, medicinal purpose for their tumor inhibiting properties, and as biofuel plants in tropics and sub-tropics of the world. In view of their growing importance hitherto unintroduced species/hybrids will be soon available for commercial production.

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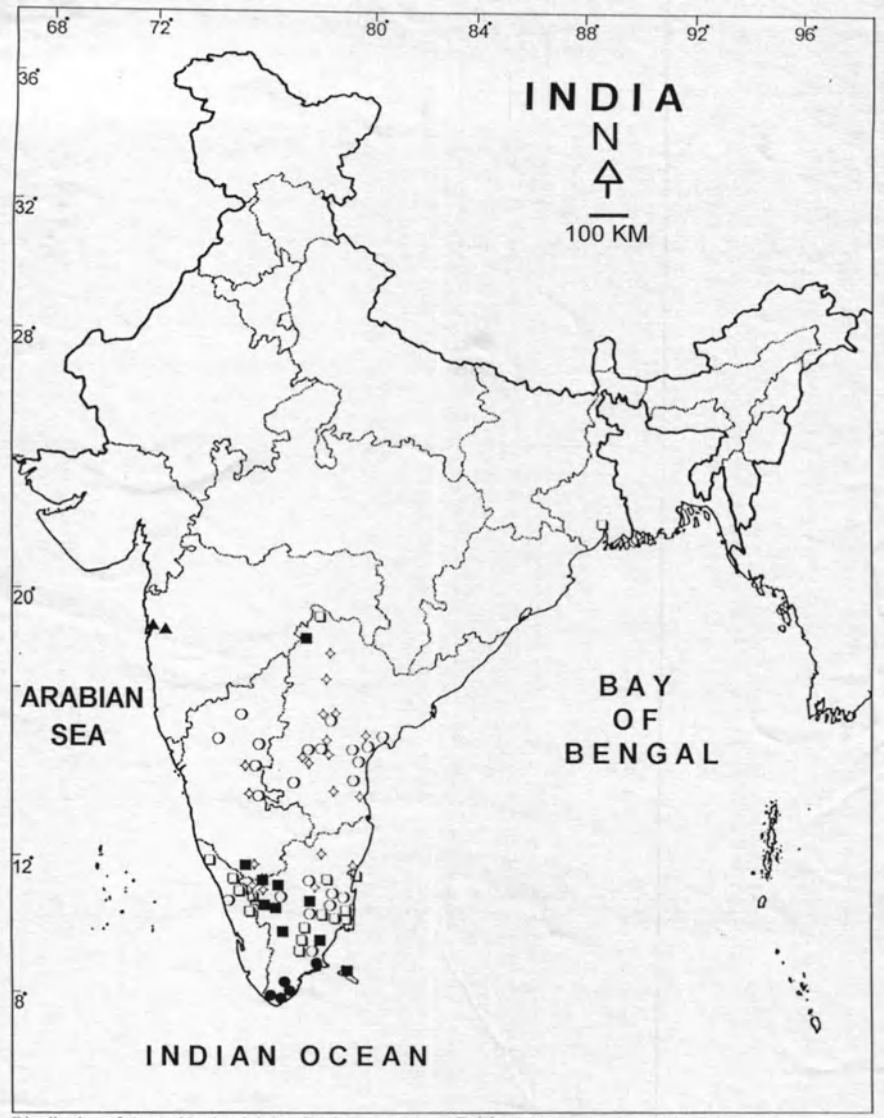

Map 10.1 Distribution of *Jatropha* species in India

***J. curcas*:** The physic nut, or petro plant species has attracted global attention as source of biofuel. Three distinct varieties have been recognized, (1) The Cape Verde variety that has spread all over the world, (2) The Nicaraguan variety with few but larger fruits, and (3) Non-toxic Mexican variety devoid of phorbol esters (Henning 2006) and is edible.

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Taxonomic History of the Genus

Linneaus (1737) proposed the genus *Jatropha* (Gen. Pl. 288). In his *Species Plantarum* (pp. 1006–7), Linneaus (1753) described seven species *viz., J. gossypiifolia* L., *J. moluccana* [= *Aleurities moluccana* (L.) Willd.], *J. curcas* L., *J. multifida* L., *J. manihot* [=*Manihot esculenta* Crantz], *J. urens* [=*Cnidoscolus urens* (L.) Arth.] and *J. herbacea* [=*Cnidoscolus herbaceous* (L.) I.M. Johnston]. Linneaus (1763) in the second edition of *Species Plantarum* (pp. 1428–1430), divided the above species into two groups: **"calyculati",** included the first four species, and the last three species treated under **"acalyculati".**

Adanson (1763), in his *familes des Plantes*, recognized two genera *viz., Curcas* and *Jatropha* and treated *Manihot* as a separate genus. Although Adanson's generic delimitation proved incompatible with that of later taxonomists (Pohl 1827; Baillon 1858; Mueller 1874; Pax 1910) and retained through consolidation and reduction of the two genera to sub-genera and several sections. McVaugh (1945) was the first to discard the two groups by correctly pointing out the unnaturalness of a classification based on corolla character alone. He therefore proposed four "homogeneous" sections for the American species (*Polymorphae*, *Macranthae*, *Adenoropium* and *Mozinna*). Dehgan and Webster (1979) accepted, incorporated and revised with minor modifications, three of McVaugh's four sections into their revised system, recognized two sub-genera, *Curcas* and *Jatropha* with ten sections, and ten subsections to accommodate all the Old and New world species.

Jatropha L.

Sp. Pl.:1006. 1753 & Gen. Pl. ed.5. 437. 1754.
Type species: *Jatropha gossypiifolia* L. [lectotype].

Shrubs or under shrubs, often rhizomatous with rhizome or tuberous woody or gouty stem, geophytes and facultative annuals, monoecious (rarely dioecious), glabrous or glandular pubescent; latex never milky, pale cloudy, yellow to distinct red, powdery when dry; laticifers articulated, non-articulated or idioblastic; bark of woody species smooth, wrinkled with fissures and cracks, or peeling. *Leaves*: alternate entire, angular-lobed, palmate 3–5, or 7-lobed, or rarely 11-lobed, palmately or rarely pinnately veined, brochidodromous; leaf size varies considerably, margins entire or conspicuously serrate, with or without peltate, panduriform, mucronate tips, sometimes the serrations with stipitate glands; stipules present or absent, simple, or branched, foliar, long filiform, glandular and often modified into serially arranged stalked capitate glands, fugacious in nature. *Inflorescences*: axillary, terminal, subterminal corymbs of cymes, or reduced to a few or solitary flowers in axils, the main branches often terminating into a female flower, male flowers many surrounding the female flower, 5-merous bracteate unisexual, (rarely bisexual). *Male flowers:* urceolate, subglobose or tubular; calyx-lobes 5, free, imbricate, entire,

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glandular, serrate or rarely divided, often foliaceous; petals 5, free or variously coherent or connate, (rarely absent), contorted either to left-or right on the same inflorescence, rarely imbricate, greenish, yellow green, white, pink or red; nectary disc glands 5, in both male and female flowers, free or connate in an annular ring, entire, dissected or lobed; stamens 8 or 10 (very rarely 6), all or inner whorl basally or rarely entirely connate often 5, shorter or subconnate at base, epipetalous; anthers linear, sagittate longitudinally dehiscent, anthers rarely with hairs. *Female flowers:* calyx-lobes 5, imbricate often glandular margined or linear foliaceous, green or crimson, enlarging in fruit, petals 5, contort either to left or right, sometimes absent, larger than sepals, free or coherent at base, recurved at apex; disc glands 5, free or united into a ring; staminodes sometimes present or minute; ovary (2) 3-loculed, ellipsoid, each 1-ovuled, glabrous or pubescent; ovules 1 per locule; styles 3, free or connated at base, forming a short column, styles crimson red, pink, green, clavate, bifid; stigmas 2-lobed, narrow, capitate or horse-shoe shaped. *Fruits:* ellipsoid, ovoid or sub-rotund, capsular, drupaceous or dry, (2) 3–4-loculed, breaking up septicidally-loculicidally into (2) often 3 cocci. *Seeds*: 2–3 oblong, ellipsoid to spherical, light brown to dark brown, testa dull, shiny, crustaceous with spots or linear streaks, carunculate, caruncle of various sizes and colour, white to pale yellow, oil bearing endosperm copious, starchy; embryo spathulate; cotyledons broad, cordate palmately veined; with 3 to 5 (7) veins, radicle short; germination phanerocotylar.

Distribution: Tropical America, Africa and Asia, ca 175 species for the world; about 11 species occur in India, 8 species are wild, indigenous with varieties under two species. Four species are cultivated in gardens in India and elsewhere.

Pollen grains inaperturate, spheroidal, typically crotonoid, with hexagonally arranged exinous pila/clava, smooth or ornamented (Punt *Wentia,* 7:1–116, 1962; Bahadur et al. 1997, 2000, 2012). Pollen grains binucleate (Webster and Rupert *Evolution,* 27:524–531, 1973; Brewbaker 1967).

Seed germination phanerocotylar (i.e. cotyledons are freed from the seed coat) in majority of the species (Duke *Ann. Mo. Bot. Gdn.* 56: 125–161. 1969; Kamilya and Paria *Acta Bot. Indica* 22: 251–256, 1994; Bahadur and Goverdhan 1996a). In *J. multifida*, the cotyledons are never completely freed from the seed coat, i.e., cryptocotylar. This feature is unique in Euphorbiaceae and is shared by *Hevea brasiliensis* (Duke 1969).

Literature: Bir Bahadur and Venkateswarlu (1976). *J. Indian Bot. Soc.* 55:30–37; Bahadur et al. (1998) *J. Swamy Bot. Club,* 14:45–47; Bahadur et al. (2000) *Geophytol.*, 28:67–75; and *Intl. J. Plant Sci*: 28:111–119, 2012; Bahadur and Goverdhan (1996a, b) *Proc. IAAT*, 7–10, Bahadur et al. (1997) *J. Palynol*. 33:123–127, Bahadur et al. (1998) *J. Swamy Bot. Cl.* 15:85–87, Chamundeswari et al. (2004). *Gleanings in Bot. Res.* 397–407. Chaturvedi and Jehan (1982). *Acta Bot. Indica,* 10:246–251. Dehgan (1980). *Bot. J. Linn. Soc.,* 80(3):257–278, Dehgan (1982). *Amer. J. Bot.,* 69:1283–1295. Dehgan and Craig (1978). *Amer. J. Bot.,* 65:345–352, ff. 1–22. Dehgan and Webster (1979). *Univ. Calif. Publ. Bot.,* 74:1–73, tt. 1–33. Kamilya and Paria (1994). *Acta Bot. Indica,* 22:251–256. Rao and Raju (1994). *J. Econ. Taxon. Bot.,* 18:585–589; Somboonsaru (1983) *Morphol cytol. Anat. Cytol. Investigation of Some Hydrocarbon Plants*; Reddy et al. (2002) *Rec. Progr. Med. Plants* 8: 489–500; Prabakaran and Sujatha (1999) *Genet. Resour. Crop Evol* 46: 213–218.

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Key to the species		
1a.	Stipules well-developed, divided into narrow lobes or often modified into decurrent series of stalked glands	**2**
b.	Stipules absent or minute and subulate	**6**
2a.	Leaves without stalked glands; young leaves green; peduncles glabrous	**3**
b.	Leaves with stalked glands; young leaves dark purple; peduncles hairy	**4**
3a.	Shrubs tuberous at base; leaves peltate, 5-fid for halfway down; lobes entire; sepals orange-red	(cult.) **11. *J. podagrica***
b.	Shrubs not tuberous at base; leaves not peltate, rarely slightly subpeltate, divided into numerous lobes to far below the dissected towards apex; sepals crimson-red	(cult.)**10. *J. multifida***
4a.	Lobes of leaves entire, glandular hairy along margins; flowers reddish or purplish red	**3. *J. gossypiifolia***
b.	Lobes of leaves serrate, each tooth ending in gland-tipped bristle; flowers green with pinkish tinge	**5**
5a.	Leaves deeply palmately lobed up to below the middle; stipules prominent, deeply divided into several gland-tipped segments, petals free or almost so to the base in female flowers; staminodes absent in female flowers	**2. *J. glandulifera***
b.	Leaves shortly palmately lobed up to or above the middle; stipules short, with only a few gland-tipped segments; petals in female flowers connate to one-third the length; staminodes present in female flowers, almost stamen-like but much smaller and sterile	**7. *J. tanjorensis***
6a.	Leaves oblong-lanceolate or oblong-obovate, entire	**7**
b.	Leaves broadly ovate, 3–7-angular or 3–7-lobed	**8**
7a	Flowers pink to crimson red; leaves oblong-obovate or panduriform, with 1–5 subulate acute teeth at base	(cult.) **9. *J. integerrima***
b.	Flowers yellow; leaves oblong, not panduriform, without subulate teeth at base	**5. *J. maheshwarii***
8a.	Leaves peltate, 3–7-lobed	**8. *J. villosa***
b.	Leaves not peltate, 3–7-angular or lobed	**9**
9a.	Leaves 3-lobed, wedge-shaped, obtriangular, broadly cuneate at base	**6. *J. nana***
b.	Leaves 3–7-angular or 3–7-lobed, broadly ovate, cordate at base	**10**
10a.	Large branched shrubs; stem base not tuberous; leaves not glaucous beneath; petals united for half the length	**1. *J. curcas***
b.	Unbranched stunted shrubs; stem base tuberous; leaves glaucous beneath; petals free or connate at base only	**4. *J. heynei***

Wild Species

1. *J. curcas* L., Sp. Pl: 1006. 1753; Hook. f., *Fl. Brit. India* 5: 383. 1887; Gamble *Fl. Madras* 2: 437. 1957 (Repr. ed); Surya Prakash Babu in Pullaiah & Ali Moulali, *Fl. Andhra Pradesh* 2: 872. 1997.

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Assamese: *Bongalibhotora;* Bengali: *Baghbheranda, Eranda-gachh, Kulsera, Totkabendi;* English: Barbados nut; Garo Hills: *Borbandong*; Gujarathi: *Jamalgota, Magali-erandi, Kalaerendy*, Hindi: *Bangbherenda, Jamalgota, Jungli-arundi, Ratanjota, Safed-erandi, Safedarand*; Kannada: *Adaluharalu, Bettaharalu, Maraharalu, Kadandla, Karnocchi, Turukuharalu;* Konkani: *Mogali-erandi;* Malayalam: *Katalavanakku, Kattavanakka Kattu avanakku; Kammati;* Marati: *Mogali-eranda, Rana-erandi; Ratnajot*; Nepali: *Nera-khar-shing*; Oriya: *Jahazigaba, kadam*; Sanskrit: *Dravanti, Parvatarande, Kaneraeranda;* Tamil: *Kadalamanakku, Kollamankku, Kattamanakku, Kattu-avanakku;* Telugu: *Adaviamudam, Adavi-amadam, Pedda Nepalam.*

Large shrub or small tree, 3–5 m tall, with thick branches and soapy sticky latex, glabrous or rarely more or less flocculent tomentose; bark greenish white to yellowish brown, smooth papery, pealing off in thin white flakes, wood white soft. *Leaves:* alternate triangular, ovate or orbicular, in outline (8-) 15–40 × 10–20 cm, broadly cordate at base, entire or 3–5 angled or lobed with caudate-acute lobes, 5–15 cm across, membranous, glabrous, 5–7 palmately nerved from petiole base, veins distinct on both surfaces; petioles 4–15 cm long; stipules small or absent. *Inflorescences*: cymose, laxly expanded, loosely flowered, much shorter than leaves; peduncles 3.5–5 cm long, glabrous; main branches terminating in one female flower, surrounded by many male flowers; bracts lanceolate to linear-lanceolate, entire, 4–7 mm long. *Male flowers*: sepals ovate, elliptic, 3–4 × 1–2.5 mm, entire, yellow green; petals 5–10, connate up to middle, oblong-obovate, 7–8 mm long, yellowish green, hairy inside near the middle; disc columnar, cylindrical, deeply 5-lobed, glabrous; stamens 10, lemon yellow, outer whorl filaments free, inner whorl 5, connate at base. *Female flowers*: sepals longer than in male flowers; petals as in male flowers, but free and smaller. Ovary 3-locular; styles short and connate at base. *Fruits*: fleshy, drupaceous, drying to capsule, 3-lobed, splitting into 3 valved cocci, subglobose, rugose, 2.5–3.5 cm in diam., 2.4 cm long, glaucous, pale yellow, turning to black; seeds ellipsoid, 15–25 mm long, 12–15 mm wide, dark brown or blackish, encrusted-striate (Fig. 10.1).

Fl. & Fr.: April – Dec.

Habitat: Common in tropical regions along plains and coastal regions, sea level and often up to 1,500 m altitude. Usually grown as a hedge plant, often as an escape and runs wild along road sides and river banks.

Distribution: Native to tropical America (Brazil), now in cultivation throughout the tropics of the world. Naturalised and cultivated in: Andhra Pradesh, Bihar, Goa, Gujarat, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Orissa, Rajasthan, Sikkim, Tamil Nadu, Uttar Pradesh, West Bengal, and Andaman & Nicobar Islands. Sri Lanka.

Seedlings are epigeal and phanerocotylar, the paracotyledons with 7–9 primary veins and the first two leaves serrate and provided with glands (Kamilya and Paria 1994).

2. *J. glandulifera* Roxb., *Fl. Indica* 3: 668. 1832; Hook, f., *Fl. Brit. India* 5: 382. 1887; Gamble, *Fl. Madras* 2: 937. 1957 (repr. edn.); Surya Prakash Babu in Pullaiah & Ali Moulali, *Fl. Andhra Pradesh* 2: 873. 1997.

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Fig. 10.1 Plant and flower characteristics of *Jatropha* species: *J. curcas*

Bengali: *Lalbherenda, Undirbibi;* Hindi: *Undirbibi, Jangli-arandi*; Kannada: *Totlagide*; Malayalam: *Atala, nakadanti;* Marathi: *Janglierandi;* Sanskrit: *Nikumba;* Tamil: *Sivappu, Adalai, Adalai-chedi, Eliavanakku, Vellai-kattukottai*; Telugu: *Dundigapu, Nela-amidam.*

Erect shrub to undershrub, 1–2 m tall, with pale yellowish latex/juice; stem stout, dichotomously branched, glabrous, shiny, marked by prominent leaf scars; branches stout glabrous; bark smooth, papery, peeling off in flakes. *Leaves*: simple, orbicular, deeply palmately 3–5-lobed, truncate or subcordate at base, 8–16 cm in diam., glabrous beneath; lobes obovate or oblanceolate, acute or subacuminate at apex, narrowed at base into rounded sinuses, sharply serrated with gland-tipped serrations, 3.5–8.5 × 1.5–5.5 cm, the posterior lobes smaller; main nerves 3–5, palmate, grooved above, raised beneath; secondary nerves many, oblique, 1–2 cm long; petioles slender; 5–15 cm long; stipules deeply divided into several long capiliary gland-tipped segments. *Inflorescences*: axillary, corymbose cymes, with central flower of each cyme usually female, glabrous; peduncle 8–15 cm long; branches alternate, up to 5 cm long; bracts similar to stipules, up to 1 cm long; pedicels ca 2 mm long. *Male flowers:* greenish yellow pedicels 2–3 mm long; calyx ca 2 mm long, glabrous, eglandular; lobes oblanceolate, narrowed at base, obtuse at apex, 4 × 2 mm; nectary disc of 5 round glandular lobes; stamens 8 in 2 whorls, outer whorl of 5 and inner of 3; filaments connate for half the length, 2–2.5 cm long, outer whorl smaller, inner longer; anthers oblong, ca 1 mm long. *Female flowers:* pedicels 3–4 mm long; calyx 3–4 mm long, lobed almost to base; lobes lanceolate, acute, gland-tipped, serrations along margins, 2–3 mm long; petals free or almost so to the base, oblong lanceolate, obtuse, narrowed at base, ca 5 × 2 mm, densely brown villous at base inside, pale yellowish green; nectary disc

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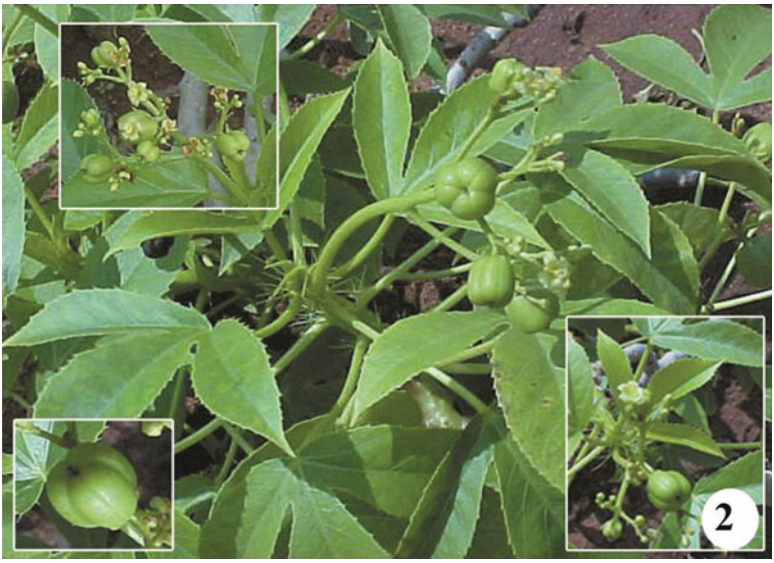

Fig. 10.2 Plant and flower characteristics of *Jatropha* species: *J. glandulifera*

consisting of 5 rounded glandular lobes; ovary oblong-ovoid, ca 2 mm long, ca 1.5 mm thick, glabrous; styles 3, ca 1.5 mm long, connate at base; stigma club-shaped. *Fruits*: oblong-ovoid, subglobose, 3-lobed, ca 1–1.5 cm long; ca 8 mm across, glabrous, rugulose, with persistent sepals; seeds oblong, ca 1 cm long, ca 5 mm thick, smooth, shiny, dark brown to black; caruncle palmately lobed (Fig. 10.2).

Fl. & Fr.: mostly during rainy seasons up to Dec-Jan.

Habitat: Scrub forests, road sides and edges of forests, in tropical semi-arid regions, up to 1,000 m altitude.

Distribution: Exotic weed, naturalized in waste lands and road sides. INDIA: Andhra Pradesh (common in Krishna and Godavari Dists.) along the east coast up to Bengal, Karnataka (common in Bellary), Kerala and most districts of Tamil Nadu. Often grows in black cotton soil, rare in Oudh and Punjab. Sri Lanka and Myanmar.

3. *J. gossypiifolia* L. Sp. Pl.: 1006. 1753 (as *'gossypiifolia'*); Hook, f. *Fl. Brit. India* 5:383. 1887; Gamble, *Fl. Madras* 2: 937. 1957 (repr. edn.); Surya Prakash Babu in Pullaiah & Ali Moulali *Fl. Andhra Pradesh* 2: 873. 1997.

Assamese: *Bhotera;* Bengali: *Lalbherenda;* English: *Bellyache Bush*; Gujarathi: *Erandi, Torspodla, Velati*; Hindi: *Bherenda, Verenda Rani-jadha*; Kannada: *Cikkakdu-haralu, Hathile-heralu, Karituruku haralu*; Malayalam: *Simayavanakku;* Marathi: *Kosni-ronda*; Tamil: *Adalai, Atalai, Siria-amanakku*; Telugu: *Amadam, Nala-amudam, Seema nepalamu, Nela-amudam, Nepalamu.*

Bushy shrub, somewhat thick, stout, woody, erect, up to 3 m high, branching young stems and leaves dark purplish red coloured; wood soft; branchlets glandular

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hairy; bark shiny, rough, with raised black patches. *Leaves*: closely spirally arranged, entire or palmately 3–5 lobed or partite up to the middle, subcordate at base, 5–13 cm in diam., densely hairy on abaxial surfaces of major veins or only at the divergence of primary veins, shortly stipitate glandular-ciliate with viscid glands along leaf margin, membranous, bronze-red when young, becoming reddish green on maturity; lobes entire, obovate, broadest at the middle, shortly acute; veins reddish when young; main nerves 3–5, basal, palmate; petioles 4–10 cm long; petioles and stipules with long often branched viscid glands. *Inflorescences*: corymbose cymes, glandular; bracts lanceolate, 9–11 mm long, glandular-ciliate; flowers 7–8 mm across; pedicels up to 5 mm long. *Male flowers*: sepals oblong-ovate, acute, ca 6×2 mm, glandular cilate; petals free, recurved, purplish or reddish; stamens 8(12), diadelphous; inner filaments longer; anthers ca 1 mm long. *Female flowers*: sepals and petals as in male; ovary globose, ca 3 mm in diam.; staminodes absent; styles ca 1.5 mm long. *Fruits*: oblong-ovoid, 3-lobed, 3-coccous, ca 1.5 cm long, reticulate-verruculose, reddish green. (Figs. 10.3 and 10.4).

Seedlings epigeal and phanerocotylar, the paracotyledons with five primary nerves and the first two leaves entire (Kamilya and Paria 1994).

Key to varieties

1a.	Young leaves dark purple or bronze red, gradually becoming reddish green on maturity, glaucous, sparingly hairy at the divergence of primary veins; all parts including stalks of viscid glands purplish; styles connate at base; capsule smooth or sparingly hirsutulous	**3.1. var. *elegans***
b.	Young leaves purplish green or greenish as they mature; densely hairy all over; all parts including stalks of viscid glands greenish; styles not forming a column, free; capsule densely hirsutulous	**3.2. var. *gossypiifolia***

3.1. var. ***elegans*** (R. Pohl) Muell. Arg., DC. *Prodr.* 15(2): 1087. 1866; Dehgan and Webster in *Univ. Calif. Publ. Bot.* 74: 55. 1979; Rao and Raju in *J. Econ. Taxon, Bot.* 18:587. 1994; Surya Prakash Babu in Pullaiah & Ali Moulali, *Fl. Andhra Pradesh* 2: 873. 1997. *Adenoropium elegans* R. Pohl, *Pl. Bras. Icon. Descr.* 1:12.t.15. 1826.

Fl. & Fr.: especially Mar. – Nov., to lesser extent other months.

Habitat: Common along road sides, railway tracks and wastelands, open eroded lands.

Distribution: India: Andhra Pradesh.

Native of tropical America (Brazil) introduced and naturalized in India.

3.2. var. ***gossypiifolia***

Fl. & Fr.: Apr. – Oct., in fact flowers all round the year.

Habitat: In plains from coastal areas to 500 m altitude, usually cultivated as hedge plant, often naturalised in wastelands and road sides.

Distribution: India: Andhra Pradesh, Assam, Bihar, Gujarat, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Orissa, Rajasthan, Tamil Nadu, Uttar Pradesh, West

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Fig. 10.3 Plant and flower characteristics of *Jatropha* species: *J. gossypiifolia* var. *gossypiifolia*

Fig. 10.4 Plant and flower characteristics of *Jatropha* species: *J. gossypiifolia* var. *elegans*

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Bengal and Andaman Islands. Native of New World, introduced, planted and often naturalised in many tropical countries.

4. *J. heynei* Balakr, in *Bull. Bot. Surv. India* 3: 40. 1961; Surya Prakash Babu in Pullaiah & Ali Moulali, *Fl. Andhra Pradesh* 2: 874. 1997. *J. heterophylla* B. Heyne ex Hook. f., *Fl. Brit. India* 5: 382. 1887 (nec Steudel 1840). Gamble *Fl. Madras* 2: 937. 1957.

Telugu: *Karuamudam dumpa,*

Rhizomatous small shrub of dry rocky slopes, 40–50 cm tall, monoecious, stout, short, erect, with, dark brown tuberous rootstock; stems terete, glabrous. *Leaves*: simple or deeply 3–5 lobed up to below the middle, cordate at base, without glands, glaucous beneath, 3-nerved at base; lobes entire, oblong to narrowly oblanceolate, cuneate at base, acute at apex, 2.5–15 × 1.2–3 cm, chartaceous; petioles stout, 1–8 cm long; stipules capillary. *Inflorescences*: terminal, panicled cymose, with a central female flower surrounded by male flowers; bracts entire; flowers ca 6 mm across. *Male flowers*: sepals ovate, obtuse, entire, free, ca 2.5 mm long, greenish yellow to white; petals free or nearly so, oblong, obtuse, ca 5 × 2 mm; stamens 8–10; filaments of inner row connate; anthers erect, ca 2 mm long. *Female flowers:* sepals and petals as in male flowers; ovary 3-loculed, each uniovulate. *Fruits*: ovoid ca 2 cm long, often pustulate, dehiscing into 3 cocci; each 2-valved, one-seeded; seeds ovoid; testa crustaceous (Fig. 10.5).

Fl. & Fr.: May–Aug. to Dec.

Habitat: Dry scrub jungles at low altitudes.

Distribution: India: Andhra Pradesh, Karnataka, Madhya Pradesh and Tamil Nadu.

Endemic to Deccan plateau.

5. *J. maheshwarii* Subram. & Nayar in *Bull. Bot. Surv. India* 6:331, ff. 1–8. 1964 (publ. 1965); Balakr. in *Bull. Bot. Surv. India* 10: 245. 1968. *Tritaxis beddomei* auct non Benth.: sensu Sundararaj in *J. Bombay Nat. Hist. Soc.* 53: 525, t.2. 1956.

A glabrous monoecious evergreen rhizomatous undershrub, erect, low branching, up to 1.5 m tall; bark pale with latex, smooth, papery. *Leaves*: oblong-ovate to ovate-lanceolate, rounded and peltate or subpeltate at base, entire, acute to acuminate at apex, basally 3–5 nerved; lateral nerves on midrib 5–7 pairs; petioles 4.5–16 cm long; stipules small, scarious, soon caducous. *Inflorescences*: terminal or rarely leaf-opposed compound cymes, the central flower of the cyme usually female; flowers yellow to orange, shortly pedicellate; bracts lanceolate, acute, 3–8 mm long. *Male flowers*: sepals 5, imbricate left or right shortly united at base, elliptic-oblong or oblanceolate, 3–4 mm long, glabrous; petals 5, contorted, shortly united at base, elliptic-lanceolate to oblanceolate, 4–5 mm long, glabrous outside, villous at base inside; nectary disc of 5 distinct orate glands around the base of staminal column; Stamens 8–9; filaments slender, united in a column; anthers linear-oblong, dorsifixed, longitudinally dehiscing, pistillode absent. *Female flowers:* sepals 5, imbricate in bud, shortly united at base, oblong-elliptic to oblong-lanceolate, 4–5 mm long, glabrous; petals 5, contorted, shortly united at base, oblong-lanceolate, 4–5 mm long, glabrous; nectary disc as in male flower; ovary ellipsoid, 3-locular, glabrous; ovule one in each locule, pendulous; styles 3, united below, each limb bilobed; stigma clavate. *Fruits*: oblong-subglobose to globose, 1.4–1.6 cm long, 1.2–1.4 cm across,

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Fig. 10.5 Plant and flower characteristics of *Jatropha* species: *J. heynei*

rough, glabrous, brownish; seeds oblong, ca 1.3×0.8 cm, crustaceous, brownish, shiny with a prominent caruncle (Fig. 10.6).

Fl. & Fr.: Feb – May. Rarely up to September.

Habitat: Coastal sandy areas.

Distribution: India: Tamil Nadu, coastal sandy areas of Tirunelveli and Kanyakumari Districts. Endemic to south eastern peninsular India.

Notes: The species epithet is named in honour of Late Prof. P. Maheshwari, F.R.S., renowned botanist of Delhi University, India. The species is allied to *J. villosa* but differs in glabrous nature of the plant, entire, oblong ovate leaves, rounded at base, petals united at base.

6. *J. nana* Dalzell & Gibson *Bombay Fl.:* 29. 1861; Hook.f., *Fl. Brit. India* 5: 382. 1887; Khanna et al. *Suppl. Fl. Madhya Pradesh*: 160. 2001; Singh and Karthikeyan *Fl. Maharashtra* 1: 2009. 2000; Samvatsar S *Fl. West. Trib. Madhya Pradesh*: 264. 1995.

Marathi: *Kirkundi.*

A small, bushy monsoon ephemeral to undershrub, 20–45 cm tall, rhizomatous, main root woody, stem terete, glabrous; branches erect. *Leaves:* ovate, entire or 3-lobed from above the middle, 8–12 cm long, and about equally broad lobes with entire naked margins, ovate, obtuse or subacute, middle lobe larger than side lobes, cuneate at base with three strong nerves; petioles 3–25 mm long; stipules minute. *Inflorescences*: terminal, few-flowered paniculate cymes; flowers pedicellate; bracts acute, not glandular along margins. *Male flowers*: calyx ca 3 mm long, glabrous, lobed for about half-way down; lobes 5, ovate, subobtuse; petals 5, free, obovate, orange cuneate, 5–6 mm long, glabrous outside, densely glandular hairy inside at

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Fig. 10.6 Plant and flower characteristics of *Jatropha* species: *J. maheshwarii*

base; stamens 8, with filaments united in lower half; nectary disc of minute glands. *Female flowers:* calyx 3–4 mm long, glabrous; lobes 5, lanceolate, acute to subacute; petals 5, free, obovate-oblong, 8–9 mm long, glabrous outside, glandular hairy at base inside; ovary obovoid, on glandular nectariferous disc, glabrous; styles ca 3 mm long, divided into three branches from the middle, each branch further divided at apex into two somewhat triangular ca 3 mm long lobes. *Fruits*: oblong to obovoid, flattened at top, 1 cm long, slightly 6-lobed, seed 9–10 mm long (Fig. 10.7).

Fl. & Ft.: July to September

Habitat: Dry stony ground and waste places in deciduous forests and scrubs, rare.

Distribution: India: Maharashtra (Bombay, Pune {around Panchgaon parvati about 12 km from Pune} and Konkan), West Madhya Pradesh (Jhabua). Endemic.

Notes: This species closely resembles *J. heynei* morphologically.

7. *J. tanjorensis* Ellis & Saroja in *J. Bombay Nat. Hist. Soc.* 58: 834, ff. 1–7. 1961 (Publ. 1962); Bennet in *J. Bombay Nat. Hist. Soc.* 62: 329. 1965; Ellis in *J. Bombay Nat. Hist. Soc.* 64:394, ff. 1–4, 1967; Ramach. & Nair *Fl. Cannanore*: 420. 1988; Babu et al. in *J. Bombay Nat. Hist. Soc.* 91:163. 1994; Prabakaran & Sujatha, *Genet. Resour. Crop Evol.,* 46: 213–218. 1999; Surya Prakash Babu in Pullaiah & Ali Moulali *Fl. Andhra Pradesh* 2: 874. 1997.

Telugu: *Nepalam, Lodhas, Hadajana*

Branched shrub to under tree with latex, 1.5–3 m high, glabrous, puberulous in young condition; stems stout, dichotomously branched; tender parts brownish. *Leaves*: simple, alternate, ovate or orbicular in outline, slightly cordate with

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Fig. 10.7 Plant and flower characteristics of *Jatropha* species: *J. nana*

a shallow sinus at base, 7.5–11.5 cm across, as broad as long, palmately 3–5 lobed above the middle; lobes broadly ovate, acuminate, distantly serrate, each serrature ending in a gland-tipped sparsely hairy on both sides, velutinous on either side of the veins and veinlets, palmately 7–9 nerved; petioles 4.5–7.5 cm long, with a few glandular hairs near the adaxial side; stipules short, ciliate, each segment ending in a glandular head. *Inflorescences*: corymbose, consisting of ca 9 cm long cymes; flowers green with pale pink tinge; bracts lanceolate, acute, 6–20 × 2–5 mm, with gland-tipped hairs along margins. *Male flowers:* shortly pedicellate; calyx 5-lobed, free, quincuncial; lobes ovate, slightly serrate, ca 4 mm long, pinkish green, pilose outside; petals 5, free, contorted, obtuse, rounded, ca 4 mm long; disc of 5 small nectary glands at the base of staminal column; stamens eight, yellow, free; filaments ca 3 mm long; anthers erect, basally attached, bearing 5–7 hairs on sides; connective prominent; pollen sterile. *Female flowers:* shortly pedicellate; calyx 5-lobed, quincuncial; lobes ovate, 5–8 mm long, pinkish green, pilose inside with gland-tipped serratures on the margin; petals connate for one-third their length at base, contort, obtuse, 5–8 mm long, veined, hairy inside; nectary disc of 5 glands around the ovary, smooth; staminodes 6–8 free; ovary glabrous, 3-locular with one pendulous ovule in each locule; styles 3, each divided into two stigmatic lobes. *Fruits:* 3-locular capsules, oblong obovoid, only one ovule developing into seed, loculicidal; pedicel pubescent; seed Oblong-ovoid, pale green with a few deep pink spots, dark brown when dry; caruncle striated, conspicuous; sepals slightly accrescent as the fruit matures (Fig. 10.8).

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Fig. 10.8 Plant and flower characteristics of *Jatropha* species: *J. tanjorensis*

Fl. & Fr.: Jan.–June.

Habitat: Open sandy places, in plains.

Distribution: India: Tamil Nadu (Thanjavur, Pudukottai, Tiruchinapalli, Chengalpattu, Dharmapuri, Coimbatore, S. Arcot, Ramanathapuram Districts), Kerala, West Bengal (Bennet lc. 1965), and Andhra Pradesh.

Endemic to Coastal India, particularly Thanjavur and adjoining places in Tamil Nadu but grows well at various locations in Tamil Nadu and Andhra Pradesh.

According to Idu et al. (2009), *J. tanjorensis* is a common weed of field crops, road side and disturbed places in the higher rainfall forest zones of West Africa including Ugbowo, Benin city, Edo State, Nigeria.

Notes: The original description states that the flowers are polygamous with male and bisexual flowers. However the stamens in the bisexual flowers are extremely diminutive and probably sterile. Ellis and Saroja (1961) in their original description stated "fruit not seen". Scientists of Directorate of Oilseeds Research, Rajendranagar, during their survey in Tanjore, Ramnad, Tiruchirapalli noted *J. tanjorensis* without fruits. They raised full grown plants with flowers from stem cuttings collected from different locations of T.N. The author (BB) also raised such plants earlier at Kakatiya and Osmania Universities, in A.P. India from cuttings for experimental purposes. It is also presently grown for experimental purposes at NBRI, Lucknow, India.

This probably is a hybrid between *J. gossypiifolia* and *J. curcas* with characters intermediate between the two, showing similarities and dissimilarities from both the parents. Recent collections from disjunct locations indicate such a possibility Sahai et al. (2009) reported floral abnormalities in Jatropha tanjorensis. Further studies are needed to clear the doubt (for details see interspecific hybridization chapter).

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8. *J. villosa* Wight Icon. *Pl. Ind. Orient.* 3: t. 1159. 1846; Balakr in *Bull. Bot. Surv. India* 3:40. 1961 (non Baill. 1863, nec. Muell. Arg. 1866); Surya Prakash Babu in Pullaiah & Ali Moulali, *Fl. Andhra Pradesh* 2: 874. 1997. *J. peltata* Wight, *Icon. Pl. Ind. Orient.* 4(1):t. 1169. 1848 (non Cerv. 1794, nec Steudel 1840). *J. wightiana* Muell.Arg. in DC., Prodr. 15(2):1080. 1866; Hook f., *Fl. Brit. India* 5: 383. 1887, Gamble, *Fl. Madras* 2: 937. 1957 (repr.ed).

Tamil: *Thanakku*

Dry stony low branching shrub 1–2 m high, pubescent, eglandular; branches rusty villous or subglabrous. *Leaves*: crowded at the ends of branches, peltate, rounded or subcordate at base, orbicular or ovate, entire or 3–5(7) lobed, 8–16 cm in diam., densely tomentose beneath, sparsely pubescent above in adult leaves, densely so in young leaves; lobes obtuse or subacute, entire; sinuses between lobes obtuse; main nerves 5–7, palmate; petioles 2–8 cm long, villous or subglabrous; stipules minute, early caducous. *Inflorescences*: terminal corymbose cymes, ca 7 cm long, with central flower of each cyme usually female, ca 7 cm long and up to 8 cm across, pubescent or subglabrous; peduncles unbranched for about 2–5 cm length, then alternately branching; bracts linear-lanceolate, entire, up to 12 mm long; flowers cream-coloured or yellowish green; pedicels up to 2 mm long. *Male flowers:* calyx 4–5 mm long, pubescent, lobed up to middle; lobes ovate-lanceolate, acute, 1.5–2.5 mm long; corolla salver-shaped, 12–13 mm long, ca 6 mm across, glabrous outside, villous inside, ca 6 mm across, lobed to about middle; lobes 5, oblong-obovate to spathulate; disc of 5 free, glandular, ovate-acute lobes; stamens 8, monadelphous; anthers oblong-ovate, ca 2 mm long, outer 5 introrse, inner 3 extrorse; filaments 5–8 mm long. *Female flowers*: calyx up to 6 mm long, lobed for three-fourth the length; corolla up to 1 cm long, subglabrous inside; disc as in male; ovary oblong-ellipsoid, 3–4 mm long, pubescent; styles 3, united into a slender column, ca 4 mm long, pubescent; stigma 2-partite. *Fruits*: capsular, oblong-globose, 2–2.5 cm long, 1.2–1.5 cm wide, rugulose, pubescent when young; seeds oblong, ca 1 cm long, ca 6 mm wide, glossy, smooth, brown; caruncle curved, white at edges like horns (Fig. 10.9).

J. villosa is endemic to peninsular India, with two distinct varieties differing in flower morphology and is allied to *J. glandulifera* Roxb., but differs in having leaves lobed above the middle, the stipules are shorter with a few filiform glandular-tipped, flowers are polygamous, and the petals connate to one-third their length at the base in bisexual flowers.

Key to varieties

1a.	Branches, leaves and petioles glabrous or subglabrous; lobes of leaves acute or subacute; petals of female flowers pubescent inside	**8.1. var. *ramnadensis***
b.	Branches, leaves and petioles densely villous pubescent; lobes of leaves obtuse; petals of female flowers subglabrous inside	**8.2. var. *villosa***

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Fig. 10.9 Plant and flower characteristics of *Jatropha* species: *J. villosa*

8.1. var. ***ramnadensis*** Ramam. in *Bull. Bot. Surv. India* 9:278, ff. 1–11. 1967.
Chromosome number 2n = 20 (Sasikala and Paramathma 2010).
Fl. & Fr.: July – Oct.
Habitat: Coastal areas.
Distribution: India: Coastal areas of Tamil Nadu (Ramanthapuram District). Endemic.

8.2. var. ***villosa***
Chromosome number 2n = 20 (Sasikala and Paramathma 2010).
Fl. & Fr.: Jan.-Dec.
Habitat: Scrubs and forests, at 400–1550 m altitude.
Distribution: India: Tamil Nadu (Coimbatore, Salem, Trichirapally, Madurai, Nilgiris). Andhra Pradesh, Karnataka. Endemic.

The distribution of various wild species in India is shown in Map 10.1. Cultivated species and wild species with wide distribution all over India are not shown.

Cultivated Species

9. *J. integerrima* Jacq Strip. *Select. Amer. Hist.* 265, t. 183, f. 47, 1763; Mc Vaugh, *Bull Torrey Bot. Club* 73: 274, 1945. *J. hastata* Jacq Strip. *Select. Amer. Hist*. 256, t. 173, f. 54, 1763. *J. panduraefolia* Andr., *Bot. Reports.* 4: t. 267. 1802; Haines, *Bot. Bihar & Orissa* 1: 105. 1925.

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English: Fiddle leaved jatropha, Spicy jatropha

Large, erect, woody shrub, 1–3 mm high; branchlets thin, flexuous, finely pendulous; young leaves purplish beneath. *Leaves:* single to trilobed, fiddle-shaped, oblong, obovate, oblong-oblanceolate, rounded, truncate or subcordate at base with 2–4 subulate acute gland-tipped teeth on either side of petiole base, the remaining margin entire or sub-entire with or without a few distant teeth, abruptly caudate-acuminate at apex, sometimes narrowed or constricted above the base, 6–16 × 2–8 cm, dark green above, purplish tinge beneath, from seedling stage onward glabrous except the puberulous midrib above; midrib slightly grooved above, raised beneath; lateral nerves 10–15 pairs, arcuate towards margin, basal veins 2–4 pairs of crowded; petioles slender, 1–7 cm long, puberulous, straight; stipules subulate, entire, up to 3 mm long. *Inflorescences*: axillary, corymbose cymes; peduncles 8–15 cm long, slender, puberulous, purple or bright crimson, greenish purple; branches puberulous, lower ones alternate, upper ones sub-opposite; bracts one at each branch, linear-lanceolate to subulate, up to 2 mm long, 1-nerved, the lower ones longer, pedicels ca 2 mm long, puberulous. *Male flowers*: calyx cup-shaped, ca 3 mm long; lobes 5, subequal, oblong, obtuse or sometimes mucronulate at apex, ca 1 mm long, separated by broad rounded sinuses, scarious, purplish red; petals 5, twisted in bud either to left or right, free, obovate, oblanceolate to spathulate, cuneate at base into a short claw, obtuse, widely patent, 10–13 × 5–6 mm, brown-hirsute at base inside, scarlet-red or crimson-pink; stamens 10; filaments flat, united into column for half the length, outer 5 shorter, 5–6 mm and inner 5 long, 8–10 mm; anthers sagittate, reddish yellow, inner often strongly curved; disc lobes 5, glandular, ovate to orbicular. *Female flowers*: calyx as in male but often larger; petals and nectary disc same as in male; ovary ovoid, rounded at apex, ca 3 mm long, glabrous; styles 3, ca 5 mm long, each bifid from middle into filiform slightly hooded branches: *Fruits*: capsule shallowly 3-lobed, ca 1 cm long, purplish green; seeds oblong, ca 8 mm long; caruncle reflexed, lacerate; hilum small (Fig. 10.10).

Fl. & Fr. Jan. – Dec.

Habitat: Cultivated in gardens all over India at low elevations.

Distribution: India: Andhra Pradesh, Karnataka, Madhya Pradesh, Orissa, Punjab, Rajasthan, Tamil Nadu, Uttar Pradesh and other states cultivated for its bright crimson flowers.

Native of Cuba now introduced and cultivated in several tropical regions around the world.

Notes: The species *J. integerrima* Jacq. and *J. hastata* Jacq. were published at the same time in the year 1763. McVaugh (1945) was the first to unite these species and choose *J. integerrima* as the name for the combined species and this has been accepted by subsequent workers. Further, *J. panduraefolia* Andr. was considered to be synonym of *J. integerrima.*

Kamilya and Paria (1994) and Bahadur et al. (1997) have described the seedlings as epigeal and phanerocotylar, the paracotyledons with three primary nerves and the first two leaves simple and subsequent leaves pandurate. Further, the cotyledons have three major veins with hypostomatic stomata.

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Fig. 10.10 Plant and flower characteristics of *Jatropha* species: *J. integerrima*

Key to varieties	
1a.	Leaves narrowly panduriform, 2–4 cm wide, much narrowed towards middle and towards petiole base, margins entire with a 2–4 dentate projections at the base of lamina on either side; petiole up to 3 cm long **9.1. var. *coccinea***
b.	Leaves not panduriform, broadly oblong-obovate or oblanceolate, usually 4–8 cm wide, not narrowed at middle; margins dentate; petioles usually up to 7 cm long **9.2. var. *latifolia***

9.1. var. *coccinea* (Link.) Balakr, in *Bull. Bot. Surv. India* 22:176. 1980 (publ.1982). *J. coccinea* Link, *Enum. Hort. Berol.* 2:406. 1822. *J. panduraefolia* Andr. var. *coccinea* (Link) Pax in Engler, *Pflanzenr. Heft* 42:50, t.19B. 1910.

9.2. var. *latifolia* (Pax) Balakr., l.c. 176. 1980 (publ.1982). *J. panduraefolia* Andr. var. *latifolia* Pax, l.c. 50, t. 19A. 1910.

Notes: However, it is rather difficult to distinguish them clearly in live plants due to intermediaries although the two varieties differ in some morphological, and micromorphological characters and hence, may not stand as taxonomically distinct. But in reality they are different.

J. panduraefolia and *J. integerrima* have been treated separately in Curtis Botanical Magazine (Vol. 35: 1464–1465, & Vol.37: 604–605, see Chaturvedi and Jehan 1982; Bahadur et al. 1998; 2000) based on dioecious and monoecious condition and differences in leaf shape, pollen and leaf, pericarp and seed microcharacters. Later, it was observed that *J. integerrima* is monoecious to dioecious and the

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leaf character also varies and this prompted Airy Shaw (1972) to include both the species under *J. integerrima*. Dehgan and Webster (1979) considered *J. panduraefolia* as synonym to var. *integerrima*.

10. *J. multifida* L., Sp. Pl.: 1006. 1753; Hook. f. *Fl. Brit. India* 5: 383. 1887; Gamble *Fl. Madras* 2: 937. 1957.

English: Coral Plant; Kannada: *Vilayathiharalu;* Konkani: *Chini-emrandi;* Malalyalam: *Aramedakam;* Marathi: *Chiniyerandi;* Sanskrit: *Bhadradanti, brihaddanti, jyotishkka, Virechani Visabhadra* Tamil: *Malaiymaramanakku, Katturervalam.*

Handsome large shrub or small tree, 1–5 m tall, branched, glabrous with tuberous roots, *Leaves:* alternate, broadly orbicular in outline, 15–35 cm across, deeply palmately divided up to about 1 cm from base into 5–13 lobes, subpeltate or cordate at base; lobes lanceolate to linear lanceolate, acute to acuminate at apex, pinnatifid or coarsely lobulate towards upper portion, rarely entire, light dull green above, glaucous green beneath; each lobe further subdivided pinnately, veins elevated abaxially, main nerves 7–13, slightly grooved above, raised beneath; lateral nerves many; petioles 10–25 cm long; latex juicy, stipules 1–2 cm long, divided into several patent long filiform eglandular segments. *Inflorescences*: terminal corymbose cymes, 3–4 cm across; peduncles 10–20 cm long, greenish red; branches alternate; pedicels 4–8 mm long, thickened upwards towards apex; bracts subulate, 1–3 mm long, entire. *Male flowers:* calyx cup-shaped, ca 2 mm long, 5-lobed; lobes oblong-ovate, obtuse at apex, ca 1 mm long, crimson-red, glabrous, twisted in bud; disc cup-shaped, glandular; stamens 8 (5 + 3) in two whorls; filaments ca 2 mm long, connate at lower half; anthers elongate, linear, sagittate, ca 1.5 mm long. *Female flowers:* calyx as in male; petals larger, 6–7 mm long; disc urceolate; ovary ovoid, ca 5 mm long, yellowish, glabrous; styles 3, ca 4 mm long, connate at base, each cleft into slender elongate segments; stigma simple, capitate. *Fruits*: schizocarpic, globose, pyriform capsule laterally oblongoid, slightly 3 or 2-lobed with 3 prominent longitudinal ribs, ca 3 cm across, subdrupaceous, indehiscent, smooth, yellow when ripe, often 2-seeded, the third locule with undeveloped or devoid of seed (Fig. 10.11).

Fl. & Fr. Jan. – Dec.

Habitat: Cultivated in tropical gardens of plains.

Distribution: India: Andhra Pradesh, Goa, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Orissa, Tamil Nadu, West Bengal and Andaman & Nicobar Islands. Native of tropical America, now introduced and cultivated in tropical gardens of several countries in Asia and Africa.

Notes: The species has the largest known seeds in the genus. Kamilya and Paria (1994) and Bahadur et al. (1999) have described the seedlings as epigeal and cryptocotylar, the hypocotyl with numerous wart-like raised spots, the cotyledons enclosed in seed coat coming out together above the soil surface along with hypocotyl and the two cotyledons never get exposed except their petioles. The first two leaves are multifid. This is probably the only *Jatropha* species known to have cryptocotylar condition. Cryptocotylar condition is unique in Euphorbiaceae and has been reported only in *Hevea brasiliensis*. Dehgan and Webster (1979) stated: "This situation poses a great deal of difficulty in germination of hybrid seeds where *J. multifida* is one of

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Fig. 10.11 Plant and flower characteristics of *Jatropha* species: *J. multifida*

the parents, because the cotyledons are never completely freed and, consequently, the food reserve becomes exhausted. Since photosynthesis does not begin until the first leaves appear, the seedlings are too weak to survive. This perhaps explains the unusually large seeds of *J. multifida* and *Hevea brasiliensis,* where sufficient food can be stored to compensate for the lack of photosynthesis by initial leaves".

11. *J. podagrica* Hook in *Curtis Bot. Mag.* 74: t. 4376. 1848; Woodrow in *J. Bombay Nat. Hist. Soc.* 12: 371. 1899; Pax in Engler *Pflanznreich* 147: 44. 1910.

English: *Gouty stemmed Jatropha, Guatemala rhubarb.*

Shrub, 0.5–1.5 m tall, peculiar gouty stem suddenly grotesquely thickened and swollen at base, bottle-shaped, widely branched dichotomously; bark peeling, greyish; branches thick, glabrous. *Leaves*: large in clusters of 6–8, broadly ovate-reniform orbicular – suborbicular, 12–30 cm across, broadly peltate, truncate to broadly rounded

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at base, palmately (shallow to deep) 3–5 lobed almost half way; lobes broad, shortly acuminate, acute or subobtuse undivided pale glaucous beneath; main nerves 5–9, primary veins not elevated abaxially palmate; lateral veins almost straight; petioles 8–20 cm, 3–4 mm thick, glabrous; long stipules pectinate into small rigid glandular segments, often persistent. *Inflorescences*: axillary, corymbose cymes, multi-forked; peduncles 16–18 cm long, 3–4 mm thick, glabrous; branches thick, dense, crowded at the top of peduncle; bracts ovate, obtuse, simple, wavy along margins, strongly thickened-keeled on midrib, ca 1 mm long, scarious; flowers in groups of 5 or 6 with male flowers surrounding generally a solitary female flower in a cluster. *Male flowers:* pedicels slender, 1–1.5 mm long; calyx cupular, 1–1.5 mm long, red, glabrous, lobed for half the length; lobes rounded, orbicular; petals oblong-obovate of oblanceolate-spathulate, obtuse at apex, narrowed to base, 5–6 × ca 2 mm, glabrous, bright red dark-orange, disc urceolate; stamens 6–8, filaments 2–3 mm long, connate at base; anthers linear-oblong, ca 2.5 mm long. *Female flowers*: pedicels 1–2 mm thick; calyx cupular, lobed almost to base, ca 1 mm long, orange – red, glabrous; lobes ovate, obtuse; petals oblong-spathulate, 6–7 mm long, ca 2 mm across, bright red; disc urceolate, connate, glandular; ovary glabrous, ovoid, ca 2 mm long; style ca 1 mm long; stigma ca 1.5 cm long, thick, bilobed. *Fruits*: oblong, truncate or obtuse, 3-lobed, ca 1.5 cm long, ca 1.2 cm across, yellow, glabrous; seeds ca 1 cm long (Fig. 10.12).

Fl. & Fr.: Feb.–Nov.

Habitat: Cultivated as ornamental plant in gardens, plains.

Distribution: India: Andhra Pradesh, Assam, Goa, Karnataka, Madhya Pradesh, Maharashtra, Orissa, Sikkim, Tamil Nadu and West Bengal. Native of tropical central America (Panama) occurring naturally in Guatemala, now introduced and cultivated in many parts of Old World including Indian gardens for ornamental in conservatories, but thrives well in rich soil exposed to sun.

This species can be easily propagated from seeds.

The seedlings are epigeal and phanerocotylar, the paracotyledons which carry nerves and the first two leaves entire and ovate-peltate.

J. podagrica Hook. var. *elegans* Muell-Arg. grown in gardens for its ornamental flowers and gouty stem.

Other Less Known Cultivated Species

J. princeps Vell., is a medium sized avenue tree native to Brazil and cultivated in Indian gardens and other tropical countries for its timber and medicinal value. The leaves are large ovate, alternate, digitately 5–7 lobed, flowers inconspicuous in paniculate cymes. Fruits large 10–12.5 cm diameter with large seeds containing agreeably flavoured kernels. The seed oil called “Anda-assy” is yellow with strong garlic odour and irritating repulsive taste. The oil is used as purgative, anthelmintic and fish poison. The bark also contains an essential oil. The milky latex from bark is used to stupefy fish.

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Fig. 10.12 Plant and flower characteristics of *Jatropha* species: *J. podagrica*

Interspecific Hybrids

Pax (1910) first reported the occurrence of natural hybrids in *J. integerrima, J. hastata* in Cuba and West Indies followed by Dehgan and Webster (1978) in south American species *J. cinerea, J.canescens*. Interpecific hybrids between cultivated and wild species have been successfully raised by Sujatha and Prabakaran (2003) and Soontornchainaksaeng and Chaiyasut (1999). To date, the hybrids have not been named. Parthiban et al. (2009) have carried out interspecific hybridization programme using wild and cultivated species to develop suitable hybrids. The cross *J. curcas* x *J. integerrima* produced successful hybrid. According to Bhagat and Kulkarni (2010), *J. nana* is suitable for future hybridization and *Jatropha* improvement programme in view of its short bushy habit and high oil content of seeds. The details of interspecific hybrids are dealt with under a separate chapter on interspecific hybridization.

Sepal Characters

The significance of various glands (sessile, branched, unbranched) in the taxonomy of *Jatropha* and *Cnidosculus* is known for long time when four sections were first recognised (see McVaugh 1944, 1945) especially the following two sections;

Section : ***Polymorphae*** Pax – leaf with few gladular teeth.

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Section : ***Adenorhopium*** Griseb, leaf, petiole, calyx and stipules with copious glands

McVaugh (1945) in his treatment of *Jatropha* in America noted the presence of glands on leaves, petioles and stipules and made use of this character in key; to identify species as briefly presented below.

1. **Species with glandular calyx:** *J. breviloba, J. bornmulleri, J. guarnitica, J. mutabilis*.
2. **Species with glands on leaf margin and rarely on stipules**: *J. pachypoda, J. papyrifera, J. ciliata, J. augusti, J. hoffmanniae, J. humboldtiana*.
3. **Species with glands on petiole**: These are of two types, *viz.,*

   (a) ***Branched glandular hairs:***
   J. gossypiifolia, J. pedatipartita, J. intercedens, J. molmeana, J. tacumbensis, J. hippocastanifolia.

   (b) ***Branched sparingly or simple/clavate:***
   J. excisa, J. flavovirens, J. thyrsantha, J. clavuligera, J. pieronoi, J. elliptica, J. intermedia, J. isabelli, J. dissecta, J. puneticulata, J. rigidifolia, J. induta, J. transiens, J. branchypoda.

The calyx in *Jatropha* is pentamerous, free, imbricate, entire, glandular, rarely divided, often foliaceous, green or dull crimson. Since the flowers are monoecious, differences in male/female calyx exist; may be urceolate, tubular or subglobose in the former and linear foliaceous, enlarging slightly in fruit and persistent in the later. McVaugh (1945) while studying the American *Jatropha* species with reference to intrageneric groups considered sepal and petal characters in distinguishing the various species and took into account male and female flowers as also the glandular margin of sepals. Dehgan (1994) noted glandular hairs in sepals in 29 out of 77 species studied by him and considered leaf marginal glands as of taxonomic value and this prompted this study on Indian species. The size, shape, sinus and glandular margin varied in various species studied (McVaugh 1945).

The calyx in the various species of *Jatropha* is 5 lobed and connate and may be small or bigger than petals. The apex is rounded in *J. podagrica, J. curcas, J. multifida, J. integerrima (J. panduraefolia)* and *J. villosa*. These species are devoid of glands and hairs on the surface or along the sepal margins; whereas *J. tanjorensis, J. gossypiifolia* possess capitate glands with a distinct stalk and globular head. The sepal epidermis in all *Jatropha* species is reticulate with paracytic stomata (Table 10.1).

J. tanjorensis: the sepal is relatively big (5×3.5 mm), the apex pointed, the glands dimorphic i.e. bigger glands alternate with smaller ones, 12 glands on either side of a sepal are present. The contents of the head of the glands appear yellowish and translucent. The sepal has 8–10 veins arising from the base whose secondaries anastomose towards apex. About 28 anastomoses are present. Epidermal cells are reticulate and devoid of hairs (Fig. 10.13a).

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Table 10.1 Summary of sepal characters in *Jatropha* species

Sl. No	Species	Sepal size length × width (mm)	Glands size in mm	Hairs	Stomata
1	*J. gossypiifolia* var. *gossypiifolia*	3 × 1.5	Capitate 10 × 2.5	Conical	Paracytic
2	*J. gossypiifolia* var. *elegans*	3 × 1.3	Capitate 8 × 1.6	Conical	Paracytic
3	*J. glandulifera*	3 × 1.3	Capitate at tip 1 × 1	Absent	Absent
4	*J. tanjorensis*	5 × 3.5	Capitate at tip 16 × 8	Conical	Paracytic
5	*J. curcas*	4 × 1.2	Absent	A bunch of hairs on both sides	Paracytic
6	*J. integerrima*	4 × 1.5	Sessile 1.2	Absent	Paracytic
7	*J. multifida*	3.6 × 1.2	Absent	Absent	Absent
8	*J. podagrica*	3 × 1	Absent	Absent	Absent
9	*J. villosa*	3.5 × 1.8	Absent	Cylindrical present in the upper region	Absent
10	*J. heynei*	2.5 × 1	Absent	Absent	–
11	*J. maheshwarii*	3.5 × 1.2	Absent	Absent	–

J. gossypiifolia var. ***gossypiifolia:*** the glands are capitate, about (8–10) 4–5 pairs, interspersed with conical hairs. Hairs are also present on the sepal surface on the outer side, sepal tip without gland. A thick mid vein arises from the base and branches into 8–10 anastomoses. In *gossypiifolia* var. *elegans*, the glands are more in number about (14–16), 6–8 pairs per sepal interspersed by a number of conical hairs, a mid vein forming 10–12 anastomoses (Fig. 10.13b, c).

J. glandulifera: the sepal is pointed with a sessile gland at the tip. The base of the sepal is broad with 4–5 primary veins forming 8–10 anastomoses (Fig. 10.13d).

J. curcas: the apex is rounded with a single vein and 14 anastomoses (Fig. 10.13e).

J. integerrima: 6 pairs of sessile glands per sepal along the dentate sepal margins. A single mid vein forms 15 anastomoses (Fig. 10.13f), while *J. panduraefolia* has a single vein anastomosing at 18–20 places (Fig. 10.13g).

J. multifida: has a single vein that anastomoses at 14 places (Fig. 10.13h).

J. podagrica: has a single vein anastomosing into branches (Fig. 10.13i).

J. villosa: has a single vein anastomosing into 7–8 branches, hairs present only at the distal region (Fig. 10.13j).

A key for the identification of different species of *Jatropha* based on sepal morphology is proposed. This will serve as an important and useful tool to identify these taxa by field botanist/taxonomist without going into the finer details.

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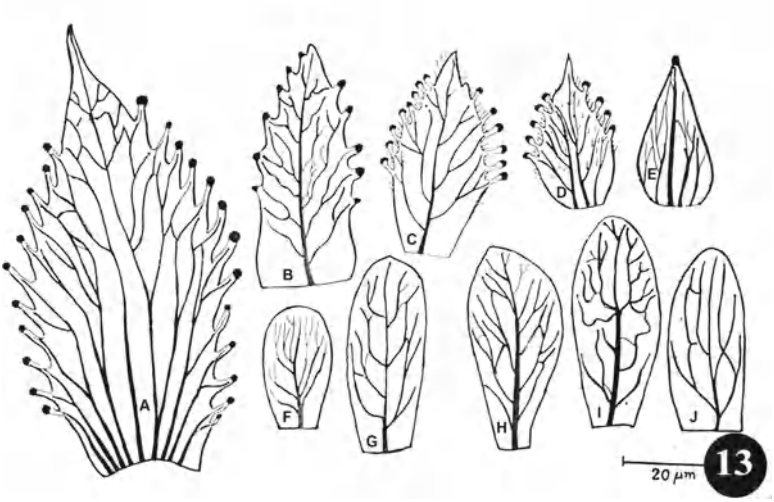

Fig. 10.13 Sepals of *Jatropha* species showing size, glands, venation pattern. (**a**) *J. tanjorensis,* (**b**) *J. gossypiifolia* var. *elegans,* (**c**) *J. gossypiifolia* var. *gossypiifolia,* (**d**) *J. glandulifera,* (**e**) *J. curcas,* (**f**) *J. integerrima,* (**g**) *J. panduraefolia,* (**h**) *J. multifida,* (**i**) *J. podagrica,* and (**j**) *J. villosa*

Key Based on Sepal Characters

1. **Sepals with glands, hairs present or absent:**

   (a) Glands with prominent stalk:
   Glands along margin, hairs present *J. gossypiifolia* var. *elegans*
   Glands along margin, hairs present *J. gossypiifolia* var. *gossypiifolia*
   Glands dimorphic along margin, hairs absent *J. tanjorensis*

   (b) Glands without stalk (sessile):
   Glands sessile, hairs absent *J. integerrima*
   Glands sessile at sepal tip, hairs absent *J. glandulifera*

2. **Sepals without glands, hairs present or absent:**

   (a) Sepals green without glands 4 mm long *J. curcas*
   Sepals green less than 4 mm long *J. villosa, J. heynei, J. maheshwarii*
   Sepals orange coloured *J. podagrica, J. multifida*
   Sepals crimson green *J. integerrima*

Petal Characters

McVaugh (1945) while studying the American *Jatropha* species with reference to intrageneric groups considered petal characters in both male and female flowers also in distinguishing the various species in respect of their connation and imbricate

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Table 10.2 Summary of petal characters in *Jatropha* species

Sl No.	Taxa	Petal size LxW in (mm)	Number of anastomoses	Size of hairs on petal upper surface (μm)	Size of hairs on petal margin
1	*J. gossypiifolia* var. *gossypiifolia*	4.5×3	50	64	40/60 μm
2	*J. gossypiifolia* var. *elegans*	4×3	28	64	30/60–64 μm
3	*J. glandulifera*	4×2	25	640	Absent
4	*J. curcas*	5×2	16	640	Absent
5	*J. tanjorensis*	7×3	19	320	50–55/70 μm
6	*J. integerrima*	11.5×5	35	640	Absent
8	*J. multifida*	6×3	19	–	Absent
9	*J. podagrica*	6×2.5	17	320	Absent
10	*J. villosa*	7×2	17	320	20–30 μm
11	*J. heynei*	5×2	–	Absent	Absent
12	*J. maheshwarii*	7×1.5	–	Absent	Absent

aestivation. Dehgan (1994) noted *Jatropha* flowers to be pentamerous, regular with petals of equal size although the petal shape and colour varies from species to species. Arnott and Tucker (1964) made analysis of petal variation, position of dichotomies in *Ranunculus repens* to be specific. Datta and Saha (1968) noted specificity of distribution of venation anastomosis pattern characteristic to be specific in various petal sectors in 4 taxa of Phaseoleae and suggested its phyletic significance. In view of this taxonomic importance petal characters of *Jatropha* species were studied and the account is as under and also in Table 10.2.

LM Observations

J. gossypiifolia var. *gossypiifolia*

The petal measures 4.5×3 mm, spathulate with a claw and distinct flattened distal portion, with notch at tip and provided with squarish to rectangular epidermal cells in linear rows. About 40 short conical hairs per petal are present along the margins of the central regions and measure 64 μm long. Paracytic stomata occur scattered all over the petal surface in different planes. A main petal vein branches and forms 50 anastomoses, confined to the distal region. Sphaero crystals present, but laticifers are inconspicuous.

J. gossypiifolia var. *elegans*

The petal measures 4×3 mm with a flattened distal region, without notch. Epidermal cells reticulate, branching less than in above species and venation pattern is simpler

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and begins at a higher level from base of petal. Anastomoses 28 and in the distal region. About 30 short conical hairs per petal measuring 64 μm present along the margins of petals. Sphaerocrystals present. Laticifers inconspicuous.

J. glandulifera

The petal measurers 4 × 2 mm. The epidermal cells reticulate. The cells of the lower region linear. Tuft of hairs present on the inner surface at the base of the petal where veins branches. Anastomoses 25 more in the distal region. Hairs cylindrical, 640 μm long, sphaerocrystals abundant, branched laticifers present.

J. curcas

The petal measures 5 × 2 mm, petal deeply notched at the tip. Epidermal cells reticulate, polygonal. Hairs abundant, long cylindrical present on lower and middle region and measure 680 μm, 3–4 veins arise from the base and subsequent branching with 16 anastomoses, laticifers present, sphaerocrystals large abundantly scattered at the base and distal region of petals.

J. tanjorensis

The petal measures 7 × 3 mm. Epidermal cells reticulate. Petal traversed by densely anastomosed laticifers. Branching of veins begins in the lower region and anastomose at the tip. Crystals present are scattered all over the petal. Hairs unicellular, cylindrical to clavate along the margin, measure 320 μm, 42 hairs per petal, sphaerocrystals abundant.

J. integerrima

The petal measures 11.5 × 5 mm, petal surface reticulate hexagonal to polygonal cells with wavy anticlinal walls. Hairs measure 640 μm, present at the base of the petal in a bunch on either side of mid vein. Anastomoses 35 in the distal region. Sphaerocrystals sparse along the margins but more in the middle region and laticifers rare, inconspicuous.

J. multifida

The petal fleshy, measures 6 × 3 mm with abundant branched laticifer system. The venation pattern shows 19 anastomoses, more at the distal region. Epidermal cells reticulate squarish to polygonal. Hairs absent, sphaerocrystals present.

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J. podagrica

The petal fleshy, measures 6 × 2.5 mm with a deep notch at the tip. Venation poor with 13–17 anastomoses. Sphaerocrystals few, present along the margins. Epidermal cells reticulate, quadrangular in linear rows, branched laticifers present. Hairs present at the base of the petal measure 320 μm.

J. villosa

The petal measures 7 × 2 mm and is villous. The main vein branches after traversing one third of the petal axis, has 17 anastomoses. Conical hairs present along the margins, filiform hairs measure 320 μm. Epidermal cells reticulate, laticifers and sphaerocrystals present.

J. heynei

The petals measures 5 × 2 mm. Epidermal cells reticulate. Hairs absent on surface and margins.

J. maheshwarii

The petals measures 4.5–7 × 1.5 mm, villous at base inside. Hairs absent on surface/margins.

An artificial key for the different species of *Jatropha* taking into consideration petal characters will serve as an important and useful tool to identify these taxa by the field botanist/taxonomist without going into the finer details.

Key Based on Petal Characters

(a) **Hairs present on petal margin and surface:**

Petal margin with 40 hairs; 50 anastomoses *J. gossypiifolia* var. *gossypiifolia*
Petal margin with 30 hairs; 28 anastomoses *J. gossypiifolia* var. *elegans*
Petal margin with 50–55 hairs; hairs on surface, 19 anastomoses *J. tanjorensis*
Petal margin with 20–30 hairs; hairs on surface, 19 anastomoses *J. villosa*

(b) **Hairs present at base of upper surface only:**

Hairs present at base of upper surface, 35 anastomoses .. *J. integerrima*
Hairs present at base of upper surface, 25 anastomoses .. *J. glandulifera*
Hairs present at base of upper surface, 16 anastomoses .. *J. curcas*
Hairs present at base of upper surface, 15 anastomoses .. *J. podagrica*

(c) **Hairs absent 19 anastomoses..** *J. multifida*, *J. heynei,*

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SEM Studies of Petals

Studies on petal surface microcharacters under SEM provide useful taxonomic markers but studies are limited – *Chlorogalum,* Liliaceae (Jernstedt 1980). To date there is no study on *Jatropha.*

McVaugh (1945) first noted the petals to be hirsute within at base in Sect. *Peltatae* and glabrous in Sect. *Macranthae*.

SEM of inner (upper surface) and outer (lower surface) petal surface in nine species of *Jatropha* were studied to ascertain their taxonomic utility. Summary of observations is as under:

J. gossypiifolia var. *elegans*

Upper petal epidermal cells reticulate, uneven, concave cells, anticlinal walls thick, wall junctions raised, with paracytic stomata distributed irregularly. Petal margin with unicellular hairs. Upper surface with sparsely scattered unicellular and uniseriate hairs. Lower epidermal cells reticulate with comparatively larger shallow epidermal cells. Stomata paracytic, epidermal cells irregular to rectangular with secondary ornamentation of crisscross interlocking pattern

J. gossypiifolia var. *gossypiifolia*

Upper petal epidermal cells reticulate with peculiar tiny spathulate hairs unevenly scattered. Epidermal cells tetra to polygonal. Conical hairs uniseriate along the margin and on upper surface. Lower epidermal surface reticulate with convex to concave epidermal cells, stomata paracytic.

Upper epidermal surface of cupulate cells shallow; anticlinal walls thick and raised. Secondary ornamentation of various striate to elongated, cristate. The lower epidermal cells comparatively larger shallow cupulate cells with thick and raised anticlinal walls. Secondary ornamentation more per cell.

J. integerrima

Upper petal epidermal cells of various sizes, cupulate, shallow with thick and raised anticlinal walls. Secondary ornamentation of striate to cristate. Lower epidermal cells shallow, comparatively deep, cupulate with thick and raised anticlinal wavy walls.

J. glandulifera

Upper petal epidermal cells reticulate, cells various, irregularly arranged. Lower epidermal cells linear to fusiform, raised with ridges and furrows.

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J. multifida

Upper petal epidermal cells reticulate, rectangular to linear with raised anticlinal walls. Epidermal cells dimorphic, lower epidermal cells reticulate, cupulate of various sizes with thick raised anticlinal walls, Secondary ornamentation irregular.

J. podagrica

Upper petal epidermal cells papillose, cells club shaped in linear rows with conspicuous bulbous head. Lower epidermal cells also papillose but comparatively smaller than the upper epidermal cells but more dense.

J. tanjorensis

Upper petal epidermal cells reticulate, with raised anticlinal walls. Cluster of many long ribbon like hairs arise at the basal region of the petals. Hairs pointed at tip and devoid of ornamentation. Lower surface not scanned.

J. curcas

Upper petal epidermal cells reticulate. Cluster of long hairs arise on the lower and middle region of petals, hairs broad and flat (ribbon shaped) with blunt tips and ornamented with verrucose striations (Dehgan 1980).

SEM observations supplement the LM observations described earlier. It may be noted that the hairs present on petal surface confirm Dehgan (1980) and noted the epidermal hairs on leaf in sub-genus *Jatropha* to be smooth and in *Curcas* verrucose. The SEM data on petal epidermal morphology such as hairs and their ornamentation appear to be reliable in *Jatropha* taxonomy but more studies representing various sections is desirable.

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Chapter 11
Economic and Medicinal Importance of Jatrophas

T. Pullaiah and Bir Bahadur

Introduction

Jatropha is a genus of approximately 175 species. The name is derived from the Greek words '*jatros*' meaning "physician", and '*trophe*' meaning 'nutrition', hence the common name physic nut. As with many members of the family Euphorbiaceae, *Jatropha* contains several compounds that are highly toxic. But detoxified seeds can be used for the animal feed. *J. curcas* came into prominence in recent years for its oil which is used as biodiesel. In 2007, Goldman Sachs cited *J. curcas* as one of the best candidates for future biodiesel production. Although there are 175 species of *Jatropha*, only a limited (less than 40 species), are used by man for various purposes. This shows that there is an urgency to explore the medicinal and other uses of as many species as possible since several *Jatropha* species are reported to be endangered and endemic.

In this paper we present the economic and medicinal importance of various *Jatropha* species both wild and cultivated. Various medicinal properties (from different parts of the plant, root, stem, bark, leaf and seeds) have been attributed to most species of the genus *Jatropha.* For instance, the fresh latex of many species belonging to this genus is used in folk medicine for the treatment of mouth blisters (Flores and Ricalde 1996), pimples (Zamore-Martinez and Pola 1992), and scabies (Manandhar 1995). Similarly leaf infusions are used to treat ulcers (Gupta et al. 1996), infected wounds (Giron et al. 1991) and diarrhea (Coee and Anderson 1996). Finally, both leaves and seeds of some *Jatropha* spp. are used as laxatives (Klinar et al. 1995). A number of biological activities have been detected in natural products from *Jatropha* spp., these include antimicrobial (Naengchomnong et al. 1986) and both antitumor

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and cytotoxic activities (Evans and Taylor 1983). Adewunmi and Marquis (1980) and Amin et al. (1972) reported molluscicidal activity of *Jatropha* species.

Medicinal Importance of *Jatropha* Species

Jatropha curcas L.

The seeds yield oil, which is a renewable source of energy and is often used for burning lamps and candle-making, which also has potential for commercial use as a lubricant. It is an efficient substitute for diesel, kerosene, LPG, furnace oil, coal and fuel wood. It has been reported by Datta (1987) that West German researchers working for the Eschborn-based foreign aid agency GTZ, have discovered in 1985, that the oil from the seeds of this "Barbados nut" can be used for fuelling internal combustion engines. At the end of 1986, the firm unveiled the first working nut-oil engines driving a generator in Cape Verde, Senegal, providing power for an electric water pump and ice-making machine. Seed oil is also used for manufacturing candles, varnishes, illumination without soot and lubrication (Sardana 1998).

The oil from seeds may be toxic or non-toxic depending on the variety used. The oil has assumed great importance as an alternative, renewable fuel. For more information the reader may refer the report : SWAFEA formal report D.21 v2 (WP2000_USFD_25/03/2010), State of the Art for Alternative Fuels and Energy Carriers I Aviation, European Commissions Directorate General for Mobility and Transport, Brussels, Accessed from (http://www.swafea.eu/LinkClick.aspx?fileticket=e54MoDYRbPs%3D and tabid=77) on 2.2.2012.

The seed resembles the castor seed and hence called as "wild castor". Different parts of the plant are useful for various purposes with many medicinal values is too well known and several publications are available. The seeds possess toxic and purgative properties because of which it is called by a common name "Purging Nut". The seed cake contains bitter and toxic principles and is unfit for use as cattle feed, yet the same cake is rich in nitrogen and phosphorous, which makes it a potential biofertilizer (Anonymous 2001).

The biopesticidal activity of *J. curcas* seed in controlling the pests, *viz.*, *Helicoverpa armigera* and adult white fly (*Bemicia tabaci*) was studied by Aravinda et al. (2009) under controlled conditions.

J. curcas plants are rich sources of bioactive compounds that can be used to develop environmentally safe pest-managing agents. *J. curcas* seed is a good source for the production of biodiesel and several tonnes of seed are utilized for this purpose during which large quantities of cake is produced which can be utilized as a source to produce good pesticide extract because the seed contains curcin, a toxalbumin.

J. curcas is a multipurpose tree, and all its plant parts can also be used for human food, animal feed, fertilizer, fuel and traditional medicine. *J. curcas* seed cake is a

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low-value by-product obtained from biodiesel production. The seed cake, however, has a high amount of protein, with the presence of a main toxic compound: phorbol esters as well as anti-nutritional factors: trypsin inhibitors, phytic acid, lectin and saponin. Saete and Suntornsuk (2011) studied the toxic compounds, antinutritional factors and functional properties of proteins isolated from detoxified *J. curcas* seed cake. The results suggest that the detoxified *J. curcas* seed cake has the potential to be exploited as a novel source of functional protein for food applications.

Latex dries to a bright reddish-brown, brittle substance, resembling shellac and used as marking ink. A dye is extracted from the leaves. Bark yields a dark blue dye used for dyeing cloth, fishing nets, and lines; also contains tannin. Seeds yield oil, 'Curcas Oil', which has low viscosity, used for manufacturing candles, soaps, and varnishes; illumination (burns without soot) and lubrication; and in wool industry. Seed oil is used for manufacturing candles. The toxicity of *Jatropha* plant extracts from fruit, seed, oil, roots, latex, bark, and leaf, to a number of species, from microorganisms to higher animals, is well established. Broadly, these extracts possess molluscicidal, piscicidal, insecticidal, rodenticidal, antimicrobial, and cytotoxic properties, and exert adverse effects on animals including rats, poultry, and ruminants.

Devappa et al. (2010) reviewed the nutritional, biochemical and pharmaceutical potential of proteins and peptides from *Jatropha*. The nutritional qualities of the kernel meal and protein concentrates or isolates prepared from seed cake are presented, enabling their efficient use in animal nutrition. In addition, (a) biologically active proteins involved in plant protection, for example, aquaporin and betaine aldehyde dehydrogenase, which have roles in drought resistance, and beta-glucanase, which has antifungal activity, as well as those having pharmaceutical properties, and (b) cyclic peptides with various biological activities such as antiproliferative, immunomodulatory, antifungal, and antimalarial activity are discussed. It is expected that the information collated will open avenues for new applications of proteins present in *Jatropha* plant, thereby contributing to enhance the financial viability and sustainability of a *Jatropha*-based biodiesel industry.

Abd-Elhamid (2003) investigated the molluscicidal and in vitro schistosocidal activities of the extract of *J. curcas*. The acetonitrile extract gave high toxicity against snails with LC_{90} value of 6 ppm and chloroform extract gave low toxicity against snails with LC_{90} value of 55 ppm. The in vitro schistosomicidal activities of the acetonitrile extract of *J. curcas* gave 91.7% mortality after exposure to 100 ppm for 4 consecutive days and 58.3% mortality after exposure to 50 ppm for 5 consecutive days.

Phytoremediation

Soil contamination by used lubricating oil from automobiles is a growing concern in many countries, especially in Asian and African continents. Phytoremediation of this polluted soil with non-edible plants like *J. curcas* offers an environmental friendly and cost-effective method for remediating the polluted soil and coal fly ash

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and even dairy sludge and biofertilizers (Agamuthu et al. 2010; Jamil et al. 2009; Santosh et al. 2009).

Phorbol ester extraction was carried out from *J. curcas* seed cake, a by-product from the bio-diesel fuel industry (Saetae and Suntornsuk 2010).

Antifungal Properties

The ethanolic extract of *J. curcas* seed cake showed antifungal activities against important phytofungal pathogens: *Fusarium oxysporum, Pythium aphanidermatum, Lasiodiplodia theobromae, Curvularia lunata, Fusarium semitectum, Colletotrichum capsici* and *Colletotrichum gloeosporiodes*. The extract contained phorbol esters mainly responsible for antifungal activities. The extract could therefore be used as an antifungal agent for agricultural applications.

Pesticidal, Insecticidal and Larvicidal Activity

J. curcas, known for its insecticidal properties, affects the insects of various families, and its ingestion inhibits the growth of several Lepidoptera species (Sauerwein et al. 1993). Its contact toxicity was described by Solsoloy (1993). The methanolic extract from *J. curcas* has insecticidal activity against *Helicoverpa armigera* (Lepidoptera) and *Sitophilus zeamais* (Coleoptera) (Solsoloy 1995), and *Culex quinquefasciatus* (Diptera) (Karmegam et al. 1997). Rahuman et al. (2008) reported the petroleum ether extracts of *J. curcas* showed larvicidal activity. Sakthivadivel and Daniel (2008) reported that petroleum ether extract of *J. curcas* showed insecticidal activity against mosquitoes. Other report on insecticidal activity include Acda (2009).

Biogas

Staubmann et al. (1997) studied the biogas formation from *J. curcas* press cake using a semi continuous upflow anaerobic sludge blanket reactor. However, the UASB reactor and the contact-process were not suitable for using this substrate. When using an anaerobic filter with *J. curcas* seed cake as a substrate, 76% of the COD was degraded and 1 kg degraded COD yielded 355 L of biogas containing 70% methane.

Medicinal Uses

Prasad et al. (2012) gave a detailed review of the medical benefits of *J. curcas*. According to Hartwell, the extracts are used in folk remedies for cancer. Reported to be abortifacient, anodyne, antiseptic, cicatrizant, depurative, diuretic, emetic, haemostatactagogue, narcotic, purgative, rubefacient, styptic, vermifuge, and

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vulnerary, physic nut is a folk remedy for alopecia, anasorca, ascites, burns, carbuncles, convulsions, cough, dermatitis, diarrhea, dropsy, dysentery, dyspepsia, eczema, erysipelas, fever, gonorrhea, hernia, incontinence, inflammation, jaundice, neuralgia, paralysis, parturition, pleurisy, pneumonia, rash, rheumatism, scabies, sciatica, sores, stomachache, syphilis, tetanus, thrush, tumors, ulcers, uterosis, whitlows, yaws, and yellow fever (List and Horhammer 1979). It is also used traditionally for the treatment of sciatica, dropsy, paralysis, rheumatism, dysentery, diarrhea and certain skin diseases (Anonymous 2001; Dymock et al. 1976; Nadkarni 1976; Duke 1985; Agarwal 1986; Ambasta 1986). Latex is applied topically to bee and wasp stings (Watt and Breyer-Brandwijk 1962). Mauritians massage ascitic limbs with the oil. Cameroon natives apply the leaf decoction in arthritis (Watt and Breyer-Brandwijk 1962). Colombians drink the leaf decoction for venereal disease. Bahamans drink the decoction for heart burn. Costa Ricans poultice leaves onto erysipelas and splenosis. Guatemalans place heated leaves on the breast as a lactagogue. Cubans apply the latex to toothache. Colombians and Costa Ricans apply the latex to burns, hemorrhoids, ringworm, and ulcers. Barbadians use the leaf tea for marasmus, Panamanians for jaundice. Venezuelans take the root decoction for dysentery. Seeds are used also for dropsy, gout, paralysis, and skin ailments (Watt and Breyer-Brandwijk 1962). Leaves are regarded as antiparasitic, applied to scabies; rubefacient for paralysis, rheumatism; also applied to hard tumors. Latex is used to dress sores and ulcers and inflamed tongues (Perry 1980). Seed is viewed as aperient; the seed oil emetic, laxative, purgative, for skin ailments. Root is used in decoction as a mouthwash for bleeding gums and toothache. Otherwise used for eczema, ringworm, and scabies (Perry 1980). Thomas et al. (2008) have in their recent mini review discussed the therapeutic biology of *J. curcas.*

Branches of *J. curcas* are used as a chewing stick in Nigeria (Isawumi 1978). Badgujar et al. (2008) reported that small stem of *J. curcas* is used as tooth brush to cure Pyorrhea and tooth ache. Singh et al. (1984) reported that an infusion from the leaves of *J. curcas* is used in the treatment of vaginal bleeding by the locals in the island of Tongga. Members of rural communities of Churu district in the Thar desert, India, used the juice from leaves to cure diseases such as dysentery and colic (Parveen et al. 2007). The leaves were also applied to the breast to promote lactation (Parveen et al. 2007). In Southeast Asia and in some regions of Africa, the leaves are used as purgative, while in the Philippines and Cambodia the leaves are applied to wounds (Staubmann et al. 1999). In Cape Verde and Cameroon, a decoction is used internally and externally against fever. In Cameroon, the leaves are also used as the remedy against rheumatism and in Nigeria against jaundice (Staubmann et al. 1999). Thomas (1989) investigations turned out that the leaves are also active in lymphocytic leukemia. Fagbenro-Beyioku (1998) investigated and reported the anti-parasitic activity of the sap and crushed leaves of *J. curcas.* In Mali, the leaves are used as treatment for malaria (Henning 2003). The leaves are used extensively in West Africa ethnomedical practice in different forms to cure various ailments like fever, mouth infections, jaundice, guinea worm sores and joint rheumatism (Irvine 1961; Oliver-Bever 1986).

In Egypt, the seed is used for the treatment of arthritis, gout and jaundice (Khafagy et al. 1977). The seed of this plant has also been used traditionally for the

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treatment of many ailments including burns, convulsions, fever and inflammation (Osoniyi and Onajobi 2003). The seed oil can be applied to treat eczema and skin diseases and to sooth rheumatic pain (Heller 1996). In South Sudan, the seeds as well as the fruits are used as a contraceptive or as abortifacient (List and Horhammer 1979). Jain (1991) reported that *J. curcas* root is used as abortifacient, latex is used for burns, and curing cancer, leaf is used in congestion of chest and inflammation, seed is used as purgative, in rheumatism, stem is used in sores, tooth troubles, wounds, bark is used for black dye, oil for lighting, twig is used as tooth brush. Tender twigs are used for tooth brushing in swollen gums and pyorrhea in India, Nepal, S. America, and S. Africa; for teething trouble in children in Sudan, Africa.

J. curcas is used to cure skin diseases and related problems in Northeastern India (Begum and Nath 2000). Acharya and Rai (2012) reported that seeds of *J. curcas* are used to cure Cholera and dysentery, good for stomach disorders, cures tooth ache and gum ache. Seeds are used as antidote for poisoning. It is effective in skin diseases and rheumatism.

Wound healing property of curcain, the protease isolated from the latex of *J. curcas* was determined in mice by Nath and Dutta (1991, 1992, 1997). Two ointments of curcain were prepared by incorporating 0.5% and 1.0% (w/w) of curcain powder into the washable ointment base. Healing of the wound by the curcain ointments was found to be better than nitrofurazone ointment and propamidine isethionate cream in mice. The drug obtained from *J. curcas* is termed as "Dravanthi" and is reported to be bitter, pungent and astringent in taste. It has anthelmintic properties. It works as cleaner for all the impurities through its purgative properties. Besides this, it has been found beneficial in chronic dysentery, thirst, urinary discharges, abdominal complaints, anaemia, fistula, ulcer and diseases of heart and skin. The leaves are considered to be rubefacient and lactogogue. Seed oil is used externally for rheumatism and paralytic affections. The milky sap is used for the treatment of different dermatomucosal diseases such as, gingivitis, wounds, hemorrhoids, herpes, sores, burns, ulcers and warts.

The plant is used in whitlow, dropsy, anasarca, convulsions, syphilis, neuralgia, pleura, and pneumonia. EtOH (50%) extract of aerial parts, CNS depress and diuretic. In Sri Lanka the species is used in healing fractures.

The leaves are galactogogue, rubefacient, suppurative and have insecticidal properties, and are useful in treatment of foul ulcers, tumours and scabies. Leaves are used in the form of decoction, and cataplasm to the mammae as a lactagouge, rubifacient.

Juice of plants is used in scabies, eczema and ringworm affections. The juice may be a source of beta blocker against cardiac diseases. Latex is used for pyorrhea, burns, athlete foot and rheumatism. The latex is styptic, purgative and haemostatic and is good for wounds and ulcers.

Curcin, the toxalbumin, which is one of the major proteins in the *J. curcas* seed, is similar to Ricin and Abrin proteins of *Ricinus communis* and *Abrus precatorius,* respectively in function, i.e., in inhibiting protein synthesis in whole cells (Lin et al. 2003) and showed antitumor properties.

Nayak and Patel (2010) reported that the alcoholic extract of root, stem and leaf exhibited systemic and significant anti-inflammatory activity in acute

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carrageenan-induced rat paw edema. From all the alcoholic extracts, the root showed significant reduction in percentage of inflammation over the other alcoholic extracts.

Leaves are used internally for jaundice; decoction antidiarrhoeal; in stomach ache and in cough, 10% aq. infusion, increased cardiac contraction in small doses, used against warts and cancers. EtOH extract of defatted leaves and twigs are active in vivo and in vitro against P-388 lymphocytic leukemia; also tumour-promoting antileukemic diterps.

Bark paste mixed with asafoetida in butter milk cures diarrhea and dyspepsia in Konkan, Goa. Stem bark used in wounds of animal bites and bark decoction used in malaria and hemorrhoids in Peru. Root bark used in sores. Sap from bark is used to dress wounds and ulcers and can also be used to stop bleeding.

The yellow oil obtained from roots has strong anthelmintic action and used as antidote to snake bite. The seed kernels give fatty oil, 2 phytosterols, a phytosterol-ing (glucoside of phytosterol), large amounts of sucrose and resinous matter having nauseating, purging, griping effect and disagreeable taste.

The seeds are powerful purgative, acid, sweet, aphrodisiac, thermogenic, digestive tonic, anthelmintic and depurative. They are useful in hemorrhoids, wounds, splenomegaly and skin diseases. Seeds contain fixed oil, myristic, stearic and probably petroselenic acid, tannin, glucose, polysaccharides and a resinous substance. The oil is used in sciatica dropsy, and externally for skin troubles, rheumatism; and also abortifacient. The oil from *J. curcas* seeds is used in helping with rashes and parasitic skin diseases. The oil has insecticidal activity against larvae of *Manduca sexta* and also ovicidal against *Phytorimacea operculalia* a severe pest of potato.

Shetty et al. (2006) reported wound healing activities of crude bark extract of *J. curcas* in albino rats. The methanolic fraction of leaves of *J. curcas* was evaluated by Balaji et al. (2009) against hepatocellular carcinoma induced by Aflatoxin B_1. The results suggest that methanolic fraction of leaves of *J. curcas* could prevent liver against the AFB-induced oxidative damage in rats, which may be due to its capability to induce the in vivo antioxidant system.

El Diwani et al. (2009) studied the antioxidant activity of ethanol and petroleum ether extracts from roots, stem, leaves and nodes of *J. curcas* by DPPH free radical method. The study revealed that the roots and stems were the strongest in all tests followed by leaves and nodes. Roots crude extract showed IC_{50}=0.521⁻mg ml^{-1}. The presence of phenolic compounds and tannins were screened in Folin-Ciocalteau assay, HPLC and X-ray diffraction studies.

Sawant et al. (2010) investigated the in vitro antioxidant activity of hydro alcoholic extract of leaves of *J. curcas* by using DPPH radical scavenging activity; nitric oxide radical scavenging activity, hydroxyl radical scavenging activity, reducing power method and hydrogen peroxide radical scavenging activity. Significant results were obtained in the estimated parameters and thus, concluded that the extract of leaves of *J. curcas* possess potential source of antioxidants of natural origin. Narayana Swamy and Balakrishnan (2011) evaluated the antioxidant activity of three medicinal plants including fruits of *J. curcas*. The aqueous extract showed 80y inhibition of DPPH radical while ethanolic extract showed 40 y inhibition of DPPH radical.

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Rug and Ruppel (2000) reported that *J. curcas* shows toxicity against intermediate snail hosts and larvae of schistosomes and could become an affordable and effective component of an integrated approach to schistosomiasis.

Jaikumar et al. (2010) investigated the effect of methanolic extract of leaves of *J. curcas* on pylorous ligation and Aspirin-induced gastric ulcers in Wistar rats. A significant dose dependent reduction in the acid parameters like gastric volume, pH, total acidity, total acid output, total proteins and ulcer index were observed after treatment with 100 and 200 mg of *J. curcas* extracts on pylorus ligation plus aspirin induced ulcers compared to the normal pylorus ligation rats. Histopathological examination of stomach mucosa showed the protective action of *J. curcas* extracts against mucosal epithelial damage caused by Aspirin.

The analgesic and anti-inflammatory effects of the methanolic extract of the leaves of *J. curcas* were investigated in mice and rats, respectively by Uche and Aprioku (2008). This effect was comparable to the observed effect with Piroxicam (0.5 mg kg^{-1}) which was used as a standard.

The in vitro antimicrobial activity of crude ethanolic, methanolic and water extracts of the stem bark of *J. curcas* were investigated by Igbinosa et al. (2009). The extracts exhibited antimicrobial activities with zones of inhibition ranging from 5 to 12, 8 to 20 and 0 to 8 mm for ethanol, methanol and water extracts, respectively. Phytochemical screening revealed the presence of saponin, steroids, tannin, glycosides, alkaloids and flavonoids in the extracts. The ability of the crude stem extracts of *J. curcas* to inhibit the growth of bacteria and fungi is an indication of its broad spectrum antimicrobial potential which may be employed in the management of microbial infections. Oskoueian et al. (2010) investigated the antioxidant, anti-inflammatory and anticancer activities of methanolic extracts of *J. curcas*. Latex of leaf extracts showed the highest antioxidant activity, root and latex extracts showed anti-inflammatory activities while root extract exhibited anticancer properties.

Oskoueian et al. (2011b) reported that methanolic and hot water extracts of kernel meal of *J. curcas* showed antimicrobial activity against both Gram positive and Gram negative pathogenic bacteria (inhibition range: 0–1.63 cm) at the concentrations of 1 and 1.5 mg $disc^{-1}$. Methanolic extract exhibited antioxidant activities that were higher than hot water extract and comparable to β-carotene. The extracts tended to scavenge the free radicals in the reduction of ferric ion (Fe^{3+}) to ferrous ion (Fe^{2+}). Cytotoxicity assay results indicated the potential of methanolic extract as a source of anticancer therapeutic agent towards breast cancer cells.

Acharya and Rai (2012) reported that seeds are used in curing cholera and dysentery. They also reported that it is effective in skin diseases and rheumatism. It cures toothache and gum ache.

Igbinosa et al. (2011) assessed the polyphenolic contents and antioxidant potential of the aqueous, ethanol and methanol stem bark extracts of *J. curcas*. The total phenol, flavonoids, flavonols and proanthocyanidin contents of the extracts were evaluated to determine their effect on the antioxidant property of this plant, using standard phytochemical methods. The antioxidant and free radical scavenging activity of ethanol, methanol and aqueous extracts of the plant were also assessed. The concentrations of different classes of phenolic compounds were higher in methanol and ethanol extracts compared to aqueous extracts. There was correlation between total phenol, total

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flavonoids, total flavonol and total proanthocyanidins, respectively. There were correlations between the amount of phenolic compounds and percentage inhibition of DPPH radicals scavenging activity of the extract (Igbinosa et al. 2011).

Ayurveda system of Medicine: **Vyagra Eranda –** Seed, seed oil used in worm infestations, ascites, constipation, oedema, leprosy, and snake bite poisoning.

Siddha system of Medicine**: Kaattamanakku (Ellamanakku) –** Root, leaf, seed and latex – Constipation, wounds, eczema, ulcers, piles, diseases of head, leucorrhoea, rat-bite poisoning, abdominal disorders and aphrodisiac.

Homeopathic system of medicine, *J. curcas* is used for the treatment of various diseases like colic, cramps, cyanosis either as pills or mother tinctures of low/high potency. For more information the reader may visit one – step on line store, Washington, Virginia, USA for further information Info@remedysource.comweb; (http://wwwremedysource.com).

J. elliptica (Pohl) Muell.-Arg.

Molluscicidal Activity

This species of north west Brazil is known for new molluscicidal natural products, the activity of the crude ethanol extract of the rhizome of *J. elliptica* was tested by Santos et al. (1999). The LC_{50} was 13.07 ppm. The fractionation and purification of the extract furnished jatrophone and a mixture of jatropholones A and B, as the main compounds. They were tested against the snail *Biomphalaria glabrata.* Jatrophone showed an LC_{50} of 1.16 ppm as a molluscicide and an LC_{50} of 1.14 ppm for the assay of egg mass, while the mixture of jatropholones A and B presented an LC_{50} of 58.04 ppm as a molluscicide and was not active against the second assay at a concentration up to 100 ppm.

Medicinal Uses

Jatrophone, a diterpene isolated from tubercle of *J. elliptica,* inhibited vascular and extra-vascular smooth and cardiac muscle preparation (Calixto and Santana 1987; Duarte et al. 1992; Silva et al. 1995).

The pharmacological effect of the hydroalcoholic extract of *J. elliptica* was analyzed in in vivo and in vitro models by Trebien et al. (1998). When given orally in mice, the extract showed a low acute toxicity (LD_{50} 5 $g\,kg^{-1}$). In a dose of 0.5 or 1 $g\,kg^{-1}$ p.o. the extract did not interfere with diuresis in the rat, but was found to be effective in blocking rat paw oedema induced by carrageenan and partially, serotonin-induced oedema. In the same dose, the extract failed to inhibit rat paw oedema induced by dextran and the increase of rat cutaneous vascular permeability caused by *Bothrops jararaca* venom, dextran, histamine, PAF-acether and serotonin. Preincubation of the isolated rat uterus and guinea-pig ileum with the extract (0.2–0.8 mg ml^{-1}), produced a concentration-related and non-competitive inhibition of contractions induced by acetylcholine and bradykinin. However, the extract was

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about two-fold more potent in inhibiting the contraction of both agonists in guinea-pig ileum than in rat uterine muscle. In rat aorta, the extract (50–100 μg ml^{-1}) caused a concentration-dependent inhibition of noradrenaline-evoked contractions, being about five-fold more potent when compared to the IC_{50} values obtained in rat uterus. Martini et al. (2000) compounds that inhibit the binding of (3 H) glutamate and (3 H) GMP-PNP in rat cerebral cortex membrane

J. glandulifera Roxb.

Medicinal Uses

J. glandulifera seed oil is used by Irular, the tribal people of Marudhamalai hills, Coimbatore, Tamil Nadu, in chronic ulcerations, foul wound, ring worm, rheumatism and paralysis. Plant juice is used to remove film from the eyes. Water extract of root is given to children suffering from abdominal enlargement (Senthilkumar et al. 2006). Root pounded with water is given to children suffering from abdominal enlargements; also purgative and reduces glandular swellings. Leaf extract and latex are used in warts and tumors. Aqueous extracts of the fresh sap possess emulsifying and get foaming properties. The gel obtained with benzyl benzoate is used as an application for skin diseases.

Seed oil is applied externally in rheumatism and paralysis (Trimen 1878). Juice of leaves is used as eye drops to cure ophthalmic diseases (Rama Rao 1914).

Callus extract of *J. glandulifera* showed over 50% inhibition of spore germination of *Fusarium oxysporum* and 70% inhibition of *Curvularia lunata* (Bahadur et al. 1997) while root extract showed partial response. Roots showed antibacterial activity against *Escherichia coli, Bacillus subtilis* and *B. polymyxa.* Comparative studies using extracts from stem, leaves, roots and callus in eight *Jatropha* species was studied by them.

Seeds yield a fixed oil about 20–22% which is used as purgative, also applied externally in rheumatism, paralytic affections and ulcerations, foul wounds and ringworm. Protein extracted from the oil cake may be used as a raw material for plastics and synthetic fibre. The oil from seeds possesses purgative properties.

J. gossypiifolia L.

Protein extracted from the oil cake may be used as a raw material for plastics and synthetic fibre.

Insecticidal Activity

Phowichit et al. (2008) evaluated the insecticidal activity of *J. gossypiifolia* leaf extracts against *Spodoptera litura*. The extract at 3,000–10,000 ppm had significant

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toxicity with LC_{50} of 6.56 mg ml^{-1} at 24 h after exposure. Bullengpoti et al. (2012) reported antifeedant activity of *J. gossypiifolia* senescent leaf extracts on *Spodoptera frugiperda*. Seed extract is active against stored grain pests.

Medicinal Uses

Jain (1991) reported that *J. gossypiifolia* seed is used in body pain, leaf in cold, stem for toothache, root in veterinary bone fracture, dry seed in incense. Saiprasad Goud and Pullaiah (1996a) reported that tribals in Kurnool district of Andhra Pradesh use latex of *J. gossypiifolia* for curing syphilis.

The dried stem bark of the plant contains an intensely bitter amorphous alkaloid, Jatrophine, which is similar to quinine in properties. Badgujar et al. (2008) reported that small stem of *J. gossypiifolia* is used as tooth brush to cure tooth ache. Leaf, stem and root extracts of *J. gossypiifolia* showed over 50% inhibition of spore germination of *Fusarium oxysporum* and callus extract showed 70% inhibition of *Curvularia lunata* (Bahadur et al. 1997). Young shoots ground into a paste and applied on itching skin, eczema and allergic rashes. The roots are used in leprosy. Boiled leaves are applied to boils, carbuncles and itches. Seed-oil cures toothache and body pain (Jain 1965). The tribes of Sriharikota island of Andhra Pradesh chew the leaves for mucous infection in mouth and apply leaf juice in eczema and scabies (Suryanarayana et al. 1999). The fruits and seeds are poisonous, causing severe vomiting. If consumed lemon juice is the antidote.

The roots are employed against leprosy. A decoction of the bark is an emmenagogue. The leaves are used to cure stomachache, venereal diseases and as blood purifier. Seed oil is used to cure leprosy. Ethanolic extract showed hypotensive and vasorelaxant effects (Abreu et al. 2003).

A recent survey of medicinal plants from Togo, found that extracts of *J. gossypiifolia* have activity against schizonts isolated from volunteers with *Plasmodium falciparum* malaria (Gbeassor et al. 1989).

Seed yields a fixed, purgative oil used in sciatica, body pains, toothache, dropsy, paralysis and externally for skin troubles and rheumatism; also abortifacient, emetic. Juice of plants used in scabies, eczema and ringworm affections. Leaves are rubefacient and lactagogue. Leaves are employed as febrifuge in intermittent fevers; also used for swollen mammae; also applied to boils and carbuncles, eczema and itches. Decoction of bark used as emmenagogue.

Root, pounded with water is given to children having abdominal enlargement; it causes purging and reduces glandular swellings. Roots are employed against leprosy. Decoction of leaves is employed as a purgative and stomachic.

Latex is used for ulcers. Plant juice acrid; ether extract of shoots is reported to have antibiotic activity against *Escherichia coli*. Bark contains alkaloid jatrophine, which is similar to quinine in properties.

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Seed fatty oil is used in indigenous medicine, as an external stimulant, in rheumatism and paralytic affections; also in skin diseases. Seldom used internally although purgative. Dried residue to MeOH extract of fruit showed molluscicidal activity.

Dichloromethane and methanol (1:1) extract of *J. gossypiifolia* showed antibacterial and antifungal properties (Kumar et al. 2006). Santos et al. (2006) evaluated the use of raw extract of *J. gossypiifolia* in healing of skin wounds in rats. In an experimental study in rats, Servin et al. (2006) investigated the effects of *J. gossypiifolia* extract on the healing process of colonic anastomosis. The histological evaluation revealed an improvement of the acute inflammatory process.

The stem latex of *J. gossypiifolia* is routinely used by local and some urban dwellers in Southern Nigeria to stop bleeding from nose, gum and injured skin. Safety of its use was investigated by Oduola et al. (2007) in different groups of wistar albino rats using different doses of the latex. Different numbers of incisions were made on the thighs of different groups of animals and different doses of the stem latex applied. The procedure was repeated on daily basis for 18 days. The control group was not incised. The animals were sacrificed and the effect of the stem latex on blood biochemistry and haematology were assessed using standard techniques. The findings of this study showed that the stem latex of *J. gossypiifolia* has no harmful effects.

Povichit et al. (2010) investigated phenolic content and free radical scavenging effect of *J. gossypiifolia* by DPPH and ABTS assays, anti-lipid peroxidation activity by TBARS and for antiglycation activity. The results revealed that the total phenolic content showed good correlation with free radical scavenging by ABTS and antilipid peroxidation by TBARS, but showed correlation with antiglycation. The extract of *J. gossypiifolia* demonstrated a moderate antioxidant effect and also showed a moderate antiglycation effect. The IC_{50} were 1.45 mg ml^{-1} for the DPPH method, TEAC value of 0.42 mg/Trolox/mg sample for the ABTS method, IC_{50} of 0.42 mg ml^{-1} for the TBARS method and IC_{50} of 8.40 for the antiglycation method.

Panda et al. (2009) investigated the anti-inflammatory and analgesic effect of methanolic and petroleum ether extracts of dried aerial parts of *J. gossypiifolia.* The methanolic and petroleum ether extracts significantly reduced carrageenan-induced paw edema in rats and analgesic activity evidenced by increase in the reaction time by eddys hot plate method and tail-flick method in albino mice. The methanolic and petroleum ether extracts showed greater anti-inflammatory and analgesic effect comparative to the standard drugs, indomethacin and diclofenac sodium, respectively.

Biradar and Ghorband (2010) studied the ethnomedicinal wisdom of tribals of Kinwat forest of Nanded district of Maharashtra in India. About 12 drops of latex of *J. gossypiifolia* mixed with 100 g jaggery is given thrice daily for 3 days to cure typhoid.

Narayana Swamy and Balakrishnan (2011) evaluated the antioxidant activity of 13 medicinal plants including leaf of *J. gossypiifolia*. Aqueous extract showed 70% inhibition of DPPH radical while ethanolic extract showed 90% inhibition of DPPH radical. Oskoueian et al. (2011a) reported that extract of root and latex of *J. gossypiifolia* plant which contained phenolics, flavonoid and saponins showed notable antioxidant, anticancer and anti-inflammatory activities. These compounds

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have been reported to be involved in biological activities (Balasundram et al. 2006).

Ayurveda: Viagra Eranda (substitute.) – Part used and uses are similar to **Vyagra Eranda** (*J. glandulifera*).

Siddha: Kaatamanakku (Eliamanakku) – Root, leaf, seed, latex – constipation, wounds, eczema, ulcers, piles, diseases of head, leucorrhoea, rat-bite poisoning, abdominal disorders, aphrodisiac.

J. heynei Balakr. (Syn.: *J. heterophylla)*

Leaf juice is applied to heal fresh cuts and wounds. The tuberous rhizomatous roots are used as fodder in Mahaboobnagar district of Andhra Pradesh (Dharmachandra Kumar and Pullaiah 1999). Phytochemical constituents of root contain saponin and steroid (triterpenoids) while the aerial parts contain alpha sitosterol, vitexin, isovextin, quercitrin 3-galactoside (Lakshmi et al. 1975).

J. integerrima Jacq.

Spicy jatropha (*J. integerrima*) is cultivated as an ornamental in the tropics for its extended blooming of crimson flowers. Latex is toxic, leaves if chewed cause squeamish, somatalgia and purgative. Two new cyclic hepatopeptides diterpenes, integerrimides A and B both at 50 μM inhibited proliferation of human ICP-298 melanoma cells and pancreatic carcinoma cells. The roots contain rhamofolane endoperoxide 2-epicaniojane together with caniojane and integerrine are possible biogenetic precursors (see Carlsblander Blog 2010).It is antiplasmodial and cytotoxic. Root extract of *J. integerrima* showed over 70% inhibition of spore germination to fungus *Curvularia lunata* (Bahadur et al. 1997).

J. isabelli Muell.-Arg.

Jatrophone isolated from *Jatropha isabelli* exhibited antiprotozoal activity (Schmeda-Hirschman et al. 1996). A new jatrophone derivative, jatrophone, jatropholone A and jatropholone B, acetyl aleuritolic acid, cyperenoic acid and a monoterpene were isolated by Pertino et al. (2007) from the rhizomes of the Paraguayan crude drug *J. isabelli*. The compounds were characterized by spectroscopic means. The gastroprotective effect of jatrophone, jatropholone A and B as well as 9 beta, 13 alpha-dihydroxyisabellione and the triterpene was assessed in the HCl/EtOH-induced gastric lesions model in mice. Jatrophone elicited a strong gastroprotective effect with no significant differences between 25, 50 or 100 mg kg^{-1} and reducing

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lesions from 88% to 93%. The jatropholones A and B showed remarkable differences in the gastroprotective assay. Jatropholone A presented a dose-related response, with maximum effect (54% lesion reduction) at the highest dose (100 mg kg^{-1}), jatropholone B showed a strong action at all the doses, reducing lesions by 83–91%. The cytotoxicity of the compounds was assessed towards fibroblasts and AGS cells. Jatrophone was toxic against both cell lines (IC_{50} values: 2.8 and 2.5 μM, respectively). Jatropholone B was not cytotoxic while jatropholone A (4) displayed a selective effect against AGS cells (IC_{50}: 49 μM). The relevance of stereochemistry in the biological effects is clear comparing the effect of jatropholone A and B against AGS cells, with IC_{50} values of 49 and >1,000 μM for the beta and alpha C-16 isomers, respectively. The results provide scientific support for the use of "*yagua rova"* as a gastroprotective and cytotoxic crude drug in Paraguayan traditional medicine (Pertino et al. 2007).

J. macrantha Muell.Arg.

This species grows in Peruvian Amazon. Local name is '*Huanarpo macho'* and is one of the most famous aphrodisiac for men. Traditionally used as a male sexual stimulant, libido enhancing and aphrodisiac; for erectile dysfunction; for renal and adrenal support, as a nervine to calm and support the central nervous system, as an antitussive (for coughs, asthma and bronchitis). Aqueous extract from stem/bark is commercially sold in the market, for more details the reader may refer (http;//.raintree.com/huanarpo-mach-extract.htm) see also Schultes (1987).

The effects of two Peruvian folk medicines, *Lepidium meyenii* and *J. macrantha,* on mouse sex steroid hormones and embryo implantation were investigated by Oshima et al. (2003). Progesterone levels increased significantly in mice that received *L. meyenii*, while testosterone levels increased significantly in mice that received *L. meyenii* as well as in those that received both *L. meyenii* and *J. macrantha.* However, there were no marked changes in blood levels of estradiol-17 beta or the rate of embryo implantation.

J. multifida L.

This is cultivated ornamental common to almost all continents with tubers. Jain (1991) reported that *J. multifida* seed oil is used as abortifacient. In Indo-China, a decoction of dried roots is given for indigestion and colic, and also as a tonic. The fruit is poisonous and causes vomiting and intense burning pain in the stomach; lime juice and stimulants are administered as antidote in case of poisoning. The leaves are used for scabies and as purgative. The latex of the plant is applied to wounds and ulcers.

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The seeds are one of the best among emetics and purgatives and the effect is stayed by a glass of urine (Rama Rao 1914). The latex can be used for treatment of wounds (Kosasi et al. 1914). The leaf paste is used externally to cure scabies.

Leaves are chewed for mucous infections in mouth and leaf-juice in eczema and scabies in Sriharikota island of Andhra Pradesh, India (Suryanarayana et al. 1999).

Narayana Swamy and Balakrishnan (2011) evaluated the antioxidant activity of 13 medicinal plants including flowers of *J. multifida*. The aqueous and ethanolic extracts showed antioxidant activity of 90% inhibition of DPPH radical.

Hexane, ethyl acetate, chloroform and methanol successive extracts of *J. multifida* yellow rootbark, red rootbark and rootwood effectively inhibited the growth of *Bacillus subtilis* and *Staphylococcus aureus* at concentrations of 200 µg/disk (Aiyelage 2001). The same author reported japodic acid, a novel aliphatic acid

The latex contains novel non cyanogenic cyanoglucoside, the multifidin, multifidol and its glucosides and therefore applied to wounds and ulcers. It shows antibacterial activity against *Staphylococcus aureus.* Saline and ether extracts of the shoots shows antibiotic activity against *Escherichia coli*.

J. multifida oil from the seeds is used in parts of Africa to treat parasitic infestations and rheumatism. The seeds, fruit and sap contain a chemical called curcin which can cause symptoms if ingested. Eating seed can cause symptoms in children (Barufa1964).

J. nana Dalz et Gibbs.

This species is endemic and endangered to Pune in Maharashtra, India and also reported to occur in Chhattisgarh, India. Locally called as *Kirkundi* in Pune, India; it is used in traditional medicine as anti-irritant in opthalmia. According to recent report the traditional healers of Chhattisgarh, India, have used juice of the plant as counter irritant in opthalmia. Few healers consider that it possesses unique medicinal properties and several uses. The traditional healers of Chhattisgarh use the latex in treatment of skin related trouble round the year as last hope when all remedies fail (*via* internet). It is used both externally and internally but the external use is preferred. This can be used alone or in combination with mustard oil. The oil is sold by various herb vendors in Chhattisgarh without disclosing the ingredients. The traditional healers of southern Chhattisgarh specialized in the treatment of different types of cancers, cancerous wounds with dramatic success use this oil (Traditional medicinal uses of less known Jatropha species Kirkundi (Jatropha nana) in Chattisgarh, India via internet). Rani and Kulkarni (2010) studied the phytochemical, antioxidant and antimicrobial activity against gram negative and gram positive microbes and even performed antimicrobial assays using seed oil, leaf and root extract and concluded that *J. nana* has potent antibacterial activity and source of new antibiotics. Phytochemical screening revealed the presence of bioactive principles, alkaloids, flavonoids, tannins, cardiac glycosides, anthraquinone and saponins.

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Rani et al. (2011) showed insecticidal properties in three *Jatropha* species, *viz., J. nana, J. glandulifera* and *J. gossypiifolia* using seed oil, root and leaf extracts in acetone (5, 10, 15, 20 mg ml^{-1}) against stored grain pests, *Corcyra cephalonica* and *Tribolium catanum.* The activity of seed oil was more and expressed as follows *J. gossypiifolia* < *J. glandulifera* < *J. nana;* the methanolic extract of leaf *J. glandulifera* < *J. nana* < *J. gossypiifolia* and root showed *J. glandulifera* < *J. nana* < *J. gossypiifolia.* Phytochemical confirmation by TLC/HPTLC showed the presence of alpha sitosterol.

The oil is good source of future energy (Rani and Kulkarni 2010) biodiesel and it has been suggested that this species should be crossed with *J. curcas* to obtain suitable hybrids that will be similar to *J. nana*, which helps in mechanical harvesting.

J. panduraefolia Andr.

This is also a common ornamental. Antioxidant activity of *J. panduraefolia* has been investigated by Adib et al. (2010) by DPPH assay. *J. panduraefolia* was found to have a moderate antioxidant activity. Methanolic extract of leaves showed free radical scavenging activity with IC_{50} value of 160 mg ml^{-1}, Hexane soluble fraction of methanolic extract was with IC_{50} value of 165 mg ml^{-1}, carbon tetrachloride soluble fraction of methanolic extract with IC_{50} value of 104 mg ml^{-1} and chloroform soluble fraction of methanolic extract had IC_{50} value of 91 mg ml^{-1}.

J. podagrica Hook.

Cultivated ornamental commonly called as '*Buddha belly'* is a succulent, gouty bonsai type with tuberous roots which are eaten after roasting. Varieties of most Asian countries have reddish orange flowers but those of Africa have yellow flowers. It would be interesting to cross these varieties and obtain hybrids to enhance the horticultural base of this species. Produces red dye, commonly used to tan leather in Mexico and south west united states.

Medicinal Uses

The plant is used as antipyretic, diuretic, choleretic, purgative and chewing sticks (Irvine 1961; Oliver-Bever 1986).

Japodagrin and japodagrone, two macrocylic diterpenoids possessing lathyrane and jatrophane skeletons, respectively, have been isolated from the root of *J. podagrica* by Aiyelaagbe et al. (2007b). Four other diterpenoids were also isolated from this plant. The compounds displayed antibacterial activity against some gram-positive bacteria.

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Decoction of roots is given for indigestion and colic. Seeds are purgative, emetic, contain a fixed oil used as an illuminant and as abortifacient. Leaves are purgative, also used in scabies. Roasted seeds are used for treating fevers and venereal diseases, but they are dangerously cathartic, used in criminal poisoning. Whole plant is used as a fish poison in Philippines. The fruit is poisonous to human beings especially children. The plant-extract has antibacterial activity.

The plant is locally medicinal. An amide alkaloid tetramethylpyrazine has been obtained from the stem of *J. podagrica* (Ojewole and Odebiyi 1981). Adewunumi and Odebiyi (1985) also noted biological activity was tested in rabbits and rats; antifungal and antimicrobial activities were also tested, schistosomicidal activity. MeOH extract of stem is antibacterial, Tetramethylpyrazine, pharmacological mechanism of hypotensive action of alkaloid both neuromuscular and cardiovascular. Anti feedant activity was noted with hexane methanol extracts (Aiyelaagbe et al. 1998).

Hexane, chloroform and methanol extracts of the rootwood and root barks of *J. podagrica* were studied for their antimicrobial activity against 18 organisms. All the extracts exhibited some broad spectrum antibacterial activity, at a concentration of 20 mg ml^{-1}. The hexane extracts were generally more active than the chloroform and methanol extracts. The hexane extract of the yellow rootbark was the most active of all the extracts and its activity was comparable to that of gentamycin but better with regard to the control of *Streptococcous aureus* and *Bacillus cereus.* Three of the extracts, hexane extract of the yellow rootbark and hexane and methanol extracts of the rootwood showed moderate antifungal activity against the yeast fungus, *Candida albicans* (Aiyelaagbe et al. 2000). Stem bark extract showed remarkable antibacterial activity (Bhaskarwar et al. 2008).

Latex of stem is used both internally and externally, as abortifacient also used over wounds and ulcers. Tetramethylpyrazine, an alkaloid isolated from stem of *J. podagrica* inhibited vascular or extra-vascular smooth muscle preparation, and possesses hypotensive activity after intravenous administration in anaesthetized rats or cats (Ojewole and Odebiyi 1981). Jopodic acid a novel aliphatic acid with insecticidal activity was isolated from *J. podagrica* (Aiyelaagbe and Gloer 2008).

J. tanjorensis Ellis et Saroja

Lodhas in India, apply latex to treat eczema, latex mixed with mustard oil in 2:1 ratio is employed as a cure for tongue sore of babies. Santals apply paste on scabies. It is useful in herbal medicine to cure hypertension and malaria. *J. tanjorensis* was probably introduced into Ile-Ife town of Southern Nigeria, West Africa for the purpose of treating diabetes and Malaria and hypertension in the town. It is commonly called there by various names; '*hospital too far'*, *catholic vegetable*, '*iyana-ipaja', 'lapalapa'* and grows as a common weed of field crops, road sides, disturbed places in the higher rainfall forests zones of Nigeria, West Africa (Idu et al. 2009; Adeboye and Olorode1999; Iwalewa et al. 2005). This species which is endemic to South India must have been smuggled out of India long ago as evident by its abundance

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there otherwise its long distance dispersal to African continent is impossible as it sets seeds very rarely. According to Olayiwola et al. (2004) the plant is routinely used as a common remedy against diabetes in the Nigeria. Phytochemical screening of *J. tanjorensis* leaves revealed the presence of bioactive principles such as alkaloids, flavonoids, tannins, cardiac glycosides, anthraquinones and saponins (Ehimwenma and Osagie 2007). According to Oboh and Masodje (2009) it is widely grown as fence in S. Nigeria and is commonly used as a source of edible leafy vegetable by *Eedo* people in Nigeria and as medicine for diabetes. It also exhibits low antioxidant and very low hemagglutination value. They also investigated the nutritional and antimicrobial value of leaves for their proteins, moisture, mineral content and antimicrobial activity. Aqueous extracts of leaves inhibited the growth of gram positive *Staphylococcus aureus* and gram negative *Escherichia coli.* Ethanolic extract of the leaves of *Jatropha tanjorensis* exhibited antimicrobial action (Sekaran 1998).

Olayiwola et al. (2004) showed that the infusion of leaves is taken orally for the treatment of diabetic symptoms. They also investigated the antidiabetic potential. To study this the hypoglycemic effect of EtOH/H_2O (1:1) leaf extract was evaluated in fasted and glucose loaded rats at the doses of 1 and 2 $g\,kg^{-1}$ in vivo while three fractions of the extract were assessed for their anti-diabetic potential in vitro to stimulate the release of insulin secretion from INS-I cells. Only 2 $g\,kg^{-1}$ of the extract possesses significant glucose lowering activity in glucose loaded rats while the insulin secretion ability in vitro was limited to the ethyl acetate fractions.

The authors noted that "the folklore/ethnomedical use of this plant for antidiabetic purpose may necessitate repeated dosing of the diabetic individuals with the plant/extract to offer needed protection".

Orhue et al. (2008) have recently investigated the hematological and histopathological studies of *J. tanjorensis* leaves in rabbits (both sexes) for a 5 week period to study the possible toxicity to liver and renal function tests by graded concentrations 0, 5, 10 and 25%. After the end of the study all the rabbits survived and showed no significant alteration in body weight in the treated animals. No indication of bone marrow function and no severe histopathologic indicator were recorded. Further very low neurotoxic effect with the ingestion of up to 25% of the plant was observed. There was significant increase in haemoglobin, hematocrit, platelets and platelet cell distribution within the female group. Akhigbe et al. (2009) studied the effect of ground leaves of *J. tanjorensis* of various concentrations to evaluate the biochemical and ultrasonic changes after 30 days of administration of crude extract in rabbits. They noted no difference in size and weight of heart. Renal function tests showed significant reduction of serum in male rabbits suggesting that *J. tanjorensis* powder interferes with filtration function of rabbits kidneys. Further ultrasonographic study showed reduction in size of liver indicating hepatic toxicity but without any changes in heart and spleen.

Recently Viswanathan and Ananthi (2011) have reported antimicrobial and anti-inflammatory activity from the leaves of *J. tanjorensis*. Phytochemical screening revealed the presence of bioactive principles, alkaloids, flavonoids, cardiac glycosides, tannins, anthroquinone and saponins (see Carlasbander Blog 2010).

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Omoregie and Osagie (2011) reported that *J. tanjorensis* leaves supplement the activities of some antioxidant enzymes, vitamins and lipid peroxidation in rats.

J. aconitifolia Mill.

A frost hardy species of central Americas, Guatemala and western Mexico, commonly known as '*Tuba Tuba*' is better known for its alkaloids. Boiled leaves are eaten by the Mayan people who domesticated it for its protein, vitamin, calcium and iron value, also used as an aid in parturition. Nnam and Ngwa (2009) studied the antianaemic effect on adult female rats using extracts of leaves. The authors recommended the leaves (food and medicine) of this species could be popularized and incorporated into the local diet to fight anaemia and improve micronutrient status of the people. This is also a biofuel species also called as Energy tree.

J. antisyphylitica Speg.

Medicinally important species, as the species name suggests used in the cure of syphilis.

J. chevalieri Beille

The latex contains a cyclic peptide, Chevalierin A, B, C, Chevalierin C has been shown to possess antimalarial activity (Baraguey et al. 2000, 2001).

J. cuneata Wiggins et Rollins

A Mexican species commonly used for basket making by Seri people of Sonoro desert, Mexico, California and Arizona. The stems of '*haat*' are roasted, split and soaked through an elaborate process. Secondary metabolite compounds from root extract contain 2-epi-atrogrossine two has antimicrobial activity. Rhamnoflone diterpene and 15-epi-4E jatrogrissidioew has no biological activity. Crude extract of leaf has triterpenes, alpha amyrin and beta amyrin with antioxidant activity (Sanchez-Medina et al. 2001).

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J. gaumeri Greenm.

The root extract showed antimicrobial activity (Sanchez-Medina et al. 2001). The methanolic extracts of roots and leaves of *J. gaumeri* showed antimicrobial and antioxidant activity, respectively (Can-Ake et al. 2004). The bio-assay guided purification of the root extract resulted in the isolation and identification of 2-*epi*--Jatro grossidione a rhamnofolane diterpene with antimicrobial activity and of 15-*epi*-4 *E*-Jatro grossidentadione, a lathyrane diterpene without biological activity. Similarly the bio assay-guided purification of the crude leaf extract allowed the identification of β-sitosterol and the triterpenes α-amysin, β-amyrin and taraxasterol, as the metabolites responsible for the antioxidant activity. Roots are used in treating snake bites by Yucatan people.

J. glauca Vahl

Methanol and chloroform extracts of plant of this Egyptian species is reported to possess molluscicidal activity (Al-Zanbagi et al. 2000).

J. mahafalensis Jum. et Perr.

The species is endemic to Madagascar, latex contains Mahafacyclin, a heptapeptide which has been shown to have antimalarial activity. The oil is used for illumination potential biodiesel species. The seeds are toxic (Baraguey et al. 2000, 2001).

J. maheshwarii Subr. et Nayar

Viswanathan et al. (2004) have reported the phytochemical constituents from stem, which include friedelin, epi-friendlinol, β-sitosterol, β-sistosterol-3-B-D-glucopyranoside. Recently, antimicrobial and anti-inflammatory activity of the leaves has been reported by Viswanathan and Ananthi (2011).

J. mollissima (Pohl) Baill.

Known commonly as '*Pinhao-bravo', 'Pinhao-de-purga'* in Mexico and Brazil. Albuquerque (2006) reported that latex of *J. mollissima*, either direct or diluted in water is used externally or drunk for curing snake poison. The latex contains

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Pohilinins A, B, C which are cyclic peptides and have been shown to possess antimalarial activity especially the C type (Guette et al. 1999).

J. spathulata (Ortega) Muell. Arg.

A Mexican species famous for its red dye, used for tanning leather.

J. zeyheri Sond.

This African species is medicinally important as it contains Jaherin, a new daphnane diterpenoid, reported to possess antimicrobial properties (Dekker et al. 1986).

J. unicostata Balf. f.

This is a medicinally important species endemic to Soqotraen region. The following sterols have been identified, capestrol, stigmasterol, sitosterol and stigmastanol. The latex contains keto steroids. Fraxetin and Luteolin were isolated from ethyl acetate fraction. Mothana (2011) studied anti-inflammatory, antinociceptive and antioxidant activity. They noted that carragenin induced edema can be reduced.

J. weddelliana Baill.

A shrubby species found in Calimorphic and dry soils of high lands bearing the pantanal of Mato Grosso de Sul, Brazil. Hexane extracts of roots contain a diterpene with type lathyrane skeleton named Jatrowedione isolated from stem extract of the plant (Brum et al. 1998). Epijatrgrossidenadion is antifungal and antibacterial.

J. cathartica Terran et Berland

This species is a caudiciform perennial that grows in Rio Grande plain of Texas and Mexico and in western Nigeria called as '*lapalapa pupa'*. Oladde and Oshodi (2007) studied the nutritional potential of this species and showed the species as nutritionally promising with high nutrient value crude fat 47.13%, crude protein 38.5% and rich in nitrogen, phosphorous, magnesium and potassium, and low antinutrient level and therefore good substitute for nutrition. The species is said to be underutilized (Oladde and Oshodi 2007).

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J. capensis (L.f.) Sond

A caudiciform succulent of S. Africa commonly called as '*False Beadstring'*. Medicinally useful as purgative often used in ethnoveterinary practice.

J. cardiophylla (Torr.) Muell.-Arg.

Commonly called as '*Sangre de Cristo',* phenolic extracts of seeds of this species has good antioxidant activity.

J. dioica Sesse ex Cerv.

A species of southern Texas and Mexico, commonly called '*Dragons blood'* or '*Leatherwood'; 'tlapelexpath'* by *Aztecs.* The root extract is medicinally useful to cure skin diseases by adding the extract in warm water and rubbing with towel soaked in it. Four diterpenes *viz.,* beta-sitosterol, Jatropholone B, epoxytrione (citlalitrione) and riolozatrione isolated from the root are antibacterial (Villarreal et al. 1988).

J. neopauciflora Pax

Mexican species, medicinally useful and used by people of San Rafael, Puebla (Canales et al. 2005). The leaf extract is used for skin eruptions, wounds, toothache while the latex is directly applied on the affected area in view of its antibacterial activity against *Bacillus subtilis* and *Staphylococcus aureus* (Garcia and Delgado 2006; Canales et al. 2005). This species has highest informant concenses factor value (Canales et al. 2005).

J. costaricensis Webster et Poveda

This is a rare endangered and threatened shrubby species endemic to Costa Rica. The latex has anticancerous property and also used as food, drugs, ornamental and other economic uses. The oil is useful and the plant is used for reclamation purpose also.

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J. grossidentata Pax et Hoffm.

This shrubby species known as *Caniroja* by Ayoreo Indians of central north Paraguayan, Chaco, use the powdered roots and smoked in *Shamanic practice*. Root extract in vitro is known to show antileishmanial activity at 10 μg ml^{-1}. Alpha diterpenes (rhanofolane, jatrogrossideone, caniojane) have been isolated from roots (Schmeda-Hirschman et al. 1972). It is reported to be antibacterial and antifungal, antiplasmodial and antiprotozoal. Jatrogrossidione isolated from *Jatropha grossidentata* showed antiprotozoal activity (Schmeda-Hirschmann et al. 1996).

J. ribifolia (Pohl) Baill.

The species *J. ribifolia* is shrub of north east Brazil and is grazed by goats causing considerable mortality, confirmed clinically as due to presence of poison in the plant parts also to humans (Pimentel et al. 2012). Medicinally it is anti-inflammatory (Agra et al. 2008). According to Lyra et al. (2011) it is a potential biofuel plant along with *J. mollissima* and work on pollen viability and in vitro germination, reproductive biology is being pursued in Brazil.

J. divaricata (Sw.) Pohl

The species is known for its new diterpenes namely Cleistanthol, Spruceanol which are cytotoxic and show antitumor activity (Denton et al. 2001).

J. platyphylla Muell.-Arg.

This is drought resistant shrubby plant and considered as “Gift of Mexico” to the world in view of its immense value not only for its non-toxic nutritious round yellow seeds commonly used by Lacacpaxa people of Mexico but for its rich proteins and energy, given to malnourished children. The seeds are roasted and eaten without any side effects. The species is grown commonly in tropics for its seed oil (60%). Makkar et al. (2011) have studied extensively the physical and chemical constituents including antinutritional factors of seeds.

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J. macrorhiza Benth.

A caudiciform succulent species with enormous tough root commonly called as 'ragged nettle spurge'; 'Arizona Desert Potato', common to Arizona, New Mexico and Kansas. The root is a strong purgative. It contains various antitumor agents which possess inhibitory activity to p.388 (3PS) lymphocytic leukemia test system (Torrance et al. 1976). These authors also isolated yet another antitumor agent namely Triterpene acetylaleurilolic acid" which also showed antitumor properties against P.388 leukemia (Torrance et al. 1977).

Conclusions

From the foregoing it is obvious that *Jatropha* species described above have been used for varied applications such as, nutritional, agricultural, pharmaceuticals, molluscicidal, leishmanicidal, antibacterial, insecticidal, antifungal, antiplasmodial, antitumor, gastroprotective, cytotoxic, etc. This paper gives a brief account of about 20% of the total species for the genus. In view of their great potential there is urgent need to study the various species which are traditionally used.

According to UNESCO (1998) there is an increasing reliance on the use of medicinal plants in many industrialized societies and development of many drugs and chemotherapeutics from such plants as well as from the traditionally used herbal remedies. We need to focus our interest on underutilized and less known plants in the current scenario of population explosion, fast depletion of natural resources, growing needs of society, exploitation of plants for economic and medicinal uses is more relevant now than ever before. Genetic diversity/biodiversity in some areas are underexploited and less utilized in certain pockets of different countries throughout the world, despite the fact that there exists a symbiotic relationship between them for drug discovery. We need to tap the potential of medicinal plants for their rich diversity and heritage in ethno-medicinal uses in various communities and societies (both the primitive and advanced as Schultes 1987 put it) across the world for long time in our history and for long time to come in future.

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Chapter 12
Genetic Diversity of *Jatropha curcas* in Southern Mexico

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Abbreviations

AFLP	Amplified fragments length polymorphism
AMOVA	Analysis of molecular variance
SAMOVA	Spatial analysis of molecular variance
RADPs	Random amplified DNA polymorphism
ISSRs	Inter simple sequence repeats
SSRs	Simple sequence repeats
SCAR	Sequence characterized amplified regions
RFLP	Restriction fragment length polymorphism

Introduction

Jatropha curcas L. is a plant of the family Euphorbiaceae known in the Mesoamerican region as *piñón*. Its seeds are toxic to mammals, yet they have various traditional uses, e.g., medicinal, which may have a history of at least two millennia in southern Mexico (Leonti et al. 2003). Although its main use has been as a living fence for farms, this species has gained economic importance in the last decade due to the extraction of seed oil for biodiesel production (Gubitz et al. 1999; Openshaw 2000; Pramanik 2003; Fairless 2007; Escobar et al. 2008). Several authors have highlighted the advantageous features of *J. curcas* over other oil seeds (Martinez-Herrera et al. 2012), particularly its rapid growth and development (Sujatha et al. 2005), its adaptation to soils with marginal fertility (Jones and Miller 1992; Heller 1996; Henning 1997; Carels

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2009; Behera et al. 2010), and its tolerance to water stress – which allows for growth in semi-arid sites (Henning 1997; Zhang et al. 2008; Abou-Kheira and Atta 2009; Maes et al. 2009). It also has the ability to control soil erosion and a potential for phytoremediation (Mangkoedihardjo et al. 2008; Kumar et al. 2008).

J. curcas is fast undergoing widespread cultivation (Achten et al. 2007; Fairless 2007), and it is projected for 2015 that there will be about 15 million hectares planted worldwide, mainly in southern Asia and Latin America (Renner and Zelt 2008). The foregoing information implies major technological challenges, especially when one considers that it is not a fully domesticated plant; (1) it lacks an agronomic process; (2) there is no selected genetic material; (3) there is no precise knowledge either of its productive potential or its phenology; and (4) the number of varieties remains unknown.

Parallel to the interest of economic use as a crop plant, is the scientific interest in the biology and ecology of the species, its genetic diversity, and the geographic origin of populations currently distributed in many parts of tropical America, Africa, and South Asia. Although there is no consensus about the origin of this species, researchers suggest that Mesoamerica (Mexico and Central America) is the center of origin (Heller 1996; Openshaw 2000; Ranade et al. 2008), while others postulate it to be in South America (Martin and Mayeux 1984; Basha and Sujatha 2009; Carels 2009). It is certain that Mesoamerica is the center of diversification of the genus *Jatropha* (Dehgan and Webster 1979; Martínez et al. 2002), and that germplasm from this region has been the source for the establishment of plantations in Africa and Asia (Heller 1996).

The study of genetic variation in populations, along with an analysis of phenotypic variation, are two research priorities for *J. curcas*; since together they would contribute to the identification of loci associated with quantitative traits of productive interest. Although there is a growing body of knowledge on the diversity of *J. curcas*, there remain two important informational gaps in the literature: (1) most reports focus on diversity of accessions or individuals (Basha and Sujatha 2007; Gupta et al. 2008; Ranade et al. 2008; Sun et al. 2008; Abdulla et al. 2009; Basha et al. 2009; Kumar et al. 2009; Subramanyam et al. 2009; Tatikonda et al. 2009; Ikbal et al. 2010; Pamidiamarri et al. 2010; Shen et al. 2010; Umamaheswari et al. 2010; Wen et al. 2010), leaving out studies with a focus on populations, and (2) mostly Asian accessions have been studied, although to a lesser degree also South American (Hartmann-Neto et al. 2006; Oliveira et al. 2006; Rosado et al. 2010) and African (Basha et al. 2009; Ambrosi et al. 2010; Ricci et al. 2012); consequently, similar studies are needed in regard to Mesoamerican germplasm. Moreover, little attention has been paid to the study of phenotypic traits, such as the fatty acid composition of seeds and flower morphology, as markers to estimate genetic diversity. Studies of these characteristics would allow the gathering of basic information that at the same time could be used for selection of genotypes for planting.

Studies performed on germplasm collected in the Old World and South America have found low diversity and a narrow genetic base (Sun et al. 2008; Popluechai et al. 2009; Rosado et al. 2010; Ricci et al. 2012). An understanding of the genetic diversity and population structure in the postulated center of diversification, Mesoamerica, will permit identification of genetic material useful for future

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improvement of the species. For example, it could allow the designing of crosses between genetically distant plant groups.

Taking into consideration that in southern Mexico, particularly in the state of Chiapas, there exists the greatest extension of land in Mexico under cultivation as traditional hedgerows of *J. curcas*, our research is focused on the study of diversity in populations of that region.

Importance of the Study of *J. curcas* Diversity in Centers of Origin/Diversification

Before discussing the importance of the centers of origin, we wish to mention some concepts of biogeography, which studies the natural distribution of living beings in time and space, considering the processes that gave rise to the distribution of *J. curcas* (Morrone et al. 1996). Within this discipline, the school that states the existence of centers of origin is called dispersalist, which apparently is contrary to the school of panbiogeography. Morrone (2002) mentions that the dispersalism assumes that species originate in specific centers, from which they scatter randomly across existing geographical barriers to colonize new areas. For its part, the panbiogeography is not interested in individual taxon, but in the joint analysis of different taxa to find common patterns of distribution, assuming a spread from a center of mass to all areas with favorable conditions, without crossing geographical barriers. The appearance of physical barriers dividing a biota leads to different evolutions of each of the separate parts of this biota, which is called vicarism (Rosen 1978). Likewise, panbiogeography is not interested in centers of origin, but in distribution of sites. Some studies tend to find the so-called "centers of evolutionary radiation," "secondary centers of evolution" or "centers of diversification", which are the areas where an important part of the evolution of a group occurred, although that may not necessarily coincide with the centers of origin.

Methodological approaches to find centers of origin have been heavily criticized and considered contradictory (Croizat et al. 1974; Bremer 1992; Contreras et al. 2001). There are several proposed ways to identify centers of origin, including: (1) areas of extraordinary taxonomic differentiation (this approach considers that diversity increases with time, so the oldest area or place of origin has a greater number of related species); (2) areas with the greatest abundance of individuals of the species (this approach assumes that there exists optimal conditions for the development of the species in the center of origin); (3) areas where the most recent or apomorphic species of a group is present (involves the displacement of the ancestral species to the periphery of the center of origin by apomorphic species); (4) areas where the most primitive or plesiomorphic species is present; and (5) areas where the oldest fossil of a group were recorded and registered.

The search for centers of origin of domesticated plant species follows different rules because they are the product of recent evolutionary processes (Erickson et al. 2005). Diversification in agricultural species is not caused by the rise of geographic

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barriers, but by human-mediated processes. In this case the history of individual taxon (those who were/are under domestication) is important (Poncet et al. 1998; Pujol et al. 2005). A methodological approach that could be useful to discover patterns and to understand processes of distribution of domesticated plants is the phylogeography. This discipline studies taxa at the infraspecific level, constructing phylogenetic hypotheses and relating them to the geographical distribution (Olsen and Schaal 1999; Contreras et al. 2001).

Importance of the study of plant diversity in centers of origin/diversification relies on the supposition that variation between and within populations is highest in these areas in comparison to sites where a species is exotic. Synthesizing, centers of diversity are places where the individuals of a species have had more time to grow, multiply and evolve by means of genetic changes to adapt to a wide variety of environments. A diversity center contains a large number of varieties of one cultivated crop (CONABIO 2006).

Important Considerations for the Study of *Jatropha* spp. in Mexico

A conceptual and propositional analysis (Ford 2000) has helped to identify axioms and to propose the principles of the present investigation. Axioms are statements that are assumed to be true and which form the basis of a study. However, they are drawn so that they can be challenged or supported by the research to be carried out. The challenge to an axiom is neither direct nor complete, but rather through the corresponding postulates and the course of more than one investigation (Ford 2000).

Based on a review of the literature, we propose three axioms to study the Mexican *J. curcas* diversity: (1) *J. curcas* is native to Mesoamerica, i.e., it is naturally occurring in this region; (2) *J. curcas* is a plant in the process of domestication; and (3) in the Mesoamerican region, *J. curcas* is propagated mainly by way of cloning.

The first axiom is based on a review of researches on the systematics and phytogeography of the genus *Jatropha* (Dehgan and Webster 1979; Dehgan 1984; Dehgan and Schutzman 1994) and in general of the family Euphorbiaceae (Wurdack et al. 2005; Martínez et al. 2002; Steinmann 2002). In these investigations it has been postulated that *J. curcas* is the most primitive or plesiomorphic species of the genus *Jatropha*, an idea that has been strengthened by recent research based on molecular data (Sujatha et al. 2005; Ganesh-Ram et al. 2008; Pamidiamarri et al. 2010). On the contrary, the work of Basha and Sujatha (2009) did not find *J. curcas* as the most primitive species of ten species analyzed. However, those molecular studies have been based in neutral non-adaptive markers, as RAPDs, ISSRs and AFLPs. Those marker systems are founded on genome sampling. In addition, the mentioned authors have used phenetic methods (UPGMA) to search for relationships instead of phylogenetic methods (i.e., parsimony). To elucidate phylogenetic relationships, one commonly uses markers obtained from DNA sequencing, such as chloroplast genes, 18S ribosomal RNA gene or internal transcribed spacers (ITS) of ribosomal genes (Ford-Lloyd and Painting 1996). Those genome regions are highly conserved

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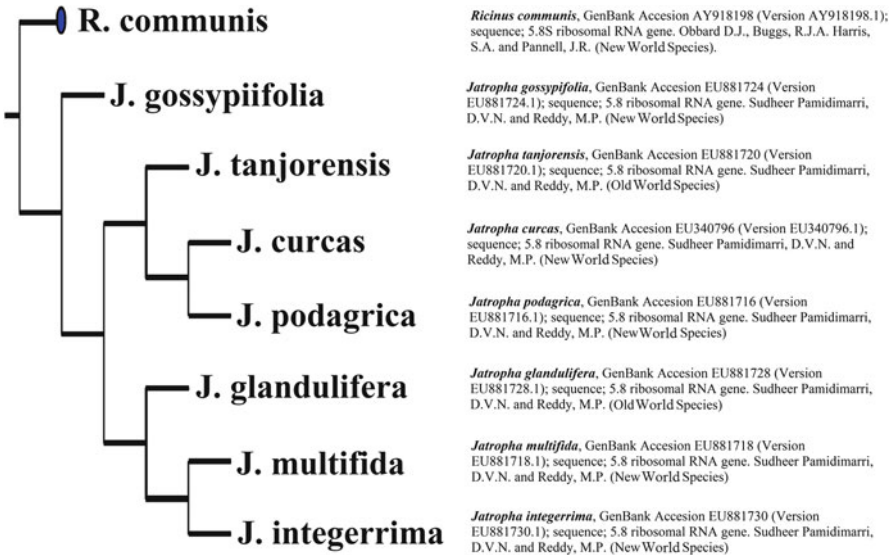

Fig. 12.1 Phylogenetic hypothesis of the ancestry of seven species of *Jatropha*, using *Ricinus communis* as sister species. The most parsimonious tree is shown, which was obtained from a heuristic search using the Nona© program (Goloboff 1999) and WinClada© 1.00.08 as interface (Nixon 2002). Data were 5.8S gene sequences obtained from GenBank©. Sequences were aligned with BioEdit© version 7.1.3.0 (Hall 1999). Length of the tree: 63 steps, consistency index: 0.88, retention index: 0.50. Tree constructed by José Alejandro Gómez Pérez (Centro de Biociencias, Universidad Autónoma de Chiapas, Mexico)

and differences in their sequences could imply evolutionary changes. Nevertheless, a work by Sudheer-Pamidimarri et al. (2009), using nuclear ribosomal DNA ITS sequence, found similar results to those obtained with dominant markers, but without establishing the phylogenetic relationships (because they used phenetic methods) among the seven species of Indian *Jatropha* investigated. Thus, we conducted a search for the most parsimonious tree of seven *Jatropha* species with *Ricinus communis* as sister species (the outgroup), using public sequences of the 5.8S ribosomal gene. This parsimonious tree did not confirme the plesiomorphism of *J. curcas* (Fig. 12.1). Unfortunately, only a few gene sequences available across several species of *Jatropha* have been published. This kind of comparative investigation will be now greatly facilitated by the online publication of the genome sequences of *J. curcas* by Sato et al. (2011). Thus, additional investigations of the molecular evolution of the genus, including species of *Jatropha* representing the majority of sections and sub-sections, both from the Old and the New World, are still needed.

It has also been found that the genus is divided into two sub-genera (*Curcas* and *Jatropha*) and ten sections (Dehgan and Webster 1979; Dehgan 1984). A fairly robust phylogenetic hypothesis is that the species of the sub-genus *Curcas*, and specifically of the section Curcas – to which *J. curcas* belongs – originated before the sub-genus *Jatropha* (Dehgan and Schutzman 1994). In this phylogenetic reconstruction the most plesiomorphic clade consisted of the species of section Curcas

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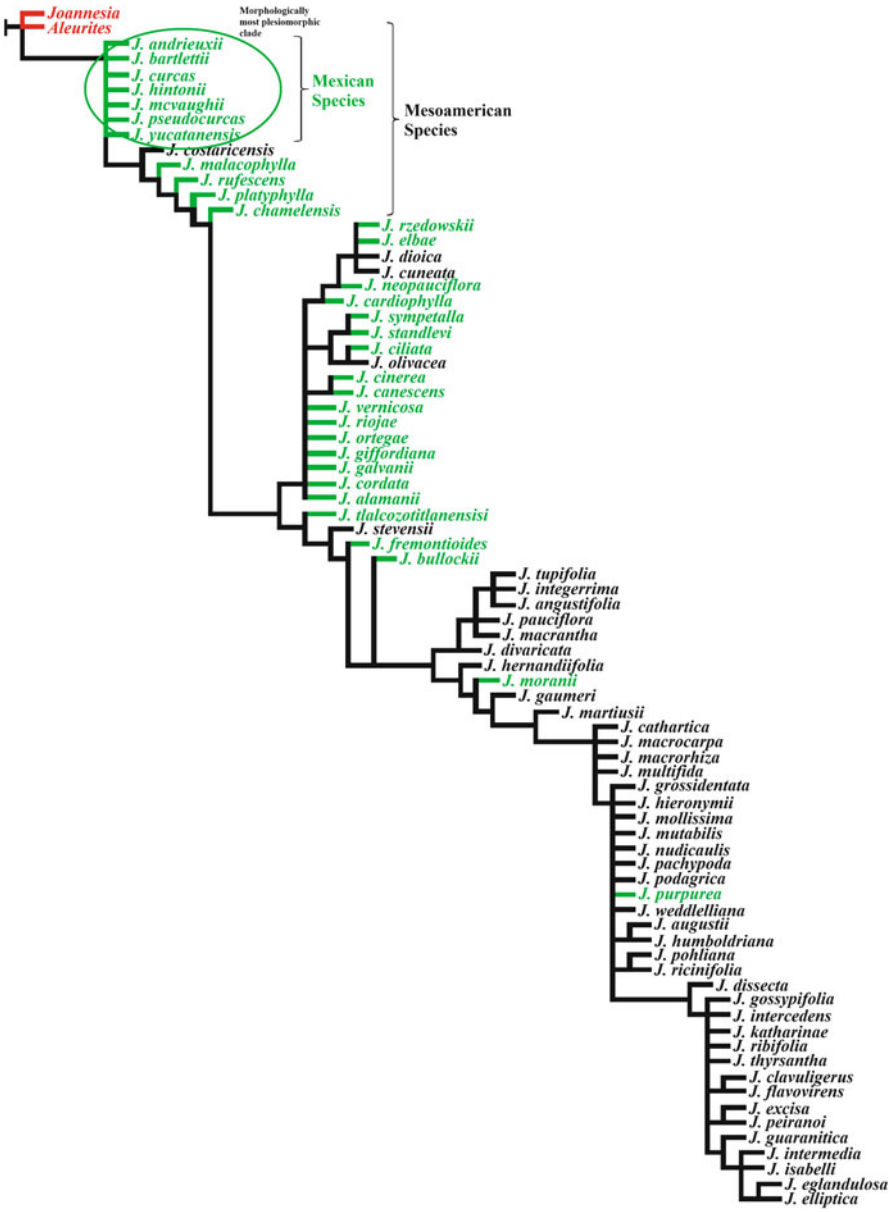

Fig. 12.2 Phylogenetic hypothesis of the ancestry of 77 species of *Jatropha*, using *Joannesia* and *Aleurites* as sister species, based in morphological characters. Tree modified from Dehgan and Schutzman (1984) in order to highlight the most plesiomorphic clade

(Fig. 12.2): *J. curcas*, *J. andrieuxii, J. bartlettii, J. hintoni, J. mcvaughii, J. pseudocurcas* and *J. yucatanensis*, the majority of which are endemic to Mexico, with all being endemic to Mesoamerica (two species of the section Curcas are from Africa:

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J. afrocurcas and *J. macrophylla*, and one is from India: *J. villosa*; Heller 1996). According to axiom 1, Mesoamerica is home to most wild relatives of *J. curcas* and is the center of diversification of the genus *Jatropha* (about 100–175 species are native to this region and in Mexico there are about 35–39 strictly endemic species; Martínez et al. 2002; Steinmann 2002). Palynological investigations of various exine features under SEM by Bahadur et al. (2000) confirms the primitiveness of *J. curcas*. However, the degree of relatedness between the endemic Mexican species of Curcas section still needs to be documented further.

With regard to the second axiom one can argue that, unlike other species of *Jatropha*, there are no populations of *J. curcas* in natural ecosystems. This plant is cultivated, both in America and in the Old World, as a living fence, and occasionally as a backyard plant (Anzueto and de MacVean 2000; Openshaw 2000; Jongschaap et al. 2007; Toral et al. 2008). In some cases plants have been found in different types of natural vegetation, but the authors that report it mention that it is probably derived from plants grown from seed that "escaped" from cultivated systems (Heller 1996). In field trips that we made on the southern Pacific coast of Mexico, *J. curcas* populations have not been found in the wild even if in some occasions, feral small populations have been registered (Fig. 12.3). This situation could be the indication of a past event of domestication of *J. curcas*, since in general, domesticated plant species are not reproductively successful without anthropic management (Elias and McKey 2000). Another symptom of domestication is the existence in Mexico of non-toxic genotypes, either cultivated or fomented, with complete absence or minimal levels of phorbol esters and other toxic molecules (Martínez-Herrera 2007; Martínez-Herrera et al. 2010; Parawira 2010). Non-toxic genotypes exist in various states of Mexico, although most of the germplasm is toxic (Ovando et al. 2009). However, the *J. curcas* does not show the so-called "domestication syndrome" which includes morphological and physiological changes (Poncet et al. 1998; Pujol et al. 2005; McKey et al. 2010). This absence of domestication syndrome is probably due to the traditional historical use of *J. curcas* for living fences and in a more limited way as a medicine and food. In various states of Mexico, roasted seeds of non-toxic varieties are eaten as well as in northern Veracruz where it is part of the local traditional cuisine (Makkar et al. 1998; Martínez-Herrera et al. 2010). As demonstrated by its widespread use as a living fence, *J. curcas* has probably superior abilities for organogenetic reproduction than its wild relatives, although no research has yet been conducted on this specific topic. The phytochemical comparison between *J. curcas* and related wild species could also shed light on the degree of domestication, as demonstrated in other taxa (Lindig-Cisneros et al. 2002). Due to the considerations made above and to the absence of any variety obtained via a process of scientific breeding, several authors consider that *J. curcas* is still in the process of domestication (Achten et al. 2010; Divakara et al. 2010).

According to the third axiom, the authors' observations in the field in southern Mexico and Central America indicate that farmers in this region only propagate *J. curcas* vegetatively. Similarly, Granados-Galván (2009) mentions that although the seeds germinate quickly and easily, living fences of *J. curcas*, which can involve hundreds of kilometers of linear plantings in Chiapas, are propagated solely by clonal spread. Nurseries have been started recently in Chiapas, other states of

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Fig. 12.3 Feral plants of *J. curcas* growing in the wild in the region "La Huacana", Michoacán, Mexico. (**a**) Plant in a site with secondary vegetation. (**b**) Plants on a riverside (Photographs by Yesenia Martínez, CIEco-UNAM, Mexico)

Mexico and Guatemala, to propagate *J. curcas* from seeds, but its plantations are easily identifiable as monoculture. In Central America, living fences of *piñón* are established from rooted cuttings (Budowski 1987).

The fact that *J. curcas* populations of Mexico are in the center of diversity of the genus *Jatropha* offers the possibility of finding greater genetic diversity in them than in its populations from other regions of the world where *J. curcas* is an exotic species.

However, the fact that *J. curcas* is a species in the process of domestication may imply less genetic diversity if farmers have undertaken an intensive selection. By contrast, the sympatry of *J. curcas* with its wild relatives in the center of diversification can be a favourable situation for genetic exchange between individuals that may lead to the production of new genotypes upon which natural and anthropic selection can operate (Jarvis and Hodgkin 1999).

Finally, clonal propagation of *J. curcas* in the Mesoamerican region could entail a reduction of genetic diversity. The absence of sexual recombination under exclusive clonality leads to loss of diversity (Obeso 2002). It should be noted that all processes of domestication are gradual and mixed, it is to say that sexual reproduction and cloning of plants are simultaneously present in the early stages of domestication of plants that are finally propagated clonally, which introduces a certain degree of variation in populations (McKey et al. 2010). Furthermore, variation can also occur through the fixation of somatic mutations or by means of heritable epigenetic changes (Gerrish and Lenski 1998). The above considerations are depicted in Fig. 12.4.

Is Mexico a Center of Origin or Center of Diversification?

It is possible that *J. curcas* effectively originated from Mesoamerica and many authors support this idea (Dehgan and Webster 1979; Heller 1996; Openshaw 2000; Ginwal et al. 2005; Abdulla et al. 2009. Basha et al. 2009; Kumar et al. 2009; Saikia

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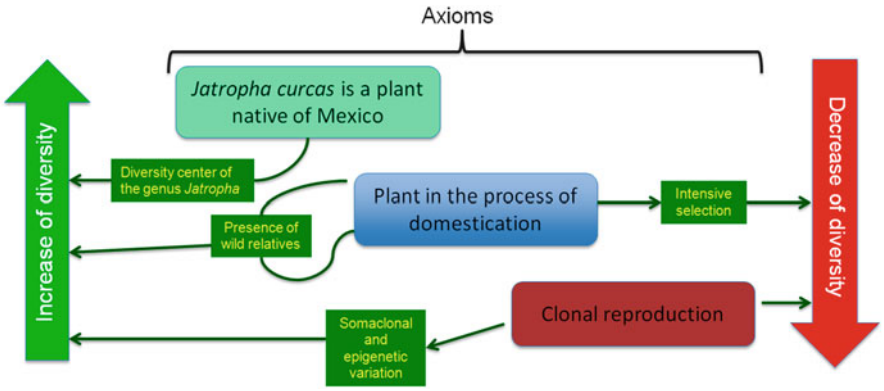

Fig. 12.4 Factors influencing the genetic diversity of populations of *J. curcas* in Mexico

et al. 2009; Sudheer-Pamidiamarri et al. 2009; Tatikonda et al. 2009; Zubieta et al. 2009; Lin et al. 2010; Parawira 2010; Umamaheswari et al. 2010). Nonetheless, other authors consider South America as the origin of *J. curcas* (Martin and Mayeux 1984; Arruda et al. 2004; Bomfim-Gois et al. 2006; Oliveira et al. 2006; Melo et al. 2006; Basha et al. 2009; Sudheer-Pamidimarri et al. 2009; Sudheer et al. 2010). Basha and Sujatha (2007), in the introduction of their article mention that *J. curcas* is native of Mexico and the Central America, but in their final comments they stated that the "true" centre of origin of *J. curcas* has not been established. Other authors prefer a more general point of view stating that the origin of the plant is "tropical America" (Ganesh-Ram et al. 2008; Ranade et al. 2008; Ambrosi et al. 2010; Divakara et al. 2010).

In 1979, Dehgan and Webster suggested *J. curcas* as the most primitive form within the *Jatropha* genus because it possesses morphological characters shared by both subgenera, *Curcas* and *Jatropha*, including palmately lobed leaves, arborescent habit, presence of a co-florescence and occasional hermaphroditic flowers. These macroscopic features are also supported by comparative microscopic examination of several anatomical and morphological features (Dehgan and Craig 1978; Dehgan 1980, 1982) that demonstrate the ability of interspecific hybridizations between the species of the two subgenera (Dehgan 1984; Sujatha and Prabakaran 2003). A first deduction could be the following: if the Mesoamerican region is the center of diversity of the genus *Jatropha* and if *J. curcas* is the most plesiomorphic species among *Jatropha* spp., with most of its wild relatives being endemic to Mexico, then *J. curcas* originates from Mesoamerica.

However, a center of diversity need not be necessarily the center of origin. An additional complication in determining the center of origin of *J. curcas* is its presence, "in the wild", in South America. Further, the existence of African species of *Jatropha*, represents an opportunity to explain the diversification of the genus by vicariance (Dehgan and Webster 1979). Dehgan and Schutzman (1994) postulated that the current distribution of *Jatropha* spp. is as a result of the separation of the ancient continent of Gondwana (*ca.* 100 millions of years ago –m.y.a.) and the subsequent spread of *Jatropha* spp. in Africa and America. Another deduction is that the center of origin

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of *J. curcas* is South America, from where it (or a similar taxon) spread to Mesoamerica (after the closure of the Isthmus of Panama, *ca*. 3 m.y.a.), a site with optimal conditions for its diversification. Supporting this view, Carels (2009) mentions that the most ancient fossil of plants of the genus *Jatropha* was registered in South America, which corresponds to the upper Eocene or lower Oligocene. Considering this, the genus *Jatropha* could have been present in South America before the closure of its connection with North America. Given the larger genetic diversity of *J. curcas* found in Mexico compared to that found in the other parts of the world (including South America), it is to be expected that *J. curcas* is a species that appeared in Mesoamerica during the radiation of the genus across the Panama isthmus. In this case, Mexico is not the center of origin, but the center of diversification of the genus.

Nevertheless, some information gaps persist: (1) the fossil record corresponding to the *Jatropha* genus is incipient. Wurdak et al. (2005) mentioned that the fossil record of the family Euphorbiaceae is of poor quality with few reliable macrofossils, thus one may ask: (2) What are the causes of a great diversity of *Jatropha* in Mexico and Mesoamerica? (3) Why the morphologically most plesiomorphic clade of the New World species of *Jatropha* is native to Mexico? (4) Will support extensive phylogenetic studies using anonymous and synonymous molecular markers the idea that *J. curcas* is the most plesiomorphic species of its genus? A more accurate conclusion may only be drawn from a complete revision of the Old and New World *Jatropha* species using both morphological and molecular characters, and conducting phylogeographic research on *J. curcas* in the American continent.

Why Study the Genetic Diversity of *J. curcas* in Mexico?

In general, studies of infraspecific diversity based on the germplasms from Asia (particularly India) and Africa have found low genetic variation using molecular markers, which is not common in an allogamous species (Bhattacharya et al. 2005; Rosado et al. 2010). However, moderate variation was detected using phenotypic traits (Kaushik et al. 2007; Saikia et al. 2009; Yi et al. 2010). The lack of variation in the Old World germplasm might have been due to possible limited introductions of *J. curcas* from Mesoamerica and, then, distributed through clonal propagation (see our review: Ovando-Medina et al. 2011a). A recent study (Yi et al. 2010) reported phenotypic variation, but with a total absence of molecular variation in populations of *J. curcas* in Africa, Asia and South America, even when various molecular, dominant and codominant markers were used. A possible explanation for the presence of phenotypic variation is the existence of epigenetic phenomena. It is noteworthy that the germplasm of South America also has low molecular diversity. In that sense, Rosado et al. (2010), using microsatellites found limited diversity in *J. curcas* in Brazil. These data indicate that South America is not a center of current diversity of *J. curcas* and there is a possibility that in this region artificial selection of the materials currently grown has been intensive. An important point is that most studies of diversity have been performed with individuals and not with a focus on populations.

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For that reasons, our group has initiated a program to analyze, under the population genetics approach, the infraspecific variation of *J. curcas* from different regions of Mexico, especially from the state of Chiapas. The purpose is to generate knowledge to answer the following overall question: How much genetic variation, characterized by neutral and adaptive characters, exists in *J. curcas* populations at the center of diversity of the genus *Jatropha*, considering it is a species in the process of domestication and is clonally propagated?

Diversity Estimation Using Phenotypic Markers

Variation in the Content and Composition of Seed Oil

The storage of oil in seeds is a pervasive feature in higher plants, as oil is the energy source for growth of the embryo before it begins to photosynthesize (Pujar et al. 2006). This stage is crucial in the success or failure of the embryo to germinate, emerge and establish itself as a seedling (Bewley and Black 1994). Therefore, the oil content of the endosperm determines, at least in part, the reproductive success of plants and this feature is expected to be a target of natural selection. For that reason, seed oil fatty acids are good candidates to estimate genetic diversity in plants.

It has been found that variation in the oil content and fatty acid composition of seed stocks for *J. curcas* populations in Mesoamerica is very high and that these chemical markers are highly heritable (Ovando-Medina et al. 2011b).

In our studies with Mexican populations, the oil content was observed to range between 12% and 44%, which is consistent with other reports for this species, whether grown in Asia or Africa (Heller 1996; Pant et al. 2006), or collected in the Mesoamerican region (Makkar et al. 1998; Martínez-Herrera et al. 2010). It has been documented that this variation is generated by both genetic and environmental factors, such as rainfall and soil fertility (Heller 1996; Escobar et al. 2008; Mishra 2009). However, other studies showed limited influence of the environment and high heritability for oil content (Kaushik et al. 2007; Gohil and Pandya 2008). Based on our investigations, the hypothesis that the environment influences oil content was put to the test for *J. curcas* in Mexico. We found that oil content has high heritability (70%) and a high genotypic coefficient of variation of 32% among accessions. That is, although this character is fixed, it has a high variability between populations. The reason for this could be the various genotypes and the environmental changes.

It is known that there are relationships between oil content and the habit and habitat of angiosperms (Levin 1974). Evolutionarily, the amount of seed oil has increased with the development of woody stems and with shade tolerance, without regard to the latitude of origin (Levin 1974). Our research used populations located in a reduced latitudinal range, which may be the cause for not finding a correlation between the amount of oil and latitude of collection sites.

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The germination temperature is an important selective factor causing seed oils of plants native to high latitudes or altitudes to have a greater proportion of unsaturated fatty acids (Linder 2000). The explanation is that in cold environments the catabolism of unsaturated fatty acids is more likely than that of saturated ones, and the seeds germinate and grow faster at lower temperatures, increasing their adequacy, even at the cost of a lower total energy available to the embryo. By contrast, in hot environments such as the tropics, seeds with more saturated fatty acids are favored because they have more energy available and need not to germinate quickly, since the conditions in the tropics are more or less stable throughout the year. *J. curcas* is a tropical species for which the Linder hypothesis does not apply because although the proportion of unsaturated fatty acids was positively correlated with altitude of collection sites, the selection has generally favored a greater proportion of unsaturated fatty acids (oleic and linoleic). Then one could postulate that, in the case of *J. curcas*, soil moisture might have exerted selection pressure for a higher proportion of unsaturated fatty acids. It is well documented that the species is tolerant to drought, but it also adapts to environments that can range from humid to semi-arid, although it is susceptible to excessive water (Dehgan and Schutzman 1994). In the Mexican tropical region the beginning of flowering and seed production coincides with the onset of the rainy season; the seeds germinate and establish quickly (for which they require a high proportion of unsaturated fatty acids) before the moisture increases to the flood. To test the hypothesis that soil moisture acts as a selective agent in the proportion of fatty acids, seed germination studies are required to detect genotypes with different average oil composition (obtained by self-pollination to avoid uncontrolled genetic variation) in media with a water activity gradient. Genotypes with a higher proportion of unsaturated fatty acids will germinate faster and in greater quantity in the media with high water activity than genotypes with more saturated fatty acids.

An indication of the high diversity of *J. curcas* populations in Mexico is the existence of genetic barriers separating populations of the Center of Chiapas and Michoacán (Fig. 12.5). Possible explanations are related to the existence of the mountain chain *Sierra Madre del Sur,* which functions as a geographic barrier; the *J. curcas* population of Center of Chiapas is the only one that is to the North of this mountain chain. Other possible reason is the geographical remoteness of the population in Michoacán from the rest of the populations.

Besides the differentiation between populations, we have found variation within populations. A cluster analysis showed that genetic diversity of Mexican *J. curcas* is high, as shown by the 7.5 units of Euclidean distance that marked the line of formation of 10 clusters (constructed with data of fatty acids in percentage). Maximum distance between groups was 33 Euclidean distance units. Most of variation was found between classes (89.3%). Unfortunately, there are no reports of similar works with which to compare the variation using fatty acids as markers. Except for the work of Wang et al. (2008), who compared the oil content and fatty acid composition in samples of *J. curcas* collected from three regions of China and India, finding 12 fatty acids and reporting differences between accessions. They concluded that attention should be given to these chemical markers to introduce new accessions in the Chinese germplasm and for the genetic improvement of the plant.

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Fig. 12.5 Map showing the two main genetic barriers (lines "*a*" and "*b*" in *yellow*) between Mexican populations of *J. curcas*, found by the algorithm of Monmonier (Barrier vers. 2.2), constructed with Fisher distances of fatty acid composition of the seed (Map prepared by FJ Pérez Racancoj, CenBio-UNACH, Mexico)

Variation in Floral Characters

Knowledge of the floral structure of plants is essential for breeding as well as for the knowledge of their taxonomy and diversity. Floral characters, because of being highly conserved and adaptive, are useful as estimators of genetic diversity of *J. curcas*. We have studied patterns of flowering and floral characters in 164 accessions of our germplasm bank of Mexican *Jatropha*, which represent eight populations of *J. curcas* from Mexico and one of Guatemala. Results have shown that floral characters of *J. curcas* from Mexico are highly variable among accessions and, at the same time, they are informative, allowing the grouping of plants at the population level.

It is well known that *J. curcas* is a monoecious species with male and female flowers in the same inflorescence (Dehgan and Webster 1979), even though some authors have mentioned the existence of dioecious plants with exclusively female inflorescences (Pecina-Quintero et al. 2011) and cases of hermaphrodite flowers (Dehgan and Webster 1979). Figure 12.6 shows the three types of flowers of *J. curcas*.

Our studies showed that populations of *J. curcas* in Mexico have a high diversity in sexual characteristics, since 93.2% of the accessions are monoecious, 4.9% are dioecious (including 60% of gynoecious or pistillate plants and 40% of androecious or staminate plants) and 2.9% are hermaphrodites (andromonoecious and sub-androecious plants). Based on an extensive search of the literature, we can

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Fig. 12.6 Type of flowers of *J. curcas* of Mexico. (**a**) Male flower (*left*). (**b**) Female flower (*center*). (**c**) Hermaprodite flower (*right*) (*Photographs courtesy:* Edilma Pérez-Castillo, CenBio-UNACH, Mexico)

Fig. 12.7 Inflorescence of a gynoecious plant of *J. curcas* (JIQ-1) showing all sites occupied by female flowers. The *upper left square* shows a recent inflorescence. This accession is part of the germplasm bank of the Center for Biosciences of the University of Chiapas (Mexico) (*Photographs courtesy:* Juan Pablo Camacho, CenBio-UNACH, Mexico)

affirm that dioecious plants have been registered only in Mexico. This kind of plants, especially the pistillate ones (Fig. 12.7), could be very useful to increase the number of fruits per inflorescence. Wu et al. (2011) classified inflorescences of *J. curcas* collected in China in three types: male-type (all flowers are male), middle-type (male and female in the same inflorescence) and female-type (all the places of female sites of the inflorescence are occupied by female flowers, but male flowers can exist). Nevertheless, Wu et al. did not mention if a particular plant produces inflorescences exclusively of a determinate type.

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Two types of floral character variations were detected in *J. curcas*: sporadic and recurrent. The first type refers to the variability between inflorescences of a same plant, which can be caused by genotype-environment interaction (Heller 1996) or epigenetic factors (Yi et al. 2010). Examples of sporadic variation in male flowers are: number of petals (four or five), sepals (four, five or six), nectar glands (four or five) and stamens (seven or ten). In female flowers sporadic variation was found in the number of ovules (two, three or four). The second type of variation refers to features that were always present in flowers of the same plant, as the abundance of trichomes and the size of the floral whorls. Only the recurrent variation was addressed in studies of *J. curcas* population diversity in Southern Mexico.

A discriminant analysis allowed the identification of the most important characters and to find relationships among the seven populations studied. The first principal component (F1) explained 46.02% of the total variation and the most important variables for their contribution to F1 were: diameter of the male flower, length of the female sepal, length of the female petal, diameter of the female flower, and pistil thickness. The second principal component (F2) accounted for 20.90% of the variance and the variables that contributed most to this factor were: pistil thickness, length of the ovary, and length of the male and female nectary.

Figure 12.8 shows the pattern of accession clustering per population, according to F1 and F2, which together account for 66.92% of the variance. It can be seen that accessions belonging to populations of Oaxaca and Border are grouped into the F1, while the accessions belonging to the Isthmus and Center populations are grouped on the F2. Guerrero's position on the extreme lower left quadrant is due to the high percentage of variance showed by the accessions of this locality and their contribution to the F1 and F2. These results are probably due to the fact that this population contains hermaphrodite plants.

It is remarkable to note the differences between populations from Border and Soconusco, although they are geographically close. Such differentiation may be due to the physical barrier represented by the mountain chain *Sierra Madre de Chiapas*. This phenomenon was also confirmed with chemical markers.

Diversity Estimation Using Dominant Molecular Markers

Genetic Variation Detected by AFLP

Very few studies on genetic diversity of *J. curcas* have had a population approach (Ambrosi et al. 2010; Wen et al. 2010; Cai et al. 2010), which shows low to moderate diversity. In our investigations (Ovando-Medina et al. 2011c) high variation and moderate structure were found in populations of *J. curcas* of Chiapas. Although clustering methods based on individuals are not useful to infer structure or other population attributes, we compared the Jaccard similarity index of accessions from Chiapas to that reported in other studies and found the lowest value (0.107) reported

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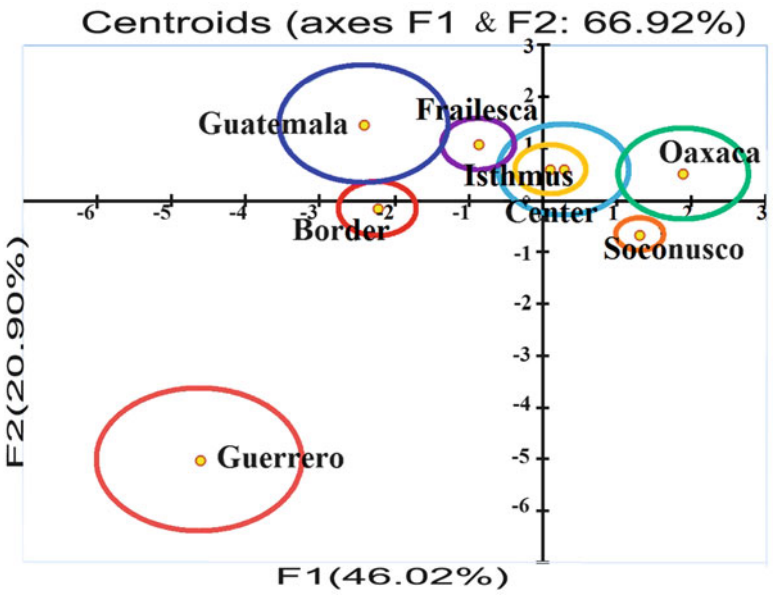

Fig. 12.8 Discriminant analysis of eight populations of Mexican *J. curcas* based on floral characters

so far (Fig. 12.9), which indicate a great diversity among these accessions. Data reported for Guatemalan accessions revealed similar values of diversity (Van-Loo et al. 2008, Fig. 12.9), strengthening the hypothesis that Mesoamerica is the center of diversification of *J. curcas*.

Since the populations showed moderate differentiation between them, the existence of isolation by distance was explored according to flower morphological markers, but the Mantel test was not significant when correlating the genetic and geographic distances. This result contrasted with the study of fatty acids, as a discriminant analysis found that populations of *J. curcas* in southern Mexico are separated according to their geographical origin according to the Mantel test that revealed a correlation between chemical and geographical distances. The lack of convergence among these results may have two possible explanations, one biological and the other technical. The first is that AFLP molecular markers, being neutral, may not reflect the actual differentiation between populations; while the chemical characters, as already argued, are adaptive and have greater power of population discrimination. The second explanation has to do with the number of populations studied in each case, since the molecular study focused on populations from Chiapas, while in the study of fatty acids were also included populations from other sites in Mesoamerica (Guatemala, Oaxaca, Guerrero and Michoacán). Considering this, the results of both studies were compared, but only for populations from Chiapas. The objective was to determine the degree of consensus produced by ordering the AFLP data matrix (binary neutral markers) and the data obtained from fatty acid ratio (continuous adaptive markers). For this a Generalized Procrustes Analysis was performed using the software InfoStat 2008 (Di-Rienzo et al. 2008). Because AFLP data

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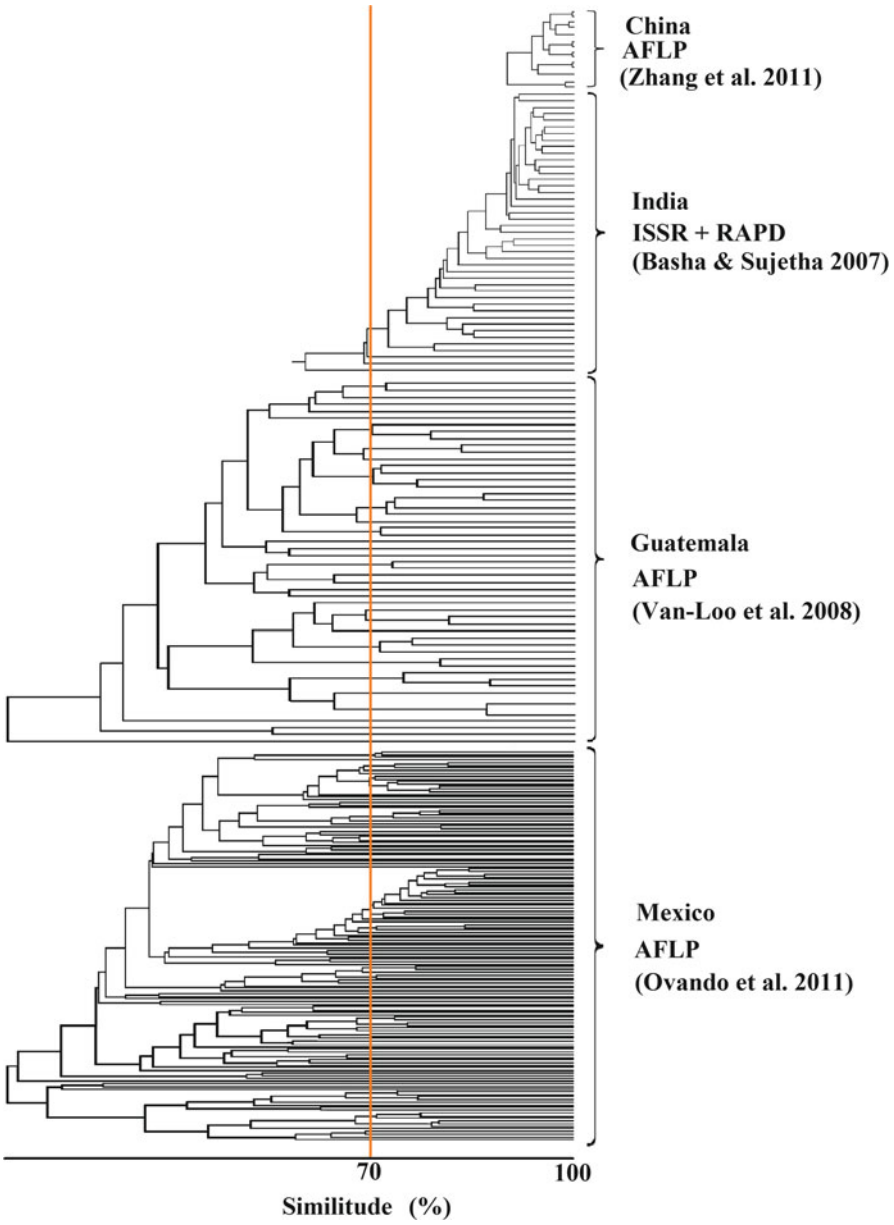

Fig. 12.9 Comparison of dendrograms of *J. curcas* from different parts of the world, obtained with dominant molecular markers (Artwork constructed by José Alejandro Gómez Pérez, CenBio-UNACH, Mexico)

were numerous (154 variables or bands) a prior reduction of variables was made by principal coordinate analysis and a 68.9% consensus between the ordering of genetic and phenotypic data was found, which can be considered quite high.

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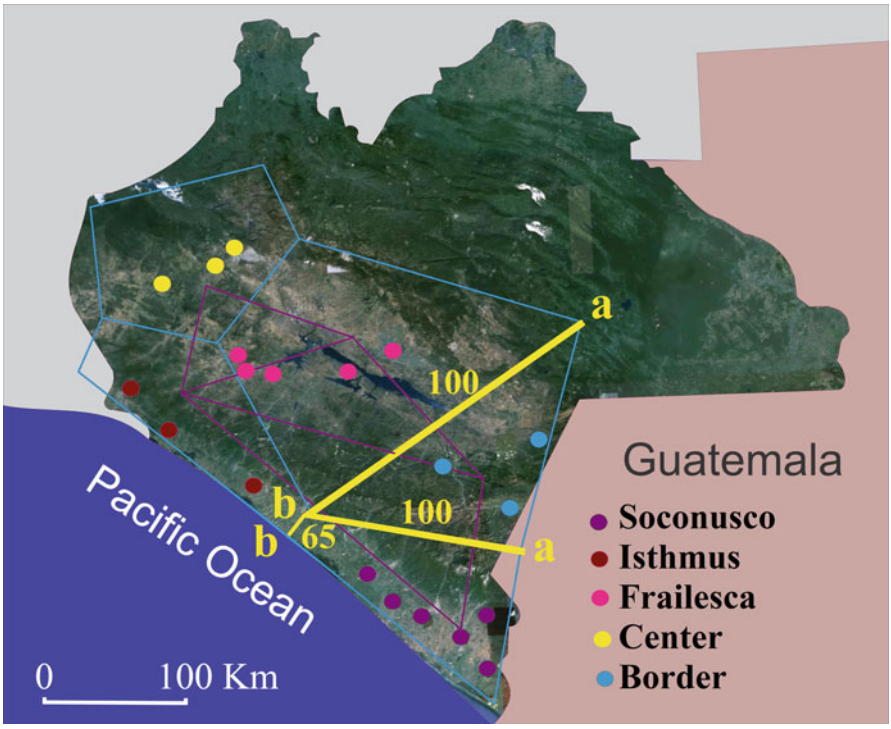

Fig. 12.10 Map showing genetic barriers between five populations of *J. curcas* of Chiapas, Mexico (Map prepared by FJ Pérez Racancoj, CenBio-UNACH, Mexico)

The study of genetic structure, revealed five genetic groups using the Structure software, which are consistent with geographic populations declared *a priori*. Mixed ancestry was obtained in most individuals, except in the Soconusco population, where there was only a small fraction of alleles from other populations to the exception of the Border one. When seeking possible genetic barriers it was found that the principal axis isolates the Border population, while the second axis separates Soconusco and Isthmus populations (Fig. 12.10).

To explain the lack of genetic recombination between Border and Soconusco, which border Guatemala and are close to a straight geographical line, it is proposed that the *Sierra Madre* has functioned as a strong physical barrier between them since their emergence. The *Sierra Madre de Chiapas* probably arose from the middle Miocene to early Pliocene, between 13 and 4.5 million years ago (Aguayo and Trápaga 1996; Burkart 1978). Moreover, *J. curcas* can have an age of approximately 70 million years (Dehgan and Schutzman 1994), assuming that it is the most plesiomorphic species of the *Jatropha* genus and that *Jatropha* spp. are found in both the Old and New World, so that the *Jatropha* genus must have existed before the separation of Africa and South America about 65 million years ago (Carels 2009). The latter suggests that the possible center of origin of *J. curcas* is the ancient continent of Gondwana, but the question remains since the most primitive clade of the genus *Jatropha* consists of endemic species in Mesoamerica.

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Two explanations for the difference between Soconusco and Border are possible, depending on the center of origin of the plant. On the one hand, if *J. curcas* originated in Mesoamerica, the existing populations in eastern Chiapas were separated by the rise of the *Sierra Madre* and have evolved differentially. On the other hand, if the species is native to South America, it could only arrive in Chiapas after the closure of the Isthmus of Panama (about 3 million years ago), so Chiapas populations colonized by at least two pathways from Guatemala: by both sides of the *Sierra Madre*. To test these two hypotheses, further studies are needed that include samples from Central and South America, using conserved markers either of slow evolution like mitochondrial DNA or chloroplast, while analyzing hypervariable markers or rapid evolution like the microsatellites. The conserved DNA can help discern the phylogeographic relationships of *J. curcas* in the Americas and identify possible lineage distribution patterns. Microsatellites would reveal where the populations had a recent origin, since the absence of high allelic variation would probably indicate that hypervariable sequences did not have time to evolve into new alleles.

The second population differentiation found with AFLP data occurred between populations from Soconusco and from the Isthmus, although there is no apparent geographical barrier that divides them. The weather could be operating as a barrier, as the climate at Soconusco is much wetter than at the Isthmus (García 1973; CNA 1998). However, this discontinuity was not validated by the data of fatty acids; data from other adaptive traits (floral morphology and physiology, for example) should be collected to verify or reject the hypothesis of Chiapas coastal climate acting as an environmental barrier. It would be desirable if an experiment of genotype-environment interaction, cultivating plants from Soconusco in the Isthmus and *vice versa*, would help identify whether natural or anthropic selection has been strong enough to influence plant performance.

Diversity Estimation Using Co-dominant Molecular Markers

Although the number of investigations on the genetic diversity of *J. curcas* has increased in the recent years, most studies have used dominant molecular marker systems (Ovando-Medina et al. 2011a). Dominant markers such as AFLP, RAPD and ISSR, have the advantage of generating a large number of DNA fragments, but have the disadvantage of not revealing the rate of homozygous/heterozygous individuals. Among several co-dominant markers, SSRs or microsatellite DNA have many advantages because of their codominant nature, abundance in genomes, high reproducibility, hyperpolymorphism, and high rates of transferability across species/genera (Yadav et al. 2011). The type of marker used can bias values of diversity index. For example, the use of SSR markers entails the risk of overestimating the diversity indexes, especially when using a low number of markers due to the high allelic variability of SSR (Xiang et al. 2007). Nevertheless, studies using SSR showed low to moderate genetic diversity in *J. curcas* germplasm of Asia and South America (Sun et al. 2008; Rosado et al. 2010; Wen et al. 2010; Sato et al. 2011; Yadav et al. 2011). It is important to note that low level of polymorphism can be the result of a low to moderate level of informativeness of the microsatellites reported for *J. curcas* (for a review of microsatellites discovered in *J. curcas* see Achten et al. 2010).

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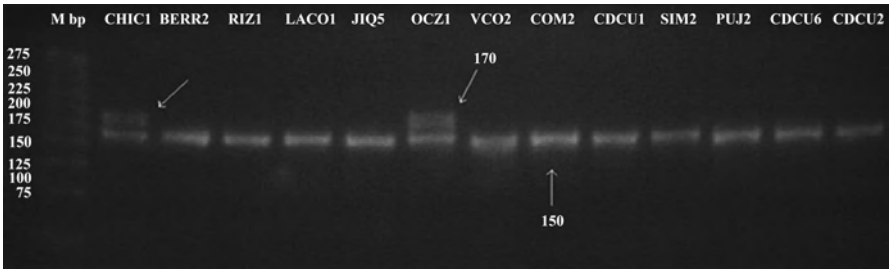

Fig. 12.11 Acrylamide gel showing alleles of 150 and 170 bp of the microsatellite jcds-24 in 13 accessions of *J. curcas* collected in Southern Mexico. Accessions CHIC1 and OCZ1 are heterozygous for this locus (*Photographs courtesy:* Julio Magaña Ramos, CenBio-UNACH, Mexico)

In order to know the genetic diversity of Mexican *J. curcas* using a co-dominant marker, we used ten microsatellites to characterize 93 individuals of six populations from the State of Chiapas. The loci (jcds10, jcds24, jcds41, jcds58, jcds66, jcps6, jcps9, jcps20, jcps21, jcms21) were selected on the basis of their polymorphism in previous studies (Achten et al. 2010). The results showed that eight out of ten loci were polymorphic and they had the ability to discriminate homozygous from heterozygous individuals (Fig. 12.11). Analysis of genetic diversity revealed that polymorphism ranged from 10% to 80%, while the average polymorphism was 45%. The effective number of alleles (Ne) was between 1.3 and 1.65 with an average of 1.513. The Shannon diversity index (I) ranged from 0.300 to 0.460 with an average of 0.321. Nei's genetic diversity (He) ranged from 0.075 to 0.244 with an average of 0.169. These results confirm the high genetic diversity among the Mexican germplasm (Ambrosi et al. 2010).

AMOVA showed that the largest proportion of total variation was only within populations (91.4%). The population differentiation index Φ_{ST} was 0.086 ($p < 0.05$), indicating moderate differentiation among populations. This result is related to the F_{IS} index, which indicates the rate of inbreeding of a population. Four populations had negative values of F_{IS} (indicating an excess of heterozygotes), while two populations had positive values (Soconusco and Isthmus, on the coast of Chiapas), indicating deficiency of heterozygotes. In general, the genetic diversity of Mexican *J. curcas* estimated with SSR was lower than that estimated with dominant markers. However, variation in SSR loci in Mexican populations was high in comparison to populations from other parts of the world.

Is the Diversity in Species Associated to *J. curcas* an Indication of its Diversity in Mexico?

Considering Mexico as the center of diversification of the genus *Jatropha*, it is possible that other groups of organisms associated to *J. curcas* display a high diversity in comparison to areas where it is an exotic species. In order to test this hypothesis,

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we are studying pollinator insects and diazotrophic rhizobacteria associated to populations of *J. curcas* from Chiapas, Mexico.

A number of flower visiting insects have been registered in plants of *J. curcas* cultivated in Asia and South America (Raju and Ezradanam 2002; Bhattacharya et al. 2005; Pinto-Juhász et al. 2009; Kaur et al. 2011). In the list of visiting insects are: *Apis mellifera*, *A. dorsata*, *A. florea*, *Eumenes conica*, *Vespa* sp., beetles, flies and dragon flies. However, it is mentioned that only species of *Apis* are effective pollinators of *J. curcas*, while the rest are foragers. In total, 16 insect species associated with populations of *J. curcas* were recorded as visitors (Achten et al. 2010).

In our studies a higher diversity of insects have been recorded visiting the flowers of *J. curcas*, which are grouped into three orders, 14 families and 17 genera, concentrating a total of 36 species (Table 12.1). Hymenoptera is the most diverse and dominant guild with 68% of the total, followed by Diptera with 25%. From the insect visitors 47.2% showed a clear affinity for the collection of nectar and pollen of *J. curcas* flowers, while 19% are efficient pollinators of this species.

All efficient pollinators were bees, especially meliponinii or stingless native bees. The Mexican *Scaptotrigona* stingless bee was the most efficient pollinator, because it transports loads of pure pollen of *J. curcas* on its head, thorax, abdomen and corbiculae. It is interesting to note that the insect species native to the Mesoamerican region are more efficient in pollinating *J. curcas* than the European bee *A. mellifera*, which may indicate concerted co-evolution between *J. curcas* and its pollinators. This result, along with additional data on the reproductive biology of *J. curcas* in Mexico (very low levels of apomixis and prevalence of xenogamy) could explain, at least in part, the great genetic diversity of this plant species in the Mesoamerican region.

On the other hand, the adaptability of this plant to marginal soils suggests that the microbial community associated to its rhizosphere plays an important role in nutrition, development and colonization of new areas. For that reason, our group has studied diazotrophic rhizobacteria (of the ecto- and endo-rhizosphere) of *J. curcas*. A total of 524 morphospecies have been isolated from 29 sites of Chiapas, Mexico. Studies of the sequence of the 16 S rRNA gene have shown that strains affecting positively the growth of *J. curcas* (because of their production of indolic phytohormones) include *Bacillus subtillis*, *B. pumilus*, *Bacillus* spp., *Pseudomonas fluorescens*, *Pseudomonas* sp., *Acinetobacter* sp. and *Enterobacter* spp. Unfortunately, similar works with *J. curcas* germplasm from other areas are not available for comparison.

Conclusions

Our research on genetic diversity of Mexican *J. curcas* has two main contributions: first it was found that *J. curcas* collected in the Mesoamerican region shows a high level of chemical, floral and molecular variability compared to the germplasm of the Old World and South America. This would support the hypothesis that Mesoamerica could be the center of diversity of this species. Genetic structuring of populations was also found, although the variation is located mainly within populations.

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Table 12.1 Diversity of insects registered as flower visitors of *J. curcas* of Chiapas, Southern Mexico (data collected by Laura Isabel Vargas-López, CenBio-UNACH, Mexico)

Order	Family	Genus	Species	Forage type	Result
Hymenoptera	Apidae	*Apis*	*mellifera*	Nectar and Pollen	OP
		Trigona	*fulviventris*	Nectar and Pollen	EP
		Trigona	*fuscipennis*	Nectar and Pollen	EP
		Nannotrigona	*perilampoides*	Nectar and Pollen	OP
		Scaptotrigona	*mexicana*	Nectar and Pollen	EP
		Tetragonisca	*angustula*	Nectar and Pollen	EP
		Oxitrigona	*mediorufa*	Nectar	OP
		Melipona	*beecheii*	Nectar and Pollen	OP
		Melipona	*solani*	Nectar and Pollen	OP
		Ceratina	*capitosa*	Nectar and Pollen	OP
		Triepeolus	sp.	Nectar	F
	Halictidae	*Agapostemon*	*nasutu*	Nectar and Pollen	EP
		Augochlora	*quiruensis*	Nectar and Pollen	OP
		Augochlora	*aurífera*	Nectar and Pollen	EP
		Augochlora	*smaragdina*	Nectar and Pollen	OP
		Halictus	*ligatus*	Nectar and Pollen	OP
		Halictus	*hesperus*	Nectar and Pollen	EP
		Lasioglossum (Dialictus)	sp1	Nectar and Pollen	OP
		Lasioglosum (Dialictus)	sp2	Nectar and Pollen	OP
	Formicidae	*Campanotus*	sp1	Nectar	OP, F
		Crematogaster	sp1	Nectar	OP, F
		Crematogaster	sp2	Nectar	OP, F
	Sphecidae	-	sp1	Nectar	F
	Vespidae	-	sp1	Nectar	OP, F
	Vespidae	-	sp2	Nectar	OP, F

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Diptera	Diptera	-	sp1	Nectar	F
	Syrphidae	*Eristalis*	sp1	Nectar	AP, F
	Tachinidae	-	sp1	Nectar	AP, F
	Tachinidae	-	sp2	Nectar	F
	Syrphidae	-	sp1	Nectar	F
	Bombylidae	-	sp1	Nectar	F
	Tephritidae	-	sp1	Nectar	F
	Sphecidae	-	sp1	Nectar	F
Hemiptera	Sphecidae	-	sp2	Nectar	F
	Cerambycidae				
	Fulgoridae	-	sp1	Nectar	AP, F
		-	sp1	Nectar	F

OP: Occasional pollinatior, EP: Efficient pollinator, F: Forager, AP: Accidental pollinator

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Secondly, information was found that could serve in the economic use of *J. curcas* as a crop. For example, groups of individuals were identified with increased oil quantity and quality; it was found that the oil content and the proportion of unsaturated fatty acids are negatively correlated, thus facilitating genetic improvement to reduce the saturated fatty acids without reducing the amount of oil; it was found that there is a weak, but significant negative correlation between oil content and the altitude of collection site, which is consistent with that reported by Pant et al. (2006) and confirmed that an association study with outstanding genotypes grown in an altitudinal gradient, could help to design planting and breeding strategies for *J. curcas*.

The results of the molecular investigations will help the genetic improvement programmes of *J. curcas* using the germplasm from Chiapas. Crosses can be performed using parental individuals from divergent populations to exploit maximum variability in the offspring, for example between Soconusco and Border. Crosses between divergent genotypes from these areas will enable the hypothesis testing concerning heterosis or hybrid vigor in the offspring (Mayo 1987). In addition, the genesis of populations segregating for quantitative traits of productive interest (number of fruits, seeds and/or oil, oil quality, tolerance to different sources of stress, etc.) could be followed up with molecular markers in parents and their offspring. The long term goal is obviously to conduct selective breeding and genetic improvement of *J. curcas* assisted by molecular markers.

We provided data that will help to identify useful markers for the selective breeding of *J. curcas*. For example, some AFLP bands were identified as specific to certain populations (Table 12.2). The individuals in the Isthmus population denominated ARR3 and TON7 have a specific 189 bp fragment; the individual PIJ1 has specific bands of 282, 342 and 344 bp; and the individual ARR6 has specific bands of 320 and 369 bp. In the Border population, the individual COM13 is the only accession to have a specific band (181 bp). In the population from Frailesca, accessions PUJ7 and CCR3 have specific fragments of 204 and 309 bp, respectively. Finally, two individuals of the Soconusco population had the specific bands: PC8 (198 bp) and SCH7 (351 bp). These bands, although they are not useful in the study of genetic relationships between populations, could be candidates for the control of out-breeding contamination in parents of selected progenies.

A principal components analysis was performed by pooling the molecular data of AFLP fragments polymorphism with data of oil and saturated fatty acids content, as proposed by Van-Loo et al. (2008) and the bands showing statistically significant correlations with these characters were identified as potential markers for selective breeding. Multiple regression analyses were conducted with phenotypic characters as the dependent variables and the AFLP bands as explanatory variables (Table 12.3). A statistically significant correlation was found for the oil content, but not for saturated fatty acids (which is good indicator of quality of oil for biodiesel production).

The model obtained for multiple regression analyses predicted the expected values of oil content for the individuals investigated, which were compared using simple linear regression with the data observed in the chemical study (Fig. 12.12). The association

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Table 12.2 AFLP banding pattern found in five populations of *J. curcas* of Chiapas, Mexico

Band type	Population				
	Isthmus	Soconusco	Border	Center	Frailesca
Different bands	140	109	130	113	128
Frequent bands[a]	118	76	130	113	99
Private bands[b]	6	2	1	0	2
Local common bands[c]	16	8	16	8	13

[a]Bands with frequency >= 5%
[b]Exclusive bands of a population
[c]Bands found in 50% or less populations

Table 12.3 AFLP bands linked seed oil content of *J. curcas* Chiapas, Mexico, as deduced by multiple regression analysis

Parameter	Value	Typical deviation	Student's *t*	*p*
Intersection	32.665	2.512	13.001	<0.0001
120 bp band	5.619	3.804	1.477	0.146
121 bp band	0.239	2.620	0.091	0.928
129 bp band	5.214	2.265	2.303	0.025
131 bp band	−6.114	2.395	−2.553	0.014
163 bp band	0.843	3.055	0.276	0.784
172 bp band	5.579	4.368	1.277	0.207
174 bp band	10.754	10.032	1.072	0.289
175 bp band	−9.731	10.833	−0.898	0.373
179 bp band	−7.562	5.097	−1.484	0.144
180 bp band	−3.248	2.395	−1.356	0.181
292 bp band	−2.452	4.553	−0.538	0.593
349 bp band	−21.115	8.022	−2.632	0.011
375 bp band	−7.360	8.403	−0.876	0.385

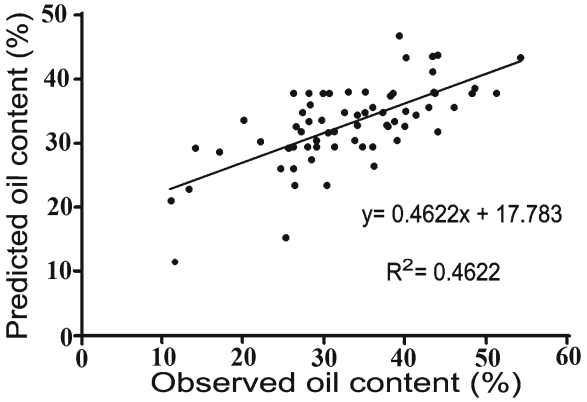

Fig. 12.12 Correlation between the oil content determined in seeds of *J. curcas* collected in Chiapas, Mexico and the oil content predicted with a multiple regression model based on 13 AFLP markers

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found was only weak (coefficient of determination r2 of 0.462), but this approach could be useful for selection at genomic level, which involves dense molecular markers (thousands of loci), such as the polymorphisms of single nucleotides obtained by massive sequencing (Goddard 2009; Crossa et al. 2010; Jannink et al. 2010).

Population specific bands (private) and bands associated with oil content should be isolated, sequenced and converted to robust markers of *sequence characterized amplified regions* (SCAR). The availability of SCAR markers would facilitate the identification of elite genotypes, since they would function as specific labels.

This research clearly shows that the genetic basis of *J. curcas* in the Mesoamerican region, specifically in Chiapas, may have been sufficiently large prior to anthropic management so that asexual propagation and domestication has not yet eroded significantly the intraspecific diversity.

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Chapter 13
Relationship of the Genetic Diversity of *Jatropha curcas* in Brazil and Worldwide

Catarina da Fonseca Lira-Medeiros, Clícia Grativol, and Paulo Cavalcanti Gomes Ferreira

Introduction

The species *Jatropha curcas* L. (physic nut) is a tropical perennial plant of great economic, ecological and environmental importance. It has gained attention in tropical and sub-tropical countries, because of its drought tolerance, easy propagation, high oil content, rapid growth, adaptation to wide agro-climatic conditions, and multiple uses of plant as a whole (Balat 2011). Although it has many different uses, it is cultivated mostly to produce biofuel in many countries around the world. Among the traits that stimulate its cultivation, one has (1) it is not grazed by animals, (2) it grows readily in poor and stony soils, (3) it is drought and disease tolerant, (4) it is multipurpose and (5) it yields high quality biodiesel (Makkar and Becker 2009). Physic nut has many alternative uses, such as protection against land erosion, degraded land reclaim, boundary fence, organic fertilizers, insecticide, medicinal properties, anti inflammatory, antimicrobial, wound-healing and even cancer prevention (Openshaw 2000; Makkar and Becker 2009). The use of its press cake as animal feed is still limited by toxic or antinutritional compounds, but it is in development (Becker and Makkar 2008).

On a global scale, the area of degraded land where physic nut can potentially be cultivated is much larger than the 1.4 billion ha under food crop cultivation and increases by approximately 10 million ha every year (Makkar and Becker 2009). The use of physic nut on some of these degraded areas could make them profitable through the production of biodiesel and co-products neither compete with cultivation of food crops nor cause degradation of natural areas.

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Actually, physic nut is being cultivated broadly around the world, but its present global production of seeds is still marginal. It is believed that approximately 25–30 million ha are currently being established in different parts of the world, largely with toxic genotypes (Makkar and Becker 2009). Thus, its productivity could be enhanced greatly in the next few years (Fairless 2007). The seed yield reported for physic nut varies in the range of 0.5–12 t/year/ha, but more typically between 1 and 2 t/year/ha – depending on soil, nutrient and rainfall conditions. The tree has a productive life of over 30 years (Francis et al. 2005; Arruda et al. 2004). In the hypothesis of selective breeding, the potential productivity of physic nut seems to be in the average of about 4–5 t/ha/year seeds in optimal agricultural conditions. Globally, there are huge areas of degraded former crop lands available for planting physic nut. India and China alone report up to 150 million ha of degraded lands and establishment of physic nut plantation on such areas not only should reduce *greenhouse gas* (GHG) emissions, but also create opportunities for farmers and rural workers (Becker and Makkar 2008).

The largest biodiesel producers are the European Union (EU), United States, Brazil and Indonesia with a combined production of edible oil for biodiesel manufacturing of about 8.6 million tons in 2007 compared to a global edible oil production of 132 million tons (Balat 2011). Currently, biodiesel is mainly prepared from edible oils from food crops, such as rapeseed, soybean, sunflower and oil palm with the consequence of raising competing interests between food and fuel (Balat 2011). Brazil has an expressive bioethanol production since the beginning of biofuel use for transportation around the world. The ethanol produced from sugarcane in Brazil is a model of biofuel production, distribution and use for other countries (Nass et al. 2007). With the expansion of biofuel production in Brazil promoted by the Brazilian government, this country is now one of the largest producer and consumer of biofuel around the world. The annual production in 2010 was 2.4 billions liters, with the capacity to produce around 5.8 billions liters per year (ANP 2011). The biofuel production in Brazil is mostly based on plant crops, such as soybean, oil palm, sunflower, babassu, peanut, castor beans and physic nut.

Many countries have tested biokerosene mixtures made from different sources in Boeing and Airbus aircrafts from different airlines. For example, kerosene mixed with 50% of biokerosene produced from the oil of Brazilian plantations of physic nut has been successfully tested on an Airbus from TAM (Subbaraman 2010). Physic nut has gained popularity in recent years as a plant of great potential for biodiesel production because of the biofuel quality it allows to produce (Devappa et al. 2010) and the ecological traits that allow cost-effective cultivation in arid or semi-arid areas.

While physic nut is seen as a very promising option for producing biofuel from degraded areas, generating rural employment, increasing environmental quality and providing primary energy carriers to energy deficient areas, the adoption and implementation of the concept have advanced comparatively slowly so far. Barriers include: (1) insufficient information on its suitability for specific areas; (2) lack of species improvement through organized selection and breeding programmes; and (3) limited agronomic studies on its responsiveness to farming practices, productivity under various climatic conditions, pathology, economic studies concerning market potential, acceptability and applicability of physic nut products (Francis et al. 2005).

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Because of its great potential, *Food and Agriculture Organization* (FAO) and *International Fund for Agricultural Development* (IFAD) consider physic nut as a promising crop for biofuel production benefiting poor farmers. In 2008, 760,000 ha were planted in Asia, 120,000 in Africa and 20,000 in Latin America. The expectation is 12.8 million hectares of physic nut planted around the world by 2015. In Asia, it is estimated that Indonesia will be the largest producer. In Africa, the main producers will be Ghana and Madagascar. In Latin America, Brazil will be the leader.

With the advent of the *Brazilian Biodiesel Program* and the emergence of strong demand for vegetable oils, physic nut has been promoted as the principal alternative of plant cultivation for biofuel production nowadays. However, some scientists and technicians are concerned by the limited technical information available on long term plantation of physic nut in large areas around the world. Some of the problems quoted by them are: (1) the absence of tradition in planting physic nut to confirm its productivity and profitability over the years; (2) the lack of scientific investigations concerning its productivity in effective cultivated areas; (3) the low level of domestication and the very few breeding program running at moment; (4) the lack of information available on its best production system according to the locality; (5) pests and diseases and their impact on productivity according to the locality; (6) the non-synchronous seed maturation and its consequences for seed harvesting; (7) the absence of a market for farmers to sell the raw product; and (8) the difficulty of capitalization by financial institutions that cannot invest in physic nut because of the absence of technical guarantees.

In Brazil, the planting of physic nut was initially promoted in the Northeast region, where there are reports that this plant is well adapted to local climatic conditions (Arruda et al. 2004). In recent years, physic nut has been grown and commercialized in different regions of Brazil, but still remains marginal in comparison to soybean. In this country, physic nut is encountered in almost all regions, adapting to the most variable soil and climatic conditions, mainly in the Central West and Northeast States, specially Goiás and Minas Gerais (www.pinhaomanso.com.br – accessed in 2011, November 25th). In general, this species grows in abandoned and uncultivated land, but it does not stand in places with dense vegetation, with which it can hardly compete.

One of the major limitations to large scale cultivation of physic nut in Brazil is that the knowledge concerning its level of genetic variability as well as large scale selective breeding and seed production are still lacking (Grativol et al. 2011). So far, all physic nut cultivated in Brazil is derived from seeds of commercial sellers and local farmers.

In order to reach the level of an industrial crop in Brazil, it is crucial to determine the genetic variability of the available accessions of physic nut and to introduce elite plants in breeding programs in order to issue varieties able to sustain high crop production in different agro-climatic regions (Becker and Makkar 2008; Ranade et al. 2008; Gupta et al. 2008). Because of its semi-wild status, the morphological characters, oil content and other chemical constituents of the oil vary considerably among different provenances of physic nut. The precise definition of character variation is important from a breeder's point of view for the establishment and performance of selective breeding.

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Genetic Diversity of Physic Nut in Brazil

Many molecular markers for population studies have been developed, especially after the advent of *polymerase chain reaction* (PCR) technology. Over the last 15 years, PCR has led to the development of a number of simple and quick techniques, such as *randomly amplified polymorphic DNA* (RAPD), *inter-simple sequence repeat* (ISSR) and *amplified fragment length polymorphism* (AFLP) markers, which have been used by various workers for characterization of genetic diversity in forest tree species (Singh et al. 2010). Many of these DNA molecular markers and some other novel approaches, such as *simple sequence repeat* (SSR), *single-primer amplification reaction* (SPAR) and *methylation-sensitive amplified polymorphism* (MSAP) have been used in genetic diversity assessment of physic nut in recent years.

In many countries, investigations have been conducted to assess the genetic diversity of physic nut using different molecular markers. In India, for example, physic nut accessions has been studied mainly using RAPD (Basha and Sujatha 2007; Gupta et al. 2008; Basha et al. 2009; Sudheer-Pamidiamarri et al. 2009), ISSR (Basha and Sujatha 2007; Gupta et al. 2008; Basha et al. 2009), AFLP (Sun et al. 2008; Sudheer-Pamidiamarri et al. 2009; Tatikonda et al. 2009) and SSR (Sun et al. 2008; Basha et al. 2009; Sudheer-Pamidiamarri et al. 2009). In Malaysia, ISSR and SNP markers were used to compare populations from America, Africa and islands in the Pacific Ocean (Noor-Camellia et al. 2012; Ricci et al. 2012).

Some of the studies quoted above are released controversial indicating the necessity for more robust and worldwide approaches to assess physic nut genetic diversity. The Indian accessions of physic nut showed a rate of polymorphism ranging from 15% (Sudheer-Pamidiamarri et al. 2009) to 88% (Tatikonda et al. 2009) according to the type of molecular marker. A worldwide assessment using accessions from 13 countries showed 62% of polymorphism using RAPD, 26% using ISSR and 72% using SSR (Basha et al. 2009).

These highly divergent results can be the consequence of the different DNA targets and analytic procedures addressed by these methodologies, but also from sample heterogeneity between experiments. Aiming to identify specific markers for toxic and non-toxic varieties, Sudheer-Pamidimarri et al. (2009) used only six different accessions of physic nut, four from India and two from Mexico. Moreover, these authors screened RAPD, AFLP and SSR loci for polymorphic bands between toxic and non-toxic varieties with, as a result, very low overall genetic diversity, i.e., 16.49%, 15.09% of polymorphism for RAPD and AFLP markers, respectively. Interestingly, the majority of the accessions clustered according to their geographical provenance. Microsatellite markers had higher polymorphism (7 out of 12 loci were polymorphic) showing better applicability of these markers in breeding programs (Sudheer-Pamidiamarri et al. 2009). The easy identification of toxic and non-toxic varieties using molecular markers is important for a diagnosis of possible toxic adulteration of animal food composition in the future (Gupta et al. 2008).

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The genetic diversity of physic nut populations from India investigated with EST-SSR markers was found to be low, although the markers had high transferability between species within the family of Euphorbiaceae (Yadav et al. 2011). The physic nut accessions classified according to these EST-SSR loci clustered independently from their geographical origins, possibly because the functional signature of this kind of markers is based on EST sequences.

When AFLP and SSR markers were used in combination in the analysis of diversity in 56 Chinese and 2 Malaysian accessions of physic nut, the reported diversity in Chinese accessions was also low. The dominant marker AFLP revealed 14.3% of polymorphism and only 1 from 17 SSR markers was polymorphic (Sun et al. 2008). Since SSR and AFLP are highly polymorphic and reproducible markers, one may conclude that India and China accessions were already homogenized after many years of cultivation in these countries.

Basha and Sujatha (2007) also found low levels of diversity in physic nut accessions from India using RAPD and ISSR markers (42.0% and 33.5% respectively). Although these authors found unique bands for some of the populations and varieties, accessions collected from the same region as well as different regions in India were clustered in closely related groups, which clearly indicates that the geographic differentiation of Indian physic nut germplasm is not extensive. However, as expected, Indian and Mexican varieties showed high genetic distance.

Malaysian accessions also revealed low genetic diversity (40% of polymorphism) in 16 physic nut accessions, suggesting that Malaysian accessions were introduced from similar sources, most likely from India (Noor-Camellia et al. 2012). A low genetic diversity was also observed by Ricci et al. (2012) within populations from mainland (Brazil, Mozambique, and Senegal) and island (Cape Verde and Cuba) areas using SSR primers to tag SNP variations, but these populations formed two groups by PCA and dendrogram analysis. Thus, the authors believe that island populations suffered short-scale evolutionary processes due to some bias in vegetative propagation or selection by both natural and anthropic pressures, such as management system by local farmers, for example. By contrast, these factors may have contributed only a little to genetic variability of physic nut populations in mainland countries, such as India, China and Brazil (Ricci et al. 2012).

In another study carried out on Kenyan populations of physic nut, Machua et al. (2011) reported a moderate level of genetic diversity using RAPD markers (55.33% of polymorphism). Since it was higher than the one reported in the studies quoted above, physic nut germplasm from Kenya has a broader genetic base, which is more favorable for selective breeding programs.

Always considering India, a high level of genetic diversity corresponding to 88%, 84% and 77% of polymorphism was found in a set of 48 accessions from different geographical regions analyzed with AFLP (Tatikonda et al. 2009) and in a group of 13 genotypes from 4 different populations using RAPD and ISSR (Gupta et al. 2008), respectively. In addition, Kumar et al. (2011) found 75.83% of polymorphism using ISSR and DAMD markers in 36 genotypes from India. In Brazilian accessions, ISSR markers showed an even larger diversity corresponding to 91% of polymorphism (Grativol et al. 2011). These results indicate that many germplasm

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collections may not include truly diverse material as attested by Ranade et al. (2008). Actually, many collections lack data on accession provenance and pedigree. However, some Indian accessions that showed high level of genetic diversity and great potential to start a breeding program in this country should be considered.

Major dominant markers, such as RAPD and ISSR were also used for congeneric diversity evaluation in the genus *Jatropha*. The percentage of polymorphic loci between congeneric species is always high, varying between 80.2% and 98.6% (Ganesh Ram et al. 2008; Basha and Sujatha 2009), which demonstrate at molecular level the importance of interspecific hybridization to import genetic variability within physic nut. However, even when using ISSR markers there are always some physic nut accessions with a lower rate of polymorphic loci, i.e., 31.4% (Tanya et al. 2011). When comparing physic nut accessions with congeneric species, there was always a clustering of physic nut accessions, dividing this species from the other related ones in the same genus. These results show the possibility to unambiguously molecular identification of alien DNA transferred from congeneric parents (♂) into physic nut background (♀), which open encouraging perspectives for the molecular characterization of interspecific hybrids (Ganesh Ram et al. 2000) and the successful introgression of useful traits from the *Jatropha* background into physic nut.

Chinese accessions analyzed by EST-SSR and genomic SSR markers showed high polymorphism (84.72%), indicating that the collections from Yunnan (China) could also be used to enrich the genetic background of physic nut in China (Wen et al. 2010).

Other methodologies, such as DIVA-GIS, were also used for the characterization of genetic diversity both in Indian (Sunil et al. 2009) and Malaysian (Shabanimofrad et al. 2011) accessions. According to these markers, Malaysian accessions grouped in three clusters while Indian accessions were more diverse forming no clear clusters. Based on these data, the authors identified geographical areas where the germplasm would be more suitable for selective breeding of quantitative characters like oil content and number of fruits, for example.

Analyzing genetic diversity in individuals from the wild or identifying the provenance of accessions and collections has great importance for the development of a viable biodiesel program on a worldwide basis. As expected, the wild genotypes generally have greater polymorphism compared to cultivated genotypes because unique alleles present in wild genotypes may be lost during selective breeding or extensive cultivation. The detection of wild genotypes or genotypes that have larger polymorphism is of great importance for breeding programs because it allows the measure of the potential genotype value on a much larger basis than normally assessed with alleles most commonly detected (Varshney et al. 2007). Following Ranade et al. (2008), germplasm collections without records of provenance of wild accessions and pedigree may not be the best source of genetic material for cultivation or for parent selection in breeding programs.

In Brazil, physic nut accessions were analyzed using ISSR markers, with the objective to identify its level of overall genetic diversity and also to diagnose the provenience of some local cultivars (Grativol et al. 2011).

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Fig. 13.1 Map of Brazilian regions and states. Physic nut accessions were obtained from Rondônia (RO), Pernambuco (PE), Rio Grande do Norte (RN), Minas Gerais (MG), São Paulo (SP), Mato Grosso (MT), Mato Grosso do Sul (MS) and Goiás (GO)

The Brazilian accessions were obtained from farmers that use their plantations for commercial purposes from different regions and states of Brazil (Fig. 13.1), i.e., North region (Rondônia – RO); Northeast region (Pernambuco – PE and Rio Grande do Norte – RN); Southeast region (Minas Gerais – MG and São Paulo – SP); Central West region (Mato Grosso – MT, Mato Grosso do Sul – MS and Goiás – GO) (see Grativol et al. 2011).

In the analysis of Brazilian physic nut accessions from different localities, it was observed that a RN accession and one sample of unknown origin (UN) had the highest percentage of polymorphic loci and also the highest genetic diversity indices of Nei and Shannon, suggesting that these accessions have maintained a great part of their original genetic diversity, as they were less affected by the homogenization process due to cultivation (Grativol et al. 2011). The accessions from SP and MT were the most genetically uniform accessions, probably because of more intensive cultivation in the Southeast and Center West of Brazil (Grativol et al. 2011).

Brazilian accessions had 91% of polymorphism for ISSR markers (Grativol et al. 2011), on average, which is similar to the reports on Indian accessions by Gupta et al. (2008), Tatikonda et al. (2009) and Ranade et al. (2008); the similar polymorphism levels between these two countries shows that a large genetic diversity is, indeed, available in Brazil.

Grativol et al. (2011) also showed that Brazilian accessions of physic nut are grouped in three clusters (Fig. 13.2): cluster I (accessions from SP and RO); cluster II (accessions

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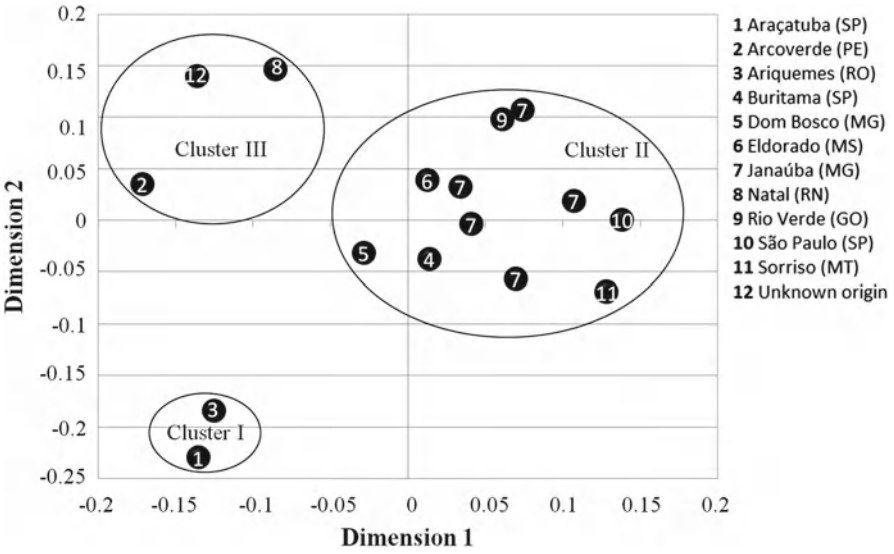

Fig. 13.2 Multidimensional scaling (MDS) analysis based on ISSR data showing three main clusters dividing 332 accessions of physic nut from 12 localities in Brazil (Source: Grativol et al. 2011)

from MS, MG, SP, GO and MT); cluster III (accessions from RN, PE and UN). There is a clear separation between north-eastern and southern accessions, but central-western and south-eastern accessions are mixed (Fig. 13.3) probably as a result of the trading policy between farmers that commercialize physic nut seeds in Brazil. We also believe that Brazilian accessions originate from different genetic sources.

Another study using RAPD and SSR markers found low genetic diversity within Brazilian accessions (Rosado et al. 2010). The interpretation by Rosado et al. (2010) was based on individuals and noted limited diversity between them. On the other hand, Grativol et al. (2011) analyzed commercial seeds as populations of individuals and found some diversity, especially in the northeast region of Brazil. These two investigations show that, besides having limited genetic diversity between individual accessions, some of them can be grouped mainly in relation to their geographical areas. Despite these works, there are many doubts about the genetic diversity available in Brazilian accessions.

Comparing these two Brazilian studies, we can observe that domestication processes of physic nut have already initiated with the aim to capitalize on the large genetic diversity that is still available in this country, this is probably because of its proximity to the supposed center of origin/diversity of the species in Mexico. Grativol et al. (2011) believed that accessions from RN State (city of Natal, cluster III) would be more appropriate for use in the genetic improvement of physic nut in Brazil given its particular isolation compared to Center-Western and Southern Brazilian germplasm; a fact that has been already noted by Martin and Mayeux (1984). We believe North-Eastern Brazilian accessions may eventually have original genetic value, which indicate great potential for this germplasm to be used in breeding programs of other countries.

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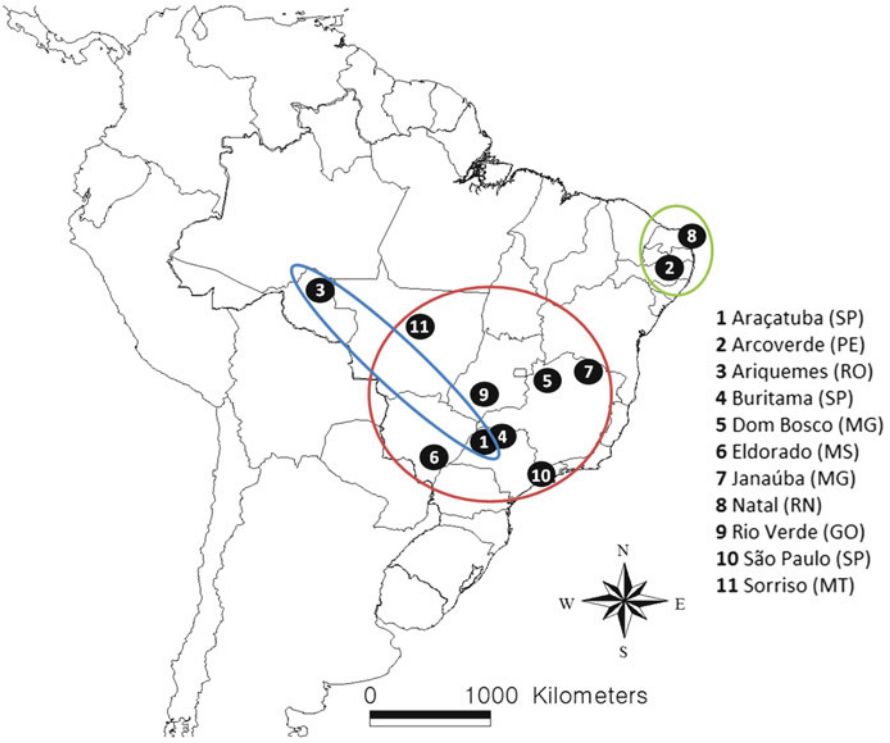

Fig. 13.3 Map of Brazil showing the geographical position of the clusters formed by the accessions from SP, MG, MT, MS and GO (*red group*), RN and PE (*green group*) and SP and RO (*blue group*). Name of the cities of provenance are shown in the map: Araçatuba, Buritama and São Paulo from São Paulo state (SP), Arcoverde from Pernambuco state (PE), Ariquemes from Rondônia state (RO), Dom Bosco and Janaúba from Minas Gerais state (MG), Eldorado from Mato Grosso do Sul state (MS), Natal from Rio Grande do Norte state (RN), Rio Verde from Goiás state (GO) and Sorriso from Mato Grosso state (MT) (Source: Grativol et al. 2011)

Diversity at Centre of Origin

According to Heller (1996) the origin of physic nut is very controversial. Some authors such as Martin and Mayeux (1984) and more recently Arruda et al. (2004) identified Brazil as center of origin however accessions from other countries such as Cambodia clustered with Brazilian accessions, which suggests the existence of a process of physic nut dispersion (Freitas et al. 2011). Dehgan and Webster (1979) defined Mexico as a center of origin and some other authors state that *Jatropha* species would have come from Central America.

Today physic nut is found in forest and non-forest areas of almost all tropical and subtropical countries and since it is not browsed by cattle it is also cultivated as protection hedges around gardens and fields (Sun et al. 2008; Henning 2009; Singh et al. 2010). The true centre of origin has not yet been elucidated, but the consensus

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among researchers is that physic nut originated from Central America and more particularly from Mexico where it occurs naturally in the forests of coastal regions (Aponte 1978). From Mexico, accessions were most probably introduced to other Latin American countries (including Brazil) and later Africa and Asia. According to data from herbaria specimens collected in the Americas, *Jatropha* species occurred in areas of native forests, woods and under stories. In Asia and Africa, only cultivated specimens are found, which were probably brought by Portuguese seafarers from the Caribbean via Cape Verde Islands and Guinea Bissau (formerly Portuguese Guinea) (Burkill 1966; Heller 1996).

Although genetic diversity of physic nut in native Central America is not well established, it is suggested that germplasm for genetic improvement programs should be introduced from its native area rather than from other introduced populations (Sun et al. 2008).

Physic nut grows well in tropical dry and humid equatorial zones as well as in semi-arid regions and can withstand long periods of drought. It is adaptable to a wide range of climatic conditions with temperatures ranging between 18 °C and 28 °C, average rainfall from 480 to 2,380 mm and growth between 0 and 1,000 m above sea level (Arruda et al. 2004). In Brazil, cultivation of physic nut is broadly distributed in all the regions: North, Northeast, Midwest and Southeast of Brazil. We consider that Brazil could be an important country in the process of diversification and distribution of physic nut varieties from its centre of origin in the Americas to the rest of the world.

The genetic diversity found in Brazilian accessions of physic nut and the narrow relationships among clusters (Grativol et al. 2011; Rosado et al. 2010) could indicate that the seeds have come from the same source with few introductions, as described by Basha et al. (2009) when analyzing accessions of different countries in Asia, Africa and Central America. Nevertheless, the clusters II and III show a large degree of separation that may have been provoked by the accumulation of genetic differences fixed by adaptation to different environments and also by geographical isolation (due to lack of gene flow; Fig. 13.2). A similar level of genetic separation was observed in clusters I and II that could indicate that the Brazilian accessions were spread from the Northeast, where physic nut has been planted for some time, to other regions in Brazil. This is corroborated by the lack of clustering between accessions from central-western and south-eastern Brazilian regions also noted by Rosado et al. (2010). In Brazil, an initial domestication process of physic nut seems to be happening, which can be seen in the reduced number of polymorphic loci and genetic diversity of accessions from Ariquemes (RO), Araçatuba (SP), São Paulo (SP) and Sorriso (MT). The low genetic diversity could reflect selection from local small farmers to obtain a more uniform crop in terms of productivity and several breeding programs are now being undertaken to increase the genetic diversity in Brazilian accessions.

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Chapter 14
Towards the Domestication of *Jatropha*: The Integration of Sciences

Nicolas Carels

Introduction

Fossil oil is becoming increasingly rare and expensive. In the near future, biofuels are expected to represent ~10% of the world's energy production and to become strategic resources in countries like India, which currently depends mostly on the importation of fossil oil for its national energy consumption. However, the increasing world population is correlated with global climate changes, as well as a scarcity of arable land and water. Expansion of the amount of land used for food production is becoming limited by public pressure in favor of wildlife sustainability. Biofuels may eventually represent a small part of the edible oil segment, in association with temporary excesses of food products, as occurs with the absorption of excess sugar by the ethanol industry. However, the demand for biofuels as a replacement energy source has led to serious concerns about the sustainability of the global production of food from crop plants in the long term. Consequently, the diversification of oil sources, areas for production and conditions of cultivation are becoming increasingly important to the biofuel industry (Carels 2011). In this respect, selective breeding through the use of genome-assisted breeding and transgenic technologies will be needed to increase the number of alternative oleaginous species available for biofuel production. At present, biodiesel production relies mainly on soybean, rapeseed and palm oil.

Because of its productivity, oil quality and ability to thrive in lands not suited for food crops, *Jatropha curcas* L. (Plantae; Embryophyta; Spermatopsida; Malpighiales; Euphorbiaceae) has been proposed as a new plant source to diversify biodiesel production in tropical climates (see Carels 2009 for a review). The genus *Jatropha* belongs to the tribe *Jatropheae* in the Euphorbiaceae family and contains

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approximately 170 known species. Recent phylogenetic studies in the genus *Jatropha* based on random amplified polymorphic DNA (RAPD), amplified fragment length polymorphisms (AFLP, Vos et al. 1995) and nuclear ribosomal-DNA internal transcribed spacer (nrDNA ITS) markers (Sudheer et al. 2009a) showed that *J. curcas* is genetically more similar to *J. integerrima* Jacq. than to any other species of genus *Jatropha*.

J. curcas is a stem-succulent deciduous tree of ~5 m in height that produces seeds rich in oil (27–40%). Crude oil of *J. curcas* meets the fuel quality standards of rapeseed and can be easily converted into biodiesel that meets US and European standards by transesterification.

J. curcas is currently considered as undomesticated; its physiology and agronomy remain to be clarified, and thus seed yield is poorly predictable (Ginwal et al. 2004). However, in the interval of just 3 years from 2007 to 2010, great efforts have been made by the scientific community, particularly in India, to gather data on the genetic diversity and interspecific hybridization of this species, as well as from analyses using the complete range of available biotechnologies, including *in vitro* propagation, tissue culture, genetic transformation, transcriptome description and genome sequencing. Forward and reverse genomics approaches are now beginning to be employed in *J. curcas* research.

The twenty-first century is witnessing an unprecedented level of scientific integration, which in the case of biology, has led to the concept of *systems biology*. This concept can be defined as the study of the interactions between the components of biological systems and how these interactions give rise to the functioning and behavior of that system. In the case of genetics, the system also includes the environment of the biological entity under consideration because it conditions the spectrum of stimuli that are used to score the genome performance. As a consequence, systems biology implies high-throughput technologies of data collection, information technology for data management and mathematical modeling.

Genetic correlations can initially be investigated by examining domesticated crops as an entity that represents a particular mode of the expression of a plant species. Related to this notion, there is a common suite of traits known as the "domestication syndrome" (Doebley et al. 2006; Tang et al. 2010), which represents the kind of complexity that is appropriate to the new perspective that plant breeding must be addressed with the support of systems biology.

Here, I propose to discuss the current knowledge and emerging technologies available from model crops with the underlying prospect of their application to the selective breeding of *J. curcas*.

Mating System

J. curcas is monoecious, with separate male and female flowers, and is a predominantly outbreeding species, as shown by its high abortion rate, low seed-ovule ratio, low fecundity rate, high pollinator attractiveness, delayed stigma receptive

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period and morphological differentiation (Bhattacharya et al. 2005). Despite these outbreeding features, Juhász et al. (2009) found that when self-pollination was processed manually, the fruit weight, seed size and seed number per fruit were not significantly different from what is observed in natural cross-pollinated plants. However, under natural conditions in Brazil, the same authors found that the rate of fruit formation was reduced by a factor two when natural self-pollination was forced because male and female flowers rarely open at the same time in the same inflorescence. Similar conclusions were reached by Abdelgadir et al. (2008) under African conditions. These data suggest that *J. curcas* is well adapted for the purpose of inbreeding.

Knowledge about rates of outcrossing versus selfing in breeding populations is essential for maintaining adequate levels of genetic variability and continuous gains over generations. Mating patterns can be influenced by differential crop fecundity and rates of pollination. In *J. curcas*, there is a genomic uniformity generally recognized to be strong, particularly among commercial varieties (Ambrosi et al. 2010a), and the high degree of homozygosity detected by molecular markers is consistent with prevalent autogamy, even if the mating system promotes allogamy. Additionally, the low genetic variability observed could be due to a past event of a population bottleneck, which would have caused the loss of polymorphism.

The inflorescence in these plants is a panicle, with the female flowers (approximately 10–20% of the total) being located at the apices of the main stem and branches of the inflorescence. Male flowers are more numerous (approximately 80–90%) than female flowers and occupy subordinate positions on the inflorescence (Raju and Ezradanam 2002). Male flowers open for a period of 8–10 days, whereas female flowers open only for 2–4 days. The continuous flowering in this species results in a sequence of reproductive developmental stages from yellow mature fruits at the base of the branch, to green fruits in the middle, and flowers at the top. After pollination, the inflorescences form bunches of ~10 green fruits that are 2–3 cm long and have an ovoid shape. Each fruit typically has three carpels and the potential for two seeds per carpel (Kochhar et al. 2008). The sigma becomes receptive 2 h after anthesis. The pollen-ovule ratio is 539:1, and pollination is carried out by insects, mainly from the Hymenoptera and Coleoptera. Fifty percent of female flowers set fruits, with a 53% fecundity rate and a 2:3 seed-ovule ratio (Bhattacharya et al. 2005; Luo et al. 2007). As noted by Ambrosi et al. (2010a), the apomixis reported by Bhattacharya et al. (2005) and Luo et al. (2007) has not been fully documented, and a lack of either a 4C or 5C endosperm peak being detected by flow cytometry suggests that gametophytic apomixis is unlikely. Additionally, the presence of only a high 2C embryo peak and a smaller 3C endosperm peak (ratio 2:3) is consistent with an obligate sexual reproductive system. However, adventitious embryogenesis from individual cells in ovule tissues contiguous to the embryo sac cannot be ruled out. Such phenomenon known as sporophytic apomixis commonly results in diploid embryos (Loureiro et al. 2006). It has not been demonstrated in *J. curcas*, but somatic embryogenesis from ovules was found to be possible by tissue culture (Jha et al. 2007).

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Genetic Diversity

Marker-based genetic distances were measured in an attempt to assess the structure of breeding populations. Thus far, molecular markers have been used to reveal the extent of genetic variation in natural populations, germplasm collections and accessions from different origins for assisting in interspecific hybridization.

In India, the genetic variability in *J. curcas* is considered to be sufficient to allow for selective breeding (Kaushik et al. 2007; Sunil et al. 2008, 2011). Similar conclusions were obtained in Brazil, where it was also concluded that the accessions considered could be clustered into only a few distinct similarity groups and that the heritability is (1) high for grain yield, height, number of days to flowering, and plant height of the first inflorescence and (2) low to intermediate for the total number of branches, stem diameter, canopy volume, and canopy projection (Laviola et al. 2010). As in India (Basha and Sujatha 2007; Jubera et al. 2009; Sudheer et al. 2010a), the accessions of *J. curcas* available in Brazil (Rosado et al. 2010) and China (Sun et al. 2008) show only modest levels of genetic variation. Wider variation was found between the genotypes from India and Central America (Basha and Sujatha 2007; Popluechai et al. 2009). However, the assessment of genetic variability by RAPD, AFLP and combinatorial Tubulin Based Polymorphism (cTBP) in 38 *J. curcas* accessions from 13 countries, including countries in Central America, India, Asia and Africa, revealed low genetic diversity (Popluechai et al. 2009), with 75% similarity being found among the global *J. curcas* accessions.

Simple sequence repeats (SSR) are frequent in the genome of *J. curcas* (289–410 Mb) and were estimated to occur at a rate of one per 7 kb, with (AT)n, (AAT)n, and (AAAT)n accounting for 71% of di-nucleotides, 60% of tri-nucleotides, and 58% of tetra-nucleotides, respectively (Sato et al. 2011). The tri-nucleotide SSRs, particularly (AAG)n and (AGC)n, are preferentially found in exons, whereas (AT)n, (AG)n, (AAT)n are more frequent in 5′ and 3′ UTRs, and (AC)n repeats are more frequent in introns. Despite the high SSR frequency, the method that best reveals genetic polymorphism in *J. curcas* is AFLP (Sudheer et al. 2010a). Additionally, multilocus profiles are better for comparing results between laboratories.

The investigations outlined above indicate that the genetic diversity of *J. curcas* is narrow worldwide and that the species probably went through a population bottleneck in its past; this hypothesis is in agreement with the small population size observed for this species today. Its precise center of origin within the intertropical Americas has not yet been established (Sujatha et al. 2008). However, genetic diversity has been found to be higher in genotypes from Central America than from other regions.

Recently, a global study compared the genetic variability between commercial accessions from Central America, South America, Africa, India and Southeast Asia using SSR techniques (Ambrosi et al. 2010a). Three groups of genetic diversity were recognized: (1) South America, Africa and Southeast Asia, (2) Central America, and (3) India. The different clustering of Indian and South American accessions found by Ambrosi et al. (2010a) was not reported by Wen et al. (2010), who also studied a mixture of accessions from Brazil, India, Indonesia, and China. It is likely that the

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differential clustering of Indian accessions found in the Ambrosi et al. (2010a) investigation actually results from a sampling bias; in fact, one Brazilian accession clusters with the Indian group in this study. If this is found to be the case, the worldwide heterogeneity of *J. curcas* would be reduced to only two major groups.

If the finding of Berry (1929) that certain fossil remains in geological formations from Peru corresponding to the early Tertiary period are effectively for the *Jatropha* genus holds true, it will indicate that the genus radiated from this area. If Central America is, indeed, the center of origin of *J. curcas*, the species must be yonger than 3 My since the isthmus of panama formed around that period. *J. curcas* would have appeared during the migration of the genus toward Central America in the last 3 My. The answer to that hypothesis should be found by analyzing gene divergence among species within the genus *Jatropha* and comparing today's distribution of wild species relatives, worldwide.

The low genetic variability reported for this species based on anonymous probe analysis contrasts with the polymorphism found using gene-specific probes. Recently, expressed sequence tag (EST)-SSRs from cassava were tested in *J. curcas* (Wen et al. 2010), and 187 out of 419 (44.6%) EST-SSRs, compared to 54 out of 182 (29.7%) genomic (G)-SSRs from cassava were found to be polymorphic among the *J. curcas* accessions. On the basis of the distribution of 183 polymorphic alleles, 45 accessions were classified into six groups in which the genotype showed a correlation with geographic origin. The estimated mean genetic diversity index was 0.5572.

However, most of the genetic variability in *J. curcas* was shown to be essentially epigenetic (~25 times higher than the genetic polymorphism) within and among populations. More than half of the CCGG sites present in this species were found to be methylated in a survey based on methylation-sensitive fluorescence AFLP (MfAFLP), with a significant difference in inner cytosine and double cytosine methylation among populations. A principal coordinates analysis based on Nei's epigenetic distance showed that accessions from Tanzania and India form a group that does not overlap with the group formed by the accessions from China, Indonesia and Suriname. As SSR markers would cluster these accessions into only one group (see above), this structure is due to epigenesis alone. Interestingly, 30 out of 39 polymorphic markers (77%) were found to be heritable and followed Mendelian segregation (Yi et al. 2010).

Another epigenetic phenomenon that may affect gene expression in *J. curcas* is paramutation, which is mediated by the interaction between two alleles of a single locus, resulting in a heritable change in the gene expression of the paramutable allele induced by the paramutagenic allele (Stam 2009).

Breeding

Because of its semi-wild state, *J. curcas* will require a minimum of 15 years of breeding before it reaches a level of domestication comparable to that of other industrial crops (Achten et al. 2010). The most important traits for the selection of

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candidate plus phenotypes of *J. curcas* are seed yield, seed size and oil yield (Mishra 2009). In the case of mechanical harvesting, dwarfing and uniform ripening will also become important (Gressel 2008). Additional traits that should be considered for selective breeding are: energy storage, fatty acid synthesis, disease resistance, toxic compound synthesis, flowering synchronization, dioecy, fruit size and tree structure. Traits such as the amount of free fatty acids (FFA), unsaponifiables, acid number and carbon residues show a wide range of variation, which indicates that oil quality is dependent on the interaction of environmental and genetic factors (Kaushik et al. 2006, 2007).

Methods that are used for naturally outbreeding populations to improve QTL definition, such as association mapping, do not make sense in the case of *J. curcas*, where a relatively large amount of linkage disequilibrium is to be expected given its low genetic variability. Marker assisted selection (MAS) and large numbers of progeny will be necessary to reach sufficient recombination and statistical consistency.

The process of selective breeding is purifying and leads to the reduction of genetic variability by capturing both additive and non-additive effects in elite clones through selection and clonal propagation; the larger the variability within the genetic base, the better the outcome of the selective process. At present, the domestication process occurs through the selection of promising individuals, germplasm collections and interspecific hybridization.

The best available practice for selecting breeding material is to use the best-performing trees in the location of interest. Trees with an annual yield above 2 kg of dry seeds and seed oil content higher than 35% can be considered to be good accessions. Profiling by polymerase chain reaction (PCR) for analyzing diversity among a collection of accessions is currently a common practice. *J. curcas* individuals exhibit a high degree of phenotypic interaction with the environment, which makes genomic DNA probes with reproducible polymorphisms essential. Probes for polymorphic sequences are needed for rapid identification of specific population abilities. This is typically assessed by correlating populations to specific phenotypic traits (Sunil et al. 2008). In fact, accessions from distinct populations form clusters according to their genotypic polymorphisms that match their geographic provenance and, therefore, their membership within a given population. Genotype clustering can be detected by an unweighted pair group method through arithmetic mean (UPGMA) analysis and ultimately revealed by a dendrogram (Ranade et al. 2008; Tatikonda et al. 2009; Cai et al. 2010; Sudheer et al. 2010a, b, c; Wen et al. 2010).

Because the current state of research on *J. curcas* is characterized by the assessment of its genetic base and physiology, it will be beneficial to proceed with gene pool enlargement by inter-specific hybridization. The introgression of negative traits into elite populations would, in fact, undermine any long-term investments in the genetic progress accumulated in these populations. Additionally, interspecific hybridization promotes instability and shuffling in the hybrid genome, which must be assessed prior to selective breeding. In wheat and related species, genomic changes due to interspecific hybridization occur on a genome-wide scale, involving all chromosomes and diverse sequences, including both coding and noncoding loci (Liu et al. 1998). This pattern of changes mainly includes three processes: loss of

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parental fragments, gain of novel fragments and simultaneous loss/gain. This suggests that the rapid genomic changes accompanying allopolyploidy are as follows; (1) largely repeatable, (2) directed, (3) can be retained over evolutionary time-scales, (4) likely to be of evolutionary significance and (5) are probably positively selected (Liu et al. 2009a).

The first attempts of interspecific hybridizations were carried out by Reddy et al. (1987). These authors performed crosses among *Ricinus communis* L. and six species in the genus *Jatropha*, i.e. *J. curcas*, *J. glandulifera* Roxb., *J. multifida* L., *J. hastata* Jacq., *J. podagrica* Hook. and *J. gossypiifolia* L. Cross-incompatibility was observed in all inter-specific combinations with however differences in pollen germination showing that *J. curcas* was closer to *J. glandulifera* and *J. gossypiifolia*. Later on, experiments of interspecific hybridization between *J. curcas* and *J. integerrima, J. multifida, J. maheshwarii* Subram. & Nayar. (Basha and Sujatha 2007; Popluechai et al. 2009) or *J. gossypiifolia* L. (Karanam and Bhavanasi 2010) were pursued with successful progenies. *Nandan-4*, the F_1 hybrid of *J. curcas* (♂) × *J. gossypiifolia* (♀), is fertile and possesses male and hermaphrodite flowers, which could be an indication of alterations in the level of endogenous cytokinins (Pan and Xu 2010). Similar to the parent species *J. gossypiifolia*, this hybrid is more tolerant to diseases and drought than *J. curcas*.

Other experiments on interspecific hybridization involving *J. curcas* as the female parent and *J. integerrima*, *J. podagrica* Hook., *J. villosa* Wight., *J. tanjorensis* Ellis and Saroja, *J. gossypiifolia, J. glandulifera* Roxb., *J. multifida,* or *J. maheshwarii* (see a description of these species in Krishnan and Paramathma 2009) as pollen donors were carried out by Parthiban et al. (2009). A cross between *J. curcas* and *J. integerrima* resulted in successful seed production and allowed backcrossing with *J. curcas* and the genesis of particularly interesting phenotype variability in the progeny related to its fruit shape, fruit color, seeds and oil yield. Crosses of *J. curcas* with the other more distantly related species were either partly successful or failed due to the existence of pre- and post-zygotic barriers that are under investigation.

Tissue Culture and Breeding Using Transgenic Technologies

Methodologies for the propagation and rooting of stem cuttings (Kochhar et al. 2008), as well as for tissue culture (Datta et al. 2007; Kalimuthu et al. 2007; see Sujatha et al. 2008 for a review of 14 references on the subject; Singh et al. 2010; Khemkladngoen et al. 2011; Kumar and Reddy 2010; Kumar et al. 2010a, 2011) have been developed for *J. curcas*. The possibility of *in vitro* culture presents opportunities for cloning rare individuals, such as mutants or interspecific hybrids. Chromosomal shuffling may occur in the calli or cells of hybrid lines under *in vitro* culture, and because these technique is increasingly feasible in *J. curcas* (Soomro and Memon 2007; Jha et al. 2007; Vasudevan and Ramashandran 2010), it could

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assist in understanding the genetic relationships in interspecific hybrids, in which seed multiplication may be a problem because of sterility (Parthiban et al. 2009).

Another significant outcome of *in vitro* technologies is the facilitation of genetic transformation using *Agrobacterium* (Li et al. 2008a; Kumar et al. 2010b; Pan et al. 2010; see Misra and Misra 2010 for a review) or by microprojectile bombardment (Joshi et al. 2011). Introduced transgenes are generally expressed constitutively, although there are several expression systems for the temporal, spatial and quantitative control of transgene activity. Molecular switches for controlling the expression of transgenes are usually based on tissue-specific promoters derived from different organisms (Baroux et al. 2005; Brand et al. 2006; Chaturvedi et al. 2007). Constructs for plant expression systems can also be induced by external compounds. Such compounds can include substances that normally activate the genes involved in systemic acquired resistance (SAR), resistance elicitation, safeners and wound signaling (Corrado and Karali 2009).

Unfortunately, transgenes can be silenced at both the transcriptional and post-transcriptional levels (Matzke and Matzke 2004). Methylation of a transgene promoter correlates with transcriptional gene silencing (Park et al. 1996), whereas methylation of a coding sequence is associated with post-transcriptional gene silencing (Ingelbrecht et al. 1994), but recent evidence suggests that a unifying mechanism based on RNA interference underlies both processes (Matzke and Matzke 2004; Matzke et al. 2004).

miRNAs regulate almost every aspect of plant growth and development including (1) leaf morphogenesis and polarity, (2) floral differentiation and development, (3) root initiation and development, (4) vascular development and (5) the transition from vegetative growth to reproductive growth (Jones-Rhoades et al. 2006; Chuck et al. 2009), playing roles in hormone signal transduction (Liu and Chen 2009) as well as responses to environmental stress and pathogen invasion (Chen et al. 2004; Sunkar et al. 2006). Therefore, artificial miRNAs (amiRNAs) that can silence specific gene(s) should permit the direct molecular modulation of plant traits (Liu and Chen 2010).

Genetic Mapping and QTL Analysis

Genetic Markers and Maps

MAS has been investigated in *J. curcas* using single primer amplification reactions – SPAR (Ranade et al. 2008), RAPD (Sunil et al. 2011; Sudheer et al. 2009b, 2010a, b), SSRs (Sudheer et al. 2009b, 2010c; Wen et al. 2010), inter simple sequence repeats – ISSRs (Sunil et al. 2011; Cai et al. 2010), AFLPs (Tatikonda et al. 2009; Sudheer et al. 2009b; 2010a, b) and MfAFLPs (Yi et al. 2010). AFLPs have thus far been shown to be the most informative marker system for *J. curcas* (Sudheer et al. 2010a) and have tentatively been correlated with QTLs (Sunil et al. 2011).

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In addition to anonymous probes (genomic probes), probes derived from expressed DNA, i.e., mRNA, may also be used. An advantage of probes derived from mRNA is that they can be generated from different tissues at various developmental stages, and therefore, they are highly effective for identifying genes that are differentially expressed during the life-cycle of an organism. Moreover, because ESTs are derived from coding DNA, their sequences are fairly conserved and more likely to be transportable across pedigree and species boundaries than are markers derived from anonymous sequences.

Of course, a method is needed for selecting and mapping candidate loci associated with particular ESTs. AFLPs combined with cDNA libraries can be applied to yield highly informative transcript-derived fragments (TDF) for mapping traits for which expression is time-dependent. In a first step, Suárez et al. (2000) introduced the sequencing of ESTs from such TDFs and their locations within a genetic map from cassava. Subsequently, Quin et al. (2001) demonstrated the detection of AFLP bands from restriction patterns of ESTs. This technique offers the advantage of allowing the exploitation of existing EST resources. Finally, Cato et al. (2001) developed a method that reveals high levels of probe polymorphisms from ESTs and is automatable for high-throughput purposes. This method is based on restriction length polymorphism (RFLP) and PCR analyses of genomic DNA fragments (restriction digested with *Dra* I or *Ssp* I) using an EST-specific primer and an adaptor primer. It detects both insertions and deletions and nucleotide changes in stretches of coding and non-coding DNA adjacent to expressed genes because it uses a 6% non-denaturing acrylamide gel to resolve fragments. This type of gel is able to resolve homoduplexes and heteroduplexes formed between amplified alleles containing nucleotide substitutions, as well as resolving allelic length differences.

Single nucleotide polymorphisms (SNPs) can also be investigated by denaturing gradient gel electrophoresis (DGGE, Fischer and Lerman 1983), single-stranded conformational polymorphism (SSCP, Orita et al. 1989; Hongyo et al. 1993; Bottley et al. 2006) or directly by sequencing and would allow the screening of informative ESTs associated with economically relevant agronomic traits.

Synteny is generally rather high between species of the same genus and may extend to families and even to taxa of higher levels, depending on the particular recombination history and population size of the members of a particular taxon. For instance, a high degree of microsynteny of *J. curcas* was observed with the genome of the castor bean and, to a decreasing extent, with those of cassava, soybean and Arabidopsis (Sato et al. 2011). Synteny allows the transfer of a given set of markers from one species to another with the purpose of consensus mapping, which facilitates the (1) investigation of QTLs among species, (2) validation of QTLs, (3) expression level of QTLs across variable genetic backgrounds, and (4) positioning of candidate genes co-localizing with QTLs. This property has been exploited recently by Wen et al. (2010) to transfer the use of a large number of EST-SSRs and G-SSRs from cassava to *J. curcas*. The conservation found among the SSR structure of these two species from two different genera of the same family suggests that the presence of synteny will allow the extension of the genomic correlations known in cassava to *J. curcas*. A typical application of this phenomenon is related to the

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ability to predict the map location of QTLs or sequence of QTLs that are known in cassava (Okogbenin et al. 2008) to assist in their investigation in *J. curcas*. QTLs have not yet been comprehensively investigated in *J. curcas*, and no genetic map has been published for this species. In contrast, a genetic linkage maps does exist for cassava (Okogbenin et al. 2006; Lokko et al. 2007; Kunkeaw et al. 2010).

A draft genome sequence for cassava has been obtained by the US Department of Energy Joint Genome Institute and can be downloaded at: ftp://ftp.jgi-psf.org/pub/JGI_data/phytozome/v6.0/Mesculenta/. Another interesting source of comparison is castor bean, for which draft genome sequence is now available (Chan et al. 2010). The castor bean is closer to *J. curcas* than is cassava, with a similar average genome size and base composition (GC content), but its genetics are less advanced than those of cassava, as no genetic map is publicly available for this species. As in *J. curcas*, the genetic variability present in castor bean is small (Allan et al. 2008). Finally, rubber, another species of Euphorbiaceae with considerable economic interest, has also entered the biotechnology era with the availability of genetic maps (Lespinasse et al. 2000) and the sequencing of 10,000 ESTs (Chow et al. 2007).

QTL Analysis and Validation

Because the breeding of *J. curcas* is still in its infancy, there is a lack of pre-designed pedigrees that maximize phenotypic segregation. However, a benefit of this species is that there are no biological barriers for the development of inbred lines to allow a more precise understanding of the architecture of quantitative traits, such as seed and oil yield. A good strategy for developing segregating crosses is to breed individuals bearing traits with Mendelian segregation that can be easily targeted, such as recessive dwarfism.

To our knowledge, the first back-crosses to be reported involve *J. curcas* and *J. integerrima* (Parthiban et al. 2009). A back-cross of the F_1 inter-specific hybrid with *J. curcas* led to depression in some cases, but also to heterosis for fruit and oil yield in other cases. Because this material could very likely have immediate commercial value, it makes sense to use it for QTL research. The genetic background of this material probably offers a higher frequency of segregating characters than crosses within the species *J. curcas* given its low genetic variability. However, it might be possible to take advantage of the genetic distance within *J. curcas*, which is the largest between Mexican and South American accessions. A set of phenotypic characters of consistent heritability shows obvious differences between these sources, which allow the investigation for genetic segregation. Knowledge about genes putatively involved in the QTLs obtained in the interspecific lines would help to detect QTLs with lower heritability within the *J. curcas* background. Hypotheses arising from reverse genetics methods would likely be particularly helpful in this regard because the resolution of QTLs is usually low, typically falling in the range of 30 centimorgans (cM). The limits of mapping QTLs are related to (1) their association with

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broad genomic regions involving several hundred of genes or *cis* regulatory elements and (2) the need of large samples and marker densities to improve mapping resolution, which will hardly fall below a few cM.

Genetical Genomics

Genome-wide association analysis is currently hampered by the limited availability of high density marker systems in crops, but it has been successfully used in Arabidopsis (Atwell et al. 2010). Forward genetics approaches are not practical for genome-wide analysis, primarily due to the effort and time that is necessary for the identification of all genes involved in coding for a particular phenotype (Alonso and Ecker 2006). These limitations, as well as limitations associated with the statistics of segregating characters can be overcome through the so-called *genetical genomics,* which uses reverse genetics (or *reverse genomics*), transcriptome and genome sequences to infer the trait under investigation. In reverse genetics, gene sequences are known and mutants are screened to identify individuals with structural alterations in the gene of interest (Nagy et al. 2003). This approach is generally less time intensive than forward genetics; it was originally applied by combining upregulated genes detected by microarrays with QTL mapping associated with polymorphic probes in a segregating population. Reverse genetics strategies also include homologous recombination, *Agrobacterium*-mediated insertional mutagenesis, transposon tagging, RNA interference (RNAi) and chemical mutagenesis, i.e., targeting induced local lesions in genomes (TILLING) (Barkley and Wang 2008).

The widespread availability of gene sequences allows the quick design of reverse genetics strategies for specific gene functions. Gomes et al. (2010) proposed the investigation of *J. curcas* QTLs using quantitative real-time PCR (qRT-PCR) of ESTs homologous to the sequences of enzymes specific to the particular metabolic pathway of the QTL of interest. QTL mapping can be carried out by making use of the allelic polymorphism of the QTL candidates or by looking for SNPs in intergenic sequences that are easily tagged from the whole genome sequence (Sato et al. 2011). This route of QTL inference is now available following the sequencing of a large number of ESTs in *J. curcas* (Costa et al. 2010; Gomes et al. 2010) and the publication of its whole-genome sequence by Sato et al. (2011). The total size of the first non-redundant genomic sequence of *J. curcas* publicly available is 285,858,490 bp (Sato et al. 2011); it corresponds to the cumulative size of 22 relatively small metacentric and submetacentric chromosomes ranging from 1.71 to 1.24 μm (Carvalho et al. 2008). The difference between the size of this genome sequence and that obtained by flow cytometry (~410 Mbp) is probably due to the incomplete resolution of repeated sequence by the whole-genome sequencing approach. The genomic sequence accounted for ~95% of the gene-containing regions and showed an average GC content ~34.3% (Sato et al. 2011), which is also

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lower (~4%) than the value obtained by flow cytometry (Carvalho et al. 2008) and suggests that some GC-rich regions were not fully sequenced. A total of 40,929 complete and partial structures of protein encoding genes have been inferred from this sequence (Sato et al. 2011), which is approximately two times the number expected for a diploid plant (~25,000). A possible explanation for this redundancy factor of ~2 would be an event of polyploidization in the past history of *J. curcas*, as was suggested by Carvalho et al. (2008) on the basis of karyotype observations. This hypothesis is, supported by the size (21,225) of the unigene set obtained by pyrosequencing of cDNAs (Sato et al. 2011).

Transformation of soybeans with either *A. tumefaciens* or *A. rhizogenes* has been effective in studying gene function, although this process is not very efficient and can be limited by genotype specificity. Directed co-suppression, overexpression of genes and RNAi have all been used to gain insights into gene function and to produce desired phenotypes in transgenic soybeans. Although the use of transgenic technology is suitable for traits that can be utilized for most cultivated crops, the current regulatory environment is prohibitive related to the extension of this technology to a large number of traits. In contrast, TILLING is not subject to specific regulation or to genotype specificity. This technique was originally developed using Arabidopsis (Greene et al. 2003) and has been successfully applied to poplar, soybean, maize, sorghum, rice, barley, wheat, pea, lotus and rapeseed. TILLING is expected to be well suited for *J. curcas* because the genetic variability in natural populations of this species seems to be low. Additionally, *J. curcas* can be self fertilized, and its seeds can be stored for long periods of time.

The procedure used in TILLING was described in detail by Barkley and Wang (2008). Basically, TILLING is carried out as follows: (1) seeds are treated with a mutagen (typically EMS) and the resulting plants are subsequently self-fertilized; (2) DNA is extracted and DNA samples are normalized; (3) DNA samples are then amplified by PCR for the target gene; (4) heteroduplexes and homoduplexes are formed from the PCR products of pooled samples (consisting of mutants and wild-type individuals) by heating (denaturing) and cooling (annealing); (5) heteroduplexes are cleaved at mismatches by *CEL* I and (6) detected by DGGE.

QTL inference by reverse genomics may, of course, be complicated by the fact that gene expression varies quantitatively according to genetic background, tissue type and physiological state, indicating that gene expression is controlled by many possible key regulators (Kirst et al. 2004) with pleiotropic or epistatic effects. Heterosis can result from epistatic interactions among alleles at different loci. Therefore, it is not surprising that it is affected by many genetic loci and by genetic backgrounds (Chen 2010). Overdominance and pseudo-overdominance are the basis of heterosis or hybrid vigor, but the molecular bases of hybrid vigor remain elusive. Recent studies have determined the roles of nonadditive gene expression (Thiemann et al. 2010), small RNAs, and epigenetic regulation in hybrid vigor. Heterosis is more predominant in outbreeding than inbreeding species and inbreeding populations do not exhibit an obvious heterosis of fitness, which is expected

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because their genetic distance is lower. Inbreeding depression is predominantly caused by the cumulative effects of deleterious mutations at many loci (see Charlesworth and Willis 2009 and references therein). It has been argued that the directional changes observed in transcriptomic, proteomic and metabolomic profiles in replicate inbred lines are a cellular response induced by the expression of the genetic load (see Kristensen et al. 2009 and references therein).

Nonadditive gene expression is also controlled by posttranscriptional mechanisms via RNA-mediated pathways (Chen 2007; Ha et al. 2009) that serve as negative regulators of gene expression by targeting RNA degradation or translational repression (Bartel 2004). Combining miRNAs and their targets of different parental origins in hybrids or new allopolyploid species may reprogram their expression and their targets. Indeed, many miRNA targets are nonadditively expressed in allotetraploids, suggesting a role for miRNAs in buffering genetic conflicts between species (Ha et al. 2009).

Characters

The publication of the whole genome sequence of *J. curcas* by Sato et al. (2011), together with the KEGG resource for metabolic pathways present the possibility of carrying out reasoned reverse genetics. The considerations related to genetic genomics and QTL structure suggest that genetical genomics could be used to develop a genetic map of *J. curca* around the search of QTLs for agronomic traits. Thus, the immediate investigation of traits such as tree size, tree structure, inflorescence structure, flowering time, seed number, seed size, oil content, disease resistance and stress tolerance through genetical genomics may decrease the delay between the construction of a genetic map and the associated economic returns from the development of commercial lines. Therefore, it is worthwhile to review the genes and factors that could be modified by transgenesis and RNAi or other techniques and that could assist in the domestication of *J. curcas*.

miRNAs have only recently begun to be investigated; they play vital roles in the regulation of key components of hormone signaling pathways and stress responses (Zhang et al. 2006). Two miRNAs were confirmed to regulate the auxin response factor (ARF) (Mallory et al. 2005; Wang et al. 2005a; Wu et al. 2006; Yang et al. 2006). ARFs are auxin receptors whose targets are short-lived repressors of transcriptional activators for ubiquitin-mediated degradation through the proteasome pathway (Dharmasiri et al. 2005; Navarro et al. 2006). Several miRNAs are also induced and regulated by *abscisic acid* (ABA) (Sunkar and Zhu 2004; Reyes and Chua 2007; Liu et al. 2009b). ABA modulates a number of key growth and physiological processes in plants, including suppression of seed germination, maintenance of seed dormancy by inhibiting cell growth, induction of stomatal closure, thereby minimizing transpiration to prevent water loss, and acceleration of abscission and senescence (Finkelstein et al. 2002; Fujita et al. 2005). Other miRNAs were found to be downregulated by gibberellin (Liu et al. 2009b). Additionally, miR319 controls jasmonate biosynthesis to coordinate the

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balance between leaf growth and senescence (see Liu and Chen 2010 for a review). Jasmonic acid has been extensively characterized (Pauwels et al. 2010). It activates genes involved in pathogen and insect resistance as well as genes encoding vegetative storage proteins. Jasmonate modulates the expression of numerous genes and influences specific aspects of plant growth, development, and responses to abiotic and biotic stresses (Creelman and Mullet 1997). These examples give an idea of the level of complexity of the interactions at play in the molecular network that underlies plant functions.

Plant Architecture

Higher plants display a variety of architectures that are defined by the degree of branching, internodal elongation, and shoot determinacy (Wang and Li 2008). Plant architecture has been found to be correlated with agronomical traits and the ability of plants to survive environmental stress (Peng et al. 1999). The Arabidopsis *REVALUTA* (*REV*) gene has been identified as a key regulator of apical meristem initiation. Mutations of this gene usually lead to severe reduction of the number of branches (Wang and Li 2006). Determining the regulation of branching by the homologous gene of *J. curcas* could be interesting. The control of dwarf genes that reduce plant height and branching would benefit the production of fruit crops, as has been the case for wheat, leading to the so-called 'green revolution' (Dyson 1996). The modern varieties are shorter than the older ones, but with increased grain yield at the expense of straw biomass.

Mutants defective in the biosynthesis of gibberellin are severely dwarfed and require exogenous gibberellin application for flower and fruit development. In apple trees, the heritability of shoot and internode length was demonstrated by quantitative genetic approaches. In this species, it was found that internode length was one of the most heritable characters among the numerous variables correlated with overall tree size.

Brassinosteroids also induce dwarfed phenotypes, but the ability to produce flowers and fruits is maintained in the affected mutants. In the tomato, mutations at the *dwarf* and *dumpy* loci cause brassinosteroid deficiency, whereas mutations at the *Curl3* locus affect brassinosteroid signaling. The *dwarf* gene encodes a cytochrome P450 monooxygenase (CYP or P450) that catalyses the C-6 oxidation of 6-deoxocastasterone to castasterone, the immediate precursor of the most bioactive brassinosteroids (see Montoya et al. 2005 and references therein). At present, nine genes have been reported to be involved in their biosynthesis (Pereira-Netto 2007).

Several dwarf phenotypes have been observed in the F_1 progeny of interspecific hybrids between *J. curcas* and other species of the genus *Jatropha* (Parthiban et al. 2009), suggesting that genes of the brassinosteroid or gibberellin pathways were affected or at least silenced through epigenetic processes.

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Plant architecture is controlled by the action of several different plant hormones, including, cytokinins, gibberellins and brassinosteroids. Cytokinins positively regulate cell division and are required for meristem function and maintenance. Cytokinins also may play a role in long-distance signaling for nitrogen availability in the promotion of branching development (Sakakibara et al. 2006). The upregulation of the *LOG* gene involved in the final step of bioactive cytokinin synthesis has the potential to promote panicle and flower development (Kurakawa et al. 2007). The increased branching mutants known as *rms* (ramosus) in the pea and *max* (more axillary branches) in Arabidopsis have been shown to be involved in branching control and in cytokinin homeostasis in shoots and roots (Ma 2008). The reduced expression of *OsCKX2* causes cytokinin accumulation in inflorescence meristems and increases the number of reproductive organs, resulting in enhanced grain yield (Ashikari et al. 2005). Cytokinins are believed to mediate signaling events that control leaf senescence. This can be observed in crop cultivars with different senescent phenotypes (He et al. 2005). It was observed that there was a remarkable delay of leaf senescence when the senescence-related promoter *SAG12* from Arabidopsis was fused with the isopentenyl transferase gene from the Ti-plasmid of *Agrobacterium tumefaciens* (*ipt*) and transfected into tobacco. Isopentenyl transferase is believed to be the control point for cytokinin synthesis. The expression of *ipt* was found to be automatically initiated, and cytokinin synthesis was increased when leaves begin to undergo senescence (Jordi et al. 2000). A delay of leaf senescence was also found by altering other genes, such as *ARR2*, *AHK2* and *AHK3* (Kim et al. 2006).

Gibberellins and auxin act antagonistically to cytokinins, with gibberellins promoting cell differentiation and auxin promoting organ initiation (see Chevalier et al. 2008 and references therein). Brassinosteroids are considered to be hormones with pleiotropic effects, as they influence developmental processes, such as growth, germination of seeds, rhizogenesis, flowering and senescence (Rao et al. 2002).

Flowering and Fructification

Shifting the seasonal timing of reproduction is a major goal of plant breeding efforts to produce novel varieties that are better adapted to local environments and changing climatic conditions. Flowering represents the transition from the vegetative to the reproductive phase in the plant life cycle. In Arabidopsis, distinct pathways, including pathways related to vernalization, photoperiod, and gibberellin, as well as autonomous pathways, form a regulatory network that controls the timing of flowering (Baurle and Dean 2006; Williams et al. 2005). In this network, *FLOWERING LOCUS C* (*FLC*) plays a central role in repressing the floral transition, largely by reducing the expression of three key floral integrators: *FLOWERING LOCUS T* (*FT*), *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1* (*SOC1*) and *FLOWERING LOCUS D* (*FD*) (Michaels and Amasino 1999; Searle et al. 2006). In short, the gene system of flowering is as follows: *Constans* (*CO*) is regulated (1) at the transcriptional level by several genes that are under circadian control,

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including *Gigantea* (GI) and CYCLING DOF FACTOR 1 (*CDF1*) and (2) at the protein level by photoreceptors (phytochromes and cryptochromes) that stabilize or de-stabilize CO (Turck et al. 2008; Jackson 2009). Photoperiod control of floral transition through CO and homologous genes is widely conserved among flowering plants (Turck et al. 2008) and predates the appearance of angiosperms (Serrano et al. 2009). However, short-day plants and day-neutral plants differ from the scheme of long-day species (Turck et al. 2008). HEADINGDATE 1 (*Hd1*) promotes flowering and expression of the rice FT homolog HEADINGDATE 3a (*Hd3a*) under short-day conditions and represses Hd3a in long days. FT orthologs might also lack regulation by photoperiod but still act as promoters of flowering, as is the case for the FT-like gene SINGLE-FLOWER TRUSS (*SFT*) in the day-neutral plant tomato (Lifschitz et al. 2006). Codependent photoperiod pathway can be also bypassed by a regulatory mechanism that involves the circadian clock genes LATE ELONGATED HYPOCOTYL (*LHY*) and CIRCADIAN CLOCK-ASSOCIATED *1* (*CCA1*) (Fujiwara et al. 2008) by regulating protein accumulation of SHORT VEGETATIVE PHASE (*SVP*), a MADS-box protein that physically interacts with FLC and inhibits flowering by repression of floral integrator genes (Fujiwara et al. 2008; Li et al. 2008b). FLC represses FT in leaves and thus prevents production of FT protein, the mobile signal that moves to the shoot apex and promotes flowering by induction of AP1. The inhibitory action of FLC is antagonized by activation of FT in leaves by CO. Another FT-homologous protein, TERMINAL FLOWER 1 (TFL1), acts antagonistically to FT and may have a function in regulating the length of the inflorescence phase between induction of FT expression and conversion of the shoot apical meristem into a floral meristem. The actual formation of flower meristems requires a set of genes related to floral identity, including LEAFY (LFY), AP1, FUL and CAL, which mainly act downstream of the floral integrators. The gene system underlying flowering has been extensively studied and has been found to be largely conserved between dicots and monocots (Greenup et al. 2009). A recent review on this topic by Jung and Müller (2009) is available.

Now that many of the floral regulatory genes have been identified, their sequences can be used by breeders as functional markers for selecting favorable genotypes, for quality control of seed lots (see discussion below), or for targeted manipulation of flowering traits by genetic modification. However, such manipulation could have collateral effects on plant architecture, as associations have been found between SNPs in *CO-LIKE* and branch length in a map region of *Medicago truncatula* where QTLs were observed for both flowering date and branch length (Julier et al. 2007). Additionally, a *CO-LIKE* gene of Arabidopsis showed an effect on stem height in Arabidopsis and in potatoes transformed with this gene (Simon et al. 1996; Martinez-Garcia et al. 2002).

In *J. curcas*, the optimal flowering time may differ between native and cultivated areas. Again, the F_1 progenies developed by Parthiban et al. (2009) constitute a valuable basis to test how genes from flowering and fructification pathways are affected by interspecific hybridization. The cytokinin and flowering pathways are connected. When exogenous application (160 mg L^{-1}) of the cytokinin 6-benzyladenine (BA) was carried out, the total number of flowers per inflorescence significantly increased

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from 215 to 784, representing a 3.6-fold increase. Furthermore, BA treatment induced the production of bisexual flowers, which were not found in control inflorescences, and a substantial increase in the female-to-male flower ratio. Consequently, a 4.5-fold increase in fruit number and a 3.3-fold increase in final seed yield were observed in inflorescences treated with 160 mg L^{-1} of BA, which resulted from the greater number of female flowers and the newly induced bisexual flowers in BA-treated inflorescences. The results of this study indicate that the seed yield of *J. curcas* can be increased by manipulation of floral development and the expression of floral sex (Pan and Xu 2010). Eight orthologs of flowering-related genes, including five flowering regulators, *CONSTANS*, *FD*, *FF*, *LFY*, and *SOC1*, and three genes for floral identity, *APETALA2*, *APETALA3*, and *PISTILLATA*, were found by Sato et al. (2011).

Jasmonate may also be expected to play a role in the formation of flowers, fruits, and seeds because of the relatively high levels of this compound in reproductive tissues. Other aspects of flower, fruit, and seed development that can be modulated by jasmonate include fruit ripening, fruit carotenoid composition, and the expression of genes encoding seed and vegetative storage proteins. Jasmonate-stimulated fruit ripening most likely occurs through the production of ethylene (Czapski and Saniewski 1992). It is possible that jasmonate levels gradually increase in developing fruit, leading to enhanced synthesis of ethylene and subsequent fruit ripening (Creelman and Mullet 1997). The characteristics of hormonal and environmental signal transduction factors in fruit ripening are conserved among genes and regulatory motives. Shortly, ethylene is perceived by a family of ethylene receptors (ETR). Acting downstream of receptors is a putative MAP-kinase kinase kinase (MAPKKK), termed CONSTITUTIVE TRIPLE RESPONSE (CTR). A MAP-kinase cascade has been implicated in the mediation of the ethylene response downstream of CTR, whereby a MAPKK activates an ethylene-inducible MAPK protein. The ethylene receptor (EIN2) involved in this process may represent a common convergence point through which multiple hormone signal transduction pathways, including those of abscisic acid, auxin, cytokinin and jasmonate, may act. EIN2 would operate upstream a family of nuclear localized trans-acting proteins whose homodimers bind to a defined target site in the promoter region of the transcription factor, ETHYLENE RESPONSE FACTOR 1 (*ERF1*). ERF1 is part of a large multi-gene family of transcription factors and is important in the regulation of downstream ethylene responsive genes (Adams-Phillips et al. 2004).

miR156 and miR172 are two other important regulators that have been implicated in promoting the floral transition through the regulation of *SBP-box* genes and *AP2*-like genes, respectively. miR159 has also been found to regulate flowering time and anther development through targeting of GAMYB (an activator of LY, an important factor in floral development). Increased levels of miR159 cause a reduction in the level of *LFY* transcripts, delay flowering and perturb anther development (see Liu and Chen 2010 and references therein).

Conflicting breeding goals, such as high biomass yields and efficient seed production, could be approached by transgenic strategies. The ectopic expression of *FT* or its homologs has been very successful in converting the vegetative shoot apical mer-

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istems of tree species into inflorescence meristems. One example of this comes from poplar (*Populus trichocarpa)* trees, which normally begin flowering after 8–20 years. However, male poplar trees transformed with the *FT* homolog *PtFT1* under transcriptional control of the 35S promoter initiated the production of flower-like structures directly from *Agrobacterium*-infected stem segments within 4 weeks (Böhlenius et al. 2006). Extremely early flowering and fruiting was also observed in trifoliate oranges after the ectopic expression of an *FT* homolog from citrus (Endo et al. 2005).

Seeds

The life cycles of plants differ from those of animals in that the products of meiosis undergo mitotic proliferation to form multicellular gametophytes (the embryo sac and pollen). The embryo sac (female) contains an egg cell, which is haploid, and this is fertilized by a sperm nucleus, which is also haploid, to form a diploid embryo. A second sperm nucleus fertilizes the central cell, which is diploid, to form triploid endosperm, an extra-embryonic tissue that has a supportive role during embryogenesis.

Several genes that act as positive regulators of the seed development program have been identified. These include: *LEAFY COTYLEDON* (*LEC*), *ABSCISIC ACID INSENSITIVE 3* (*ABI3*), *BABY BOOM* (*BBM*), *AGAMOUSLIKE15* (*AGL15*), *WUSCHEL* (*WUS*), and *SOMATIC EMBRYOGENESIS RECEPTOR-LIKE KINASE* (*SERK*) (Boutilier et al. 2002; Heck et al. 1995; Laux et al. 1996; Meinke 1992; Schmidt et al. 1997). *AGAMOUS* is member of the family of MADS-box genes, which are typical homeotic genes coding for transcription factors involved in several aspects of plant development. It is of note, here, that 28 potential MADS-box genes (*JcMADS01 – JcMADS28*) have been identified in the genome sequence of *J. curcas* (Sato et al. 2011).

Three categories of proteins have been characterized as having roles in repressing the seed transcriptome in other stages of plant development. These include (1) histone modifiers (PcG and HISTONE DEACETYLASE6/19 – HDA6/HDA19), (2) chromatin remodelers (PICKLE – PKL and BRAHMA – AtBRM1), and (3) transcription factors (VP1/ABSCISIC ACID INSENSITIVE 3-LIKE – VAL) (Zhang and Ogas 2009).

The central cell and the endosperm exhibit parent-of-origin-dependent monoallelic expression, or imprinting, which is important for proper seed development. Imprinting is an epigenetic phenomenon in plants and animals that refers to the monoallelic expression of specific genes in a parent-of-origin-dependent manner. In mammals, silent alleles of imprinted loci are targeted for the methylation of cytosine residues during gametogenesis, whereas expressed alleles generally remain relatively undermethylated. Silenced alleles are, then, protected from global demethylation during early embryogenesis to achieve monoallelic expression later in development. Imprinting in mammals is a process of selective silencing, and the

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default state is activity (Scott and Spielman 2004). Imprinted genes are maternally expressed, but paternally silenced or *vice versa*. DNA methylation and histone modifications have been found to play a role in monoallelic gene expression. Once established in the germline, these epigenetic marks are maintained throughout the lifecycle. In plants, imprinting is confined to the endosperm (Jullien and Berger 2009; Makarevich et al. 2008). The imprinted genes share a common feature in that they are usually less methylated in the endosperm than the embryo, thus exhibiting endosperm-specific expression, with low expression being observed in other tissues of the plant (Day et al. 2008; Gehring et al. 2009). At present, there are at least 10 known imprinted genes in Arabidopsis, amongst which *MEDEA* (*MEA*), *FWA*, *FIS2*, *MATERNALLY EXPRESSED PAB C-TERMINAL* (*MPC*), *HOMEODOMAIN GLABROUS8* (*HDG8*), *HOMEODOMAIN GLABROUS9* (*HDG9*) and *AtMYB3R2* are maternally expressed, whereas *PHERES1* (*PHE1*), *HDG3*, and *At5G62110* are paternally expressed.

In *J. curcas*, it is the endosperm that accumulates oil, and the removal of the parental imprinting could have interesting consequences on seed and oil yield, as it has been observed to lead to the overproliferation of the endosperm in Arabidopsis (Berger 2003).

Fatty Acid Synthesis

Selection for breeding and genetic engineering resulting in elevated oleic acid levels have been reported in many oilseed crops, including safflower (Knowles and Hill 1964), sunflower (Liu et al. 2002), peanut (Jung et al. 2000; Bruner et al. 2001; Patel et al. 2004), canola (Stoutjesdijk et al. 2000; Hu et al. 2006), cotton (Liu et al. 2002) and maize (Beló et al. 2008). Significant variation of the oil content in *J. curcas* was described in Indian accessions (Popluechai et al. 2009). The chemical composition of *J. curcas* oil is rather simple, as it contains 1.4% myristic (C14:0), 10.5–15.6% palmitic (C16:0), 2.3–9.7% stearic (C18:0), 40.8–48.8% oleic (C18:1), 32.1–44.4% linoleic (C18:2) and 0.4% arachidic (C20:0) acids (Foidl et al. 1996; Nahar et al. 2005; Adebowale and Adedire 2006; Martínez-Herrera et al. 2006; Kumar and Sharma 2008; Singh and Singh 2010; Gomes et al. 2010). Seed oil content is typically a QTL, and genes related to fatty acid biosynthesis are expected to tag this trait.

In *J. curcas*, oil is stored in the endosperm, which is a triploid tissue due to the double fertilization (Dumas and Rogowsky 2008). Reference to a diploid model of allele segregation may result in errors when assessing this type of QTL because it may lead to neglecting the paternal contribution due to pollen fertilization (Kao 2004). To increase the accuracy of QTL assessment, a specific F_3 breeding scheme involving selfing or backcrosses with the parents and the F_1 is necessary (Wang et al. 2009a, b).

With respect to reverse genetics approaches, the use of probes for fatty acid pathways should be beneficial for the selective breeding of *J. curcas* for oil quality and yield, or at least to follow the rate of these traits when breeding for other traits of

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agronomical interest, such as those listed previously. In this respect, *3-ketoacyl-CoA thiolase B* is a candidate for tagging QTLs associated with oil (Gomes et al. 2010). Other interesting candidates are *acetyl-CoA carboxylase I*, *3-ketoacyl-ACP I* and *III*, *acyl-ACP desaturase*, and *acyl-ACP thioesterase II*, *ATP-citrate lyase*, *acetyl-CoA carboxylase II*, *3-ketoacyl-ACP II*, *3-ketoacyl-ACP reductase*, *3-hydroxyacyl-ACP dehydrase*, *enoyl-ACP reductase*, *acyl-ACP thioesterase I*, and *acyl-CoA dehydrogenase* (Ambrosi et al. 2010b). The expression profile of *3-ketoacyl-CoA thiolase B* actually matches the profile of fatty acid accumulation described by NMR (Annarao et al. 2008). Following Annarao et al. (2008), the oil content increases in two major steps, with a very clear transition occurring around stage IV when the oil content increases from 3% to 18%, whereas TAGs increase from 30% to 90%. The fresh weight reaches its maximum (~1,000 mg) at stage V, i.e., stage 3 of the experiment of Gomes et al. (2010), and then decreases to ~640 mg during stages VI and VII. These last two stages parallel fruit ripening, which is accompanied by a color change from green to yellowish. However, Gomes et al. (2010) found that the basal activity of this enzyme in leaves is also relatively high, which indicates that it is involved in alternative functions that could interfere with the general performance of breeding individuals.

Map-based cloning of a diacylglycerol acyltransferase (*DGAT*) that catalyzes the final step in the glycerol biosynthetic pathway allowed the selection of a new protein variant affecting the oil content and composition in maize seeds (Zheng et al. 2008). QTLs for C16:0, C18:0, C18:1, C18:2, C18:3, C20:1 and C22:1 were also described in rapeseed (Zhang et al. 2008). In soybeans, oil utilization is determined by the fatty acid composition, with commodity soybean oil typically containing 13% palmitic acid (16:0), 4% stearic acid (18:0), 20% oleic acid (18:1), 55% linoleic acid (18:2), and 8% linolenic acid (18:3).

High oleic acid content is required for biofuel production. Conventional soybean lines with 80% oleic acid accompanied by a corresponding decrease in linoleic acid were obtained based on the contribution of only two genes (*FAD2-1A* and *FAD2-1B*) by tracking mutant alleles with polymorphic markers by MAS (Pham et al. 2010). It appears that the disfunctioning of *FAD2-1A* promotes higher oil yields in the soybean. The same conclusion has been reached using TILLING (Dierking and Bilyeu 2009). Another important gene that has been reported to control the regulation of seed oil accumulation in rape is *GLABRA2* (*GL2*), a class IV homeodomain-ZIP transcription factor (Chai et al. 2010).

Disease Resistance and Tolerance to Stress

The plant immune system relies to a great extent on the highly regulated expression of hundreds of defense genes encoding antimicrobial proteins, such as defensins, and antiherbivore proteins, such as lectins. Most of these defense genes are nucleotide-binding site and leucine-rich repeat (NBS-LRR) proteins, which are classified into two groups on the basis of the presence of *Toll* and human interleukin receptors

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(TIR) at their amino termini; 42 TIR NBS-LRR proteins and 50 non-TIR NBS-LRR proteins were identified in *J. curcas* by Sato et al. (2011). The expression of many of these genes is controlled by jasmonates (~20 members). Several classes of transcription factors are known to function in the jasmonate pathway, and in some cases, these proteins provide nodes that integrate this network with other important defensive and developmental pathways (Staswick 2007). A role for jasmonic acid in protein storage in plants was suggested, in part because jasmonate levels are high in vegetative sinks. Many proteins involved in plant defense, in particular those that are sequestered in vacuoles, are ideally suited for having a role as vegetative storage proteins (Creelman and Mullet 1997).

Disease invasion represents a major biotic stress to plants. Disease resistance is linked with the natural physiological status of plants, particularly senescence. Therefore, natural tissue senescence increases susceptibility to necrotizing pathogens. Transferring the *CaMV 35S-ipt* gene into tobacco led to greatly delayed leaf senescence (Pogány et al. 2004).

Drought is one of the most important abiotic stresses for plants because of the considerable crop yield losses that may result. Plants have evolved specific strategies related to the fact that they are sessile organisms, unlike animals, which can move freely in response to environmental stresses. QTLs for drought tolerance have been identified for a variety of traits in different crops. Although *J. curcas* is known for its ability to persist in semi-arid conditions, seed yields have been observed to fall to zero under these conditions. Finding traits that represent the best compromise between resistance to drought and seed yield should certainly be a goal in this crop species. With respect to this aim, Eswaran et al. (2010) found 32 full length genes out of 20,000 yeast transformants screened for heterologous gene sequences from *J. curcas* involved in abiotic stress tolerance. Twenty three sequences of the 32 stress tolerance genes were reported to have a strong role in conferring abiotic stress tolerance in other plant species (Ashraf 2010).

With the development of comprehensive molecular linkage maps, MAS procedures have led to pyramiding desirable traits to achieve improvements in crop drought tolerance. In dicots, drought tolerance-related QTLs have been described in a number of species, including cotton (Saranga et al. 2001) and soybean (Chen et al. 2007). In cotton, 11 QTLs were found for plant productivity, 5 for key physiological traits, and 17 for fiber quality.

Due to unfavorable epistatic interactions, it is difficult to transfer favorable QTL alleles to elite background material (Podlich et al. 2004; Collins et al. 2008). The limited success that has been achieved in improving crop drought tolerance could be due to the fact that the drought tolerance trait is controlled by multiple genes having additive effects and that there is a strong interaction with genes involved in yield potential. Thus, there is a need for more efficient methods to enhance drought tolerance. In the case of the limited genetic variability of *J. curcas*, a transgenic strategy for the pyramiding of genes with similar effects would probably be desirable (Ashraf et al. 2008; Gosal et al. 2009). Knowledge of gene regulation and signal transduction through genomics and proteomics will certainly serve this purpose. However,

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optimal agronomical practices in agreement with the physiology of *J. curcas* must be characterized first.

Ashraf (2010) listed 26 genes that were used to improve plant drought tolerance through the transformation of 13 different species for factors such as organic osmolytes, transcription factors, late embryogenesis proteins, and hormones.

Many organic osmolytes are known to play a substantial role in stress tolerance, including glycine betaine (GB), which requires choline monooxygenase (CMO) and betaine aldehyde dehydrogenase (BADH) for its biosynthesis, and trehalose, a potential organic osmoticum with a substantial role in the protection of plants against stresses (Romero et al. 1997; Jang et al. 2003; Wang et al. 2005b).

The accumulation of reactive oxygen species (ROS), resulting in oxidative stress, is another process induced by drought that has been described in *J. curcas* (Eswaran et al. 2010), among other species. The overproduction of antioxidants in response to drought was found to be associated with drought stress tolerance (Pastori and Foyer 2002; Sunkar et al. 2006). For instance, the overexpression of superoxide dismutase, which reduces singlet oxygen (McKersie et al. 1996; Perl et al. 1993), or tocopherol cyclase (Liu et al. 2008) caused enhanced drought tolerance. Transgenic Arabidopsis plants expressing the helicase 1 from *Medicago sativa* exhibited improved seed germination and plant growth under drought, salinity, and oxidative stresses, which was found to be associated with higher (1) osmotic adjustment, (2) ascorbate peroxidase and superoxide dismutase activities and (3) proline content (Luo et al. 2009).

Engineering of the genes for late embryogenesis abundant proteins (LEA) in rice (Cheng et al. 2002), wheat (Sivamani et al. 2000), barley (Xu et al. 1996), lettuce (Park et al. 2005a), rapeseed (Park et al. 2005b; Dalal et al. 2009) and *J. curcas* (Eswaran et al. 2010) resulted in enhanced drought tolerance, which was probably due to the role of these proteins in the maintenance of cell membrane structure and ion balance, the binding of water, and their activity as molecular chaperones (Babu et al. 2004).

A fusion construct consisting of the *SARK* promoter and the *ipt* gene for cytokinin synthesis was introduced into tobacco plants. *SARK-ipt* expression reached maximal levels in all leaves of transgenic plants during drought stress, which was accompanied by a significant increase in *trans*-zeatin levels. This suppression of drought-induced leaf senescence resulted in exceptional drought tolerance, and the transgenic tobacco exhibited vigorous growth after a 2-week drought period that resulted in the death of control plants (Rivero et al. 2007).

As noted above, ABA is another plant hormone that is important in the response to abiotic stresses. ABA production is enhanced under limited water conditions; it can effectively protect plants against drought stress (Shinozaki and Yamaguchi-Shinozaki 2000; Alexandre et al. 2009) and activates drought stress-inducible genes (Ingram and Bartels 1996; Seki et al. 2002). ABA also activates assembly protein 1 (NAP1), a type of histone chaperone involved in chromatin remodeling (Liu et al. 2009c). The promoters of ABA-regulated genes have a highly conserved *cis*-acting *ABA-responsive element* (ABRE) (Fujita et al. 2005). Chaperones were also found to be induced by drought in *J. curcas* (Eswaran et al. 2010).

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Several transcription factors are involved in gene regulation in plants under limited water conditions (Bartels and Sunkar 2005; Vinocur and Altman 2005). The upregulation of genes for dehydration-responsive elementbinding factors (DREB) promoted tolerance to a variety of stresses, such as drought, salinity and freezing, in transgenic Arabidopsis plants (Qin et al. 2007; Bhatnagar-Mathur et al. 2007). Similarly, transformed rice plants overexpressing transcription factors from the APETALA group, such as AP37 and AP59, under the constitutive promoter *OsCc1* showed enhanced resistance to considerable drought and salinity stresses. Additionally, miRNAs also respond to drought, cold, salinity and oxidative stress (see Liu and Chen 2010 and references therein).

Toxicity

Phorbol esters are potent cancer promoters (Blumberg 1988; Goel et al. 2007) found in the seeds of *J. curcas* (Makkar et al. 1997). Low phorbol or zero phorbol accessions occur in natural populations, particularly those from Mexico (Ferrari et al. 2009), and have also been found in interspecific hybrids between *J. curcas* and *J. integerrima* (Popluechai et al. 2009). Furthermore, *J. platyphylla* Müll.Arg. is another phorbol zero species from the *Jatropha* genus that could be used for interspecific breeding (Makkar et al. 2011).

Geranyl diphosphate synthase (GPPS), farnesyl diphosphate synthase (FPPS) and geranylgeranyl diphosphate synthases (GGPPS) are key enzymes upstream the monoterpene, sesquiterpene and diterpene biosynthesis pathways, respectively (Schmidt et al. 2010). Altering the levels of the expression of *GGPPS* has helped to more clearly define the influence of GGPPS on the regulation of diterpene biosynthesis in plants (Lin et al. 2010). For example, in tobacco, suppression of the expression of the *GGPPS* gene results in a dramatic reduction of the levels of 17-hydroxygeranyllinalool diterpenoid glycosides (HGL-DTG) (Jassbi et al. 2008). These results indicated that the GGPPSs have a universal role in diterpene biosynthesis. Sato et al. (2011) found genes for GGPPS, casbene synthase, terpene hydroxylase (cytochrome P450-dependent monooxygenase) and five to seven curcin genes, which are also toxic components (Lin et al. 2003), in the genome sequence of *J. curcas*. In *Humulus lupulus*, GPPS was shown to be a heterodimer including a small subunit (SSU) and a large subunit (LSU), with only LSU being active and leading to the synthesis of GPP, FPP and GGPP (Wang and Dixon 2009). Gomes et al. (2010) showed that the GPPS gene is overexpressed by a factor ~25 in developing fruits in comparison to leaves. Because of this large difference of GPPS expression between leaves and fruits, Gomes et al. (2010) suggested that cDNA corresponding to this gene could be used as a probe in breeding for the QTL of *J. curcas* associated with phorbol synthesis. However, GPPS has been found to also be involved in the cytokinin biosynthesis pathway (Abe et al. 2007), and breeding for this trait can have unpredictable secondary effects. Now that the whole genome sequence of *J. curcas* is available, reverse genetics approaches for the expression of genes homolo-

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gous to the enzyme sequences of the KEGG repository involved in the pathway of diterpene biosynthesis have become more feasible. This type of closer investigation would likely lead to finding probes of higher specificity for phorbol QTLs. A complementary initiative would be to investigate TILLING lines (Dierking and Bilyeu 2009) for probes associated with candidate genes for phorbol-related QTLs.

From Gene Sequences to Breeding Tools

Progress in plant breeding has been made possible by the accumulation of beneficial alleles from the vast plant genetics resources existing worldwide. The rapid accumulation of sequence and expression data in genomic databases has made genetic prospecting possible through PCR strategies for useful alleles of key genes from a wide range of species conferring resistance to biotic and abiotic stresses, greater nutrient use efficiency, enhanced yield and improved quality (Latha et al. 2004). Though most mutations are deleterious, approximately 0.1% of mutations lead to alterations in gene function that may be necessary for the survival of the plant. *Allele mining* is a promising approach to dissect naturally occurring allelic variation of candidate genes controlling key agronomic traits that has potential applications in crop improvement. Many disease resistance alleles are better expressed in wild species and were lost during evolution and domestication (Kumar et al. 2010a).

Plant breeders must consider trait correlations for either improving correlated traits simultaneously or reducing undesirable side effects of selective breeding. It is useful to distinguish between a simple additive case, in which the effects of the alleles show no interactions, and more complex nonadditive cases. When nonadditive genetic effects contribute to genetic variation, these effects and the associated genetic variation can be utilized to achieve genetic gains for desirable traits through appropriately designed breeding methods (Cooper et al. 2009). Genetic correlations between traits can also be due to correlated physiological functions, such that the first trait leads to the second trait, or the second trait depends on the first trait. The progress of genomic approaches is now allowing the discrimination of intragenic linkage (due to interactions among protein domains and/or promoters) from true pleiotropy. When the goal is to simultaneously increase the trait values of two negatively correlated characters, such as grain yield and protein content, the negative correlation is undesirable from a breeder's perspective.

Dissection of pleiotropic QTLs into quantitative trait genes and, finally, into quantitative trait polymorphism (QTP) contributes to understanding the molecular basis of trait correlations. The more tightly two traits are correlated, the more QTLs they will have in common. Near isogenic lines (NILs) obtained after several generations of backcrossing are useful to eliminate *genetic noise* for studying the effect of a single gene or genome region at cM resolution on multiple traits (Chen and Lübberstedt 2010). Candidate genes for correlated traits may be based on physiological, biochemical, or functional knowledge, or on transcriptional connectivity. Ultimately, the effect of a candidate gene on correlated traits is revealed by mutations transforming an expressed dominant allele into a recessive allele. This *com-*

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plementation approach may help to resolve whether traits are correlated by pleiotropy or by linkage (Lewis et al. 2007).

Complex traits and their correlations are controlled by multiple genes and environmental factors. However, there may be only a small number of QTLs or genes that contribute significantly to trait correlations. It is common practice to consider only the locus with the most explanatory power for the expression of a trait as being representative of a QTL. Although TILLING has the potential to resolve trait correlations at both the gene and sequence polymorphism level, low mutation rates limit its applicability without substantially increasing the number of lines used in TILLING collections (Barkley and Wang 2008). Resorting to zinc-finger nucleases (ZFN) has also been proposed to introduce gene-specific modifications (Townsend et al. 2009) and replacements (Shukla et al. 2009). Combined with high-throughput DNA sequencing, this method can be used for targeting any gene (Townsend et al. 2009).

Modeling

Systems modeling and simulation to support plant breeding involves the ongoing assimilation of knowledge into a theoretical framework that can be used for quantitative predictions of crop yield. Modeling the physiology and genetics of complex traits requires a detailed framework to ensure that important physiological linkages, trait interactions, and internal plant regulatory pathways are effectively simulated. Emergent patterns of crop growth and development that reflect genetic control and biological robustness of the overall plant functioning can be described with object-oriented representation with coarse-to-fine grain zooming (Hammer et al. 2005).

Progress in molecular plant breeding is limited by the ability to predict a plant's phenotype based on its genotype, especially for complex adaptive traits. The models used by agronomists have been designed to quantify the capture and use of radiation, water, and nitrogen within a framework to allow the growth prediction of major organs. They were designed to combine accurate predictions of economic yield (e.g., grain, biomass, or sugar yield) for many crop species in response to climate and management conditions (Keating et al. 2003). A model should be able to take the physiological consequences of genetic variation into account, as this will allow the formalization of economic yield in relation to the environmental reality and the genetic basis of the species under investigation (Hammer et al. 2010).

Genetic interactions have been assessed in the context of systems biology through the description of gene network topologies, determination of their statistical properties and modeling of their genotype-to-phenotype properties (Wagner 2002; Barabási and Oltvai 2004; Bornholdt 2005). A first tentative theoretical framework for investigating the quantitative genetics of gene networks was the NK model (E(N,K)), which simulates the role of nonadditive genetic effects, such as epistasis, in breeding progress using gene networks (Kauffman 1993; Cooper et al. 2002). The simulation of the gene network is obtained according to genetic model scenarios extending from relatively simple initial conditions, indicated by high autocorrelation, to more complex genetics, indicated by low autocorrelation.

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The initial conditions set the gene number, interactions and environment types, and the outcome is breeding progress.

The current perspective obtained from investigations of genetic architecture is that traits are influenced by multiple QTLs that can interact with each other and environmental conditions (Ma et al. 2002; Malosetti et al. 2006), which brings us close to developing QTL models for physiological parameters in crop growth simulation models (Cooper et al. 2009). The idea that should be pursued is that plant growth models should be structured to capture the dynamic interactions of the physiological determinants of crop development and, therefore, be able to predict the consequences of genotype-environment-management interactions to assist in crop improvement in general and molecular breeding in particular (Chapman et al. 2002).

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Chapter 15
Karyology and Genomics of *Jatropha*: Current Status and Future Prospects

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Introduction

The increasing worldwide demand for energy, insecurity on petroleum supply and concerns over global climate change have lead to resurgent interest for alternative energies. The energy systems of the future must depend more on alternative source such as geothermal, wind, solar and bioenergy, since petroleum resources are vanishing. Of these renewable energies, bioenergy should contribute significantly to reduce petroleum consumption and *greenhouse gases* (GHG) emissions. Biodiesel, a methyl ester of fatty acids, made from edible or non-edible vegetable oils, is an appropriate alternative to petroleum diesel. Plant-based fuels are among the best renewable sources, and their use can lead to a better balance of carbon dioxide and other GHG's which are responsible for global warming. More food will need to be produced during the next 50 years than in the entire history of humankind and increasing crop yield is a major challenge for the twenty-first century. The use of

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food crops for energy production could worsen food shortages. To warrant the sustainable large scale production of biofuel without compromising the production of food, plant species capable of growing on marginal lands with minimal agricultural inputs are essential (Fairless 2007; Parawira 2010).

Jatropha curcas L. is seen as a promising option for the production of biofuel and has been brought to the miracle-tree status because of its ability to simultaneously produce biodiesel, reclaim wasteland and promote socio-economic development (Francis et al. 2005). Large scale cultivation of *J. curcas* remains the single most important issue that will ultimately decide of its success. Despite the availability of a germplasm with a wide variability for a variety of economic and agronomic characters, not much progress has been made in developing varieties or hybrids with improved performance. The information on karyology and genomics of a plant is necessary for the mapping of *quantitative trait loci* (QTL), generation of mapping population, *marker assisted selection* (MAS) and development of superior genotypes. The small genome size (Carvalho et al. 2008; Sato et al. 2011), ease of vegetative multiplication (Kumar and Reddy 2010; Singh et al. 2010) and amenability for genetic transformation (Kumar et al. 2010) are favorable features for the exploitation of biotechnological tools for *J. curcas* improvement.

In this review, efforts made to elucidate the karyology and genomics along with phylogenetic and genomic relationships of *J. curcas* with its sister taxa is discussed.

Biodiesel

Since it was established that *Jatropha* methyl esters yield biodiesel of an exceptional quality, there has been a surge of interest in *Jatropha* across the globe (Mandpe et al. 2005; Ghosh et al. 2007; Carels 2009; Parawira 2010). For example, the viscosity at 30°C of *J. curcas* oil is lower than that reported for other plant oils (Parawira 2010). The key considerations are the quality of the biodiesel, cost of manufacture, ease of conversion to biodiesel by chemical (Berchman and Hirata 2008) or biological transesterification (Modi et al. 2007). In addition to the relatively low production cost, *J. curcas* biodiesel has been reported to be non-toxic, clean and eco-friendly (Jha et al. 2007). and, addition, by-products are available to lower the production costs (Ghosh et al. 2007) For example, the cake resulting from the mechanical extraction of oil can be used as a fertilizer (Ghosh et al. 2007) and the organic waste products can be digested to produce biogas (Gubitz et al. 1999).

Biology

The genus *Jatropha* belongs to the family Euphorbiaceae, it is native to South America and widely distributed in South and Central America, Africa and Asia (Fairless 2007). *J. curcas* is a deciduous shrub of ~3 m tall and grows under a wide

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range of arid and semi-arid climatic conditions. It can be cultivated successfully in regions with scanty to moderate rainfall and can be used to control soil erosion. Normally, five roots are formed from seedlings: one at the centre and four at the periphery. The leaves are cordate with 3–5 lobed and are 10–15 cm long; stomata are hypostomatic and paracytic (*Rubiaceous*). The inflorescence is an axillary paniculate polychasial cyme. The flowers are unisexual, monoecious, of yellowish green color and form in glabrous or pubescent cymes at the end of branches (Dehgan and Webster 1978). Cross pollination occurs by entomophily through insects from Hymenoptera, Lepidoptera and Diptera orders (Rianti et al. 2010). Pollination is potentially influenced by both pollen depositions on stigma and nectar availability. Female flowers produce more nectar than male flowers. 50% of female flowers set fruit, with a 53% fecundity rate, 32% apomixes rate and 2:3 seed to ovule ratio (Bhattacharya et al. 2005). Fruits are trilocular capsules, 1.5–3.0 cm long. The seed contains 30–40% oil with 21% saturated fatty acids and 79% unsaturated fatty acids.

Karyology

The *Jatropha* genus accounts for 175 species with 12 species reported in India (Paramathma et al. 2004). Miller and Webster (1966) were the first to study the meiotic chromosomes of five species of *Jatropha* and two species of *Cindoscolus* closely related to *Jatropha.* They reported that the chromosomes are morphologically indistinguishable in all the species of *Jatropha.* Puangpaka and Thaya (2003) compared the chromosomal organization among five species of *Jatropha* (*J. curcas, J. multifida, J. integerrima, J. podagrica* and *J. gossypiifolia*). In most of the species the chromosomes were paired as bivalents at the first metaphase and separated to 11:11 at the first anaphase. The chromosomes of these taxa are 2n = 22 with a haploid number of x = 11 and were found small ranging from 1–3.67 μm (Sasikala and Paramathma 2010). Jha et al. (2007), Soontornchainaksaeng and Jenjittikul (2003) reported similar results. The observation of *J. podagrica* pollen mother cells showed a chromosome number of 2n = 22 with 11 bivalents in metaphase and confirmed that the total number of chromosome in this species is 2n = 22 (Sarkar 1989; Krishnappa and Rashme 1980). *J. maheshwarii* and *J. glandulifera* also have a total chromosome number of 2n = 22 with the 11 chromosomes arranged in equatorial plate at metaphase II of daughter cells (Kothari et al. 1981; Navaneetham et al. 1983). The course of meiosis ends up to tetrads stage and the formation of the bivalents in the eight species *J. multifida, J. podagrica, J. villosa* var. *villosa, J. villosa* var. *ramnadensis, J. maheshwarii, J. glandulifera, J. integerrima* and *J. curcas* was normal. The chromosomal arrangement of *J. curcas* is similar to that of *J. multifida* and both taxa have a meiotic configuration of seven ring II + four rod II. The red and pink flowers of *J. integerrima* have the same meiotic configuration with six ring II + five rod II while *J. podagrica* has a meiotic configuration of eight ring II + three rod II. The karyology of *J. gossypiifolia* determined from cells in the first anaphase was

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Table 15.1 Morphological characterization of the 2n = 22 mitotic chromosomes of *J. curcas* (Carvalho et al. 2008)

Chromosome pairs	Size[a]	Arm ratio[b]	Class[c]
1	1.71	0.90	M
2	1.71	0.90	M
3	1.71	1.76	SM
4	1.71	1.76	SM
5	1.57	1.07	M
6	1.57	1.07	M
7	1.52	1.92	SM
8	1.52	1.92	SM
9	1.43	1.51	SM
10	1.43	1.51	SM
11	1.24	1.18	M

[a]Average value of pairs in μm
[b]Long/short
[c]*M* metacentric, *SM* sub-metacentric

also 11:11. Actually, *J. curcas*, *J. multifida* and, *J. gossypiifolia* appeared closely related to each other when compared for their meiotic configuration and morphological similarities (Table 15.1). Soontornchainaksaeng and Jenjittikul (2003), reported that the species *J. curcas, J. multifida* and *J. gossypiifolia* appeared to be closely related since they have seven ring + four rod bivalents in the microsporocytes. *J. tanjorensis* has been considered a new species (Prabakaran and Sujatha 1999) because it has phenotypic characters intermediate to those of *J. curcas* and *J. gossypiifolia*. Meiotic studies of this species revealed abnormal divisions with the formation of univalents and trivalents at metaphase I followed by unequal separation at anaphase leading to the formation of laggards and sporads of unequal size. In addition, distinct differences were noticed in respect to the number of bivalents at metaphase I. The existence of diploid numbers of 2n = 22 and 2n = 20 in *Jatropha* spp. is of significance bringing out the fact that there are two kinds of diploids, i.e., eight species with 2n = 22 and other two species (among one of which, two varieties are included) with 2n = 20 chromosomes (*Jatropha villosa* var. *villosa*, *Jatropha villosa* var. *ramnadensis* and *J. tirucalli* L.) (IPCN 2009; Soontornchainaksaeng and Jenjittikul 2003; Nair et al. 2009). In *J. curcas*, the chromosome size ranges from 1.24 to 1.71 μm (Fig. 15.1, Carvalho et al. 2008). To summarize, most of the *Jatropha* species are diploid with 2n = 2x = 22 and the few reports on meiosis point to a regular meiotic behavior.

Among notable particularities, three species (*J. cuneata* Wiggins & Rollins, *J. dioica* Sesse and some populations of *J. heterophylla* Heyne) are tetraploid (2n = 4x = 44). Most of the artificial interspecific hybrids are generally diploid, but two triploids (2n = 3x = 33) have been reported from crosses made between the diploid *J. curcas* x *J. cathartica* Teran & Berlan and *J. curcas* x *J. podagrica* Hook (Dehgan 1984). Amphidiploids with 2n = 44 of interspecific hybrids between 2n = 22 species (*J. curcas x J. integerrima* and *J. curcas x J. gossypiifolia*)

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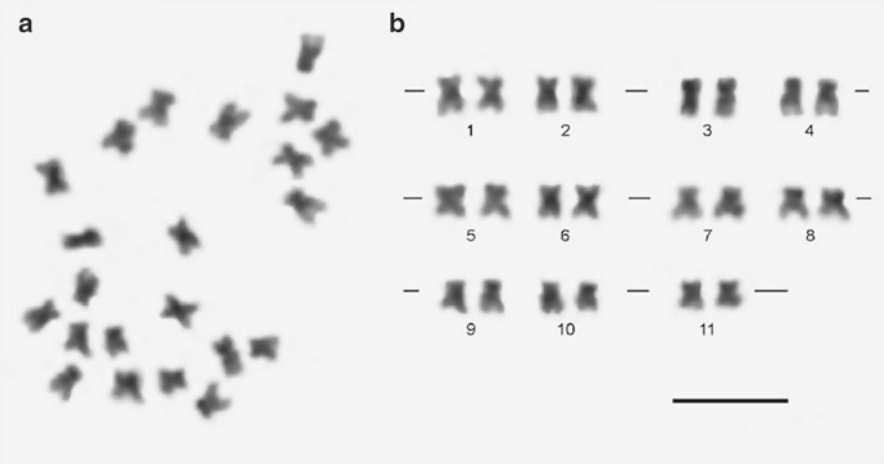

Fig. 15.1 Chromosomes from *J. curcas* root-tips. (**a**) Metaphase chromosomes, (**b**) karyogram showing five metacentric (1, 2, 5, 6 and 11) and six submetacentric (3, 4, 7, 8, 9 and 10) chromosomes. Bar = 5 mm (Reproduced with permission of Carvalho et al. 2008)

obtained by chromosome doubling will give a critical understanding of the structure (homogenetic or heterogenetic) of the homology between the genomes involved.

A systematic and critical study on the karyotype and pachytene pairing in meiosis in different species and hybrids within the genus *Jatropha* will help in a better understanding of chromosome morphology and nature of karyotype evolution in the diploid species of *Jatropha*. Karyotype analysis will provide clues as to how the basic chromosome number has evolved. It has been postulated that n = 10 arised from the basic number x =5 and that n = 11 evolved from both x = 5 and x = 6 as evidenced by the fact that the majority of today diploid species are 2n = 22 (x = 11) and the minority are 2n = 20 (x = 10). Another cytological evolutionary step is necessary to understand how the basic chromosome numbers of x = 5 and x = 6 have evolved. Karyotyping study of chromosome association in F_1s of species with 2n = 20 and 2n = 22 is a critical step to understand how to obtain interspecific hybrids for increasing the genetic variability in *J. curcas* and release stable cultivars having optimized features compatible with industrial exploitation.

Genome Size and Base Composition

The 2 C value of *J. curcas* is smaller than that of the other species of Euphorbiaceae that was reported to vary between 1.3 and 28.6 pg (Arumuganathan and Earle 1991; Bennett et al. 2000; Carvalho et al. 2008). Flow cytometry analysis revealed that the 2 C value of *J. curcas* is 0.85 pg, i.e., a genome size of 416 Mb, which is similar to

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the genome size of rice. The calculated average GC (guanine + cytosine) level of G_0/G_1 nuclei was found to be 38.7% or equivalently the AT level is 61.3%. The GC calculated for *J. curcas* is about the same as that found for *Arabidopsis* and is typical for the core dicots (Arumuganathan and Earle 1991; Carels 2005; Carvalho et al. 2008; Sato et al. 2011).

Molecular Genetics

Genetic Diversity and Phylogenetics of Genus Jatropha

Genetic diversity is one of the most valuable asset of the plant resources available to mankind. Species phylogenetics and evolution is essential not only for identification of species relationships, but also to better exploit genetic diversity through selective breeding. Assessment of diversity and phylogenetic relationship has traditionally been studied through morphological characteristics and isozyme analysis. However, such analyses have inherent drawbacks such as limited numbers of markers and are often less effective due to their variation through space (according to the environment) and time (Crawford et al. 1994; Elizabeth et al. 2000; Francisco et al. 1996; Lesica et al. 1998; Lowrey and Crawford 1985; Soltis et al. 1992). Advances in the field of molecular biology have provided many additional tools for studying genetic diversity at the genome level.

The development and use of molecular markers for the detection and exploitation of DNA polymorphism is one of the significant achievements in the field of molecular genetics, which accelerates breeding by improving the characterization of genetic variability and the measure of genetic distance among genitors (Caetano and Gresshoff 1997). DNA based markers such as: *random amplified polymorphism DNA* (RAPD), *enhanced random amplified polymorphism DNA* (ERAPD), *simple sequence repeat* (SSR), *sequence characterized amplified region* (SCAR), *amplified fragment length polymorphism* (AFLP), *inter-simple sequence repeat* (ISSR), *sequence tagged sites* (STS), and *nuclear ribosomal DNA internal transcribed spacer* (nrDNA-ITS) have been used to characterize genetic diversity, establish phylogenetic relationships, generate molecular markers for selective breeding and manage genetic resources in *J. curcas*.

Sudheer et al. (2009a) studied phylogenetic relationships among seven species of *Jatropha* (*J. curcas, J. glandulifera, J. gossypiifolia, J. integerrima, J. multifida, J. podagrica* and *J. tanjorensis*), which are widely distributed in India. Genetic diversity and phylogenetic analyses evaluated by RAPD, AFLP and nrDNA ITS led to conclude that interspecific genetic divergence between these species is 0.977, 0.972 and 0.419, respectively (Sudheer et al. 2009a, b, c). The size variation among nrDNA ITS was narrow and ranged from 647 to 654 bp explaining the poor (0.419) resolution of interspecific genetic divergence by this method. For this reason, the phylogram obtained using nrDNA ITS poorly converged with the phylograms obtained using RAPD or AFLP. However, phylogenetic trees constructed by using

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RAPD, AFLP and nrDNA ITS supported the highest genetic similarity between *J. curcas* and *J. integerrima*. The geographical distribution of *J. glandulifera* is wider, and it has distinct morphological features that separate major and minor clades. The claim that *J. tanjorensis* is a spontaneous hybrid between *J. curcas* and *J. gossypiifolia* (Prabakaran and Sujatha 1999) could not be supported by molecular data (Sudheer et al. 2009a, b). RAPD analysis confirmed the distinct genetic background of *J. glandulifera* (Ganesh Ram et al. 2008).

The overall mean *genetic distance* (GD) between species within the genus *Jatropha* was found to be 0.385. The largest interspecific GD was found between *J. glandulifera* and *J. multifida* (0.419). The smallest interspecific GD was found between *J. gossypiifolia* and *J. tanjorensis* (0.085). The largest intraspecific GD was found in *J. podagrica* (0.011) and smallest in *J. gossypiifolia* (0.002).

The genetic relationship between *J. curcas* and six sister taxa (*J. glandulifera, J. gossypiifolia, J. integerrima, J. multifida, J. podagrica,* and *J. tanjorensis*) was investigated using 49 SSR markers among which 31 showed cross species amplification in all the species studied. The overall *percentage of polymorphism* (PP) among the species studied was 38% and the mean *genetic similarity* (GS) was found to be 0.86. The highest PP (24.0%) and lowest GS (0.760) were found comparing *J. curcas* to *J. podagrica* and *J. curcas* to *J. multifida*, respectively. The lowest PP (4.4%) and highest GS (0.960) was found comparing *J. integerrima* to *J. tanjorensis* (Sudheer et al. 2009c, 2011).

Intraspecific Genetic Diversity in J. curcas

The systematic evaluation trial of *J. curcas* performance according to accession provenance is still limited. The comparison of accessions from Nicaraguan and Cape Verde germplasms revealed that the Nicaraguan accessions have fewer branches, larger pale leaves with bigger seeds, whereas, Cape Verde accessions gave a higher seed yield. In the Nicaraguan germplasm, a male sterile plant producing more fruits than the monoecious types was reported. Heller (1996) assessed the performance of 13 accessions from multi-location field trials in Senegal and Cape Verde and observed significant differences in vegetative growth.

Very few studies were carried out to understand genotype diversity using various marker systems in *J. curcas*. The majority of the investigations conducted on genetic diversity were limited to accessions available in India (Basha and Sujatha 2007; Sudheer et al. 2010a, b) with very few comparison of genotype diversity across continents (Sudheer 2008; Sun et al. 2008; Shen et al. 2010). Inter- and intra-population genetic diversity assessed in 42 accessions of *J. curcas* collected from different regions of India along with a non toxic genotype from Mexico showed 42.0% and 37.4% PP by RAPD and ISSR, respectively (Basha and Sujatha 2007). Shen et al. (2010), using AFLP found 27.0% PP comparing 38 populations of *J. curcas* that consisted of one source from Indonesia and 37 sources from the main cultivated areas of *J. curcas* in China. He et al. (2007) reported very high level of

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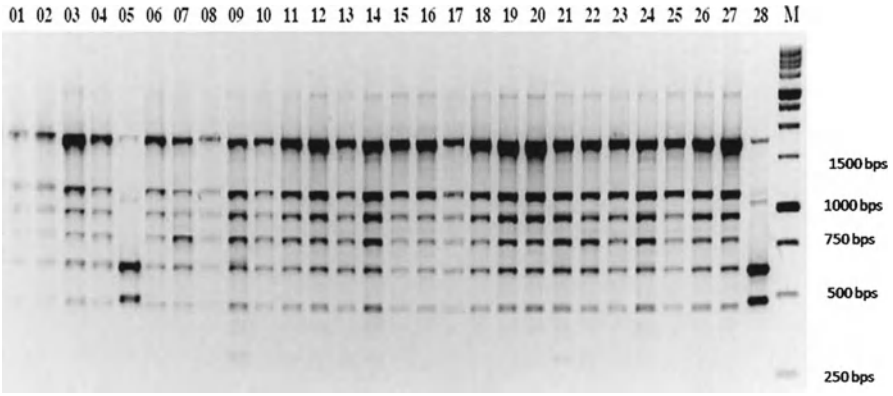

Fig. 15.2 RAPD profiles of 28 accessions of *J. curcas* L. from India with primer OPN12 (Source: Sudheer et al. 2010a)

GD among eight populations of *J. curcas* from Guangxi, Guizhou, Hainan, Sichuan and Yunnan provinces using ISSR. In contrast, Sun et al. (2008) found very low genetic diversity for the same accessions using SSR and AFLP.

The Indian investigation of *J. curcas* revealed that considering (1) five accessions from one region characterized with 18 RAPD primers (Ganesh Ram et al. 2008), (2) 22 accessions from six regions using seven RAPD and four *directed amplification of minisatellite DNA* (DAMD) primers (Ranade et al. 2008) and (3) RAPD and AFLP analyses from accession of different geographical regions of India, GD is low (Sudheer et al. 2010a, Figs. 15.2 and 15.3). Regardless of the number of accessions used, the robustness of the primer and number of markers, all accessions from India clustered together. Basha and Sujatha (2007), Ganesh Ram et al. (2008) and Tatikonda et al. (2008) also reported similar conclusions indicating the need for widening the genetic base through introduction of accessions with broader geographical background and the creation of variation through mutation and hybridization. Accessions from Andhra Pradesh characterized by AFLP were found to be scattered in different groups and demonstrated a higher frequency of unique/rare fragments associated with greater variation in oil content (Leela et al. 2009). Gupta et al. (2008) using ISSR primers grouped individuals from 17 seed sources in two different genotype clusters. Basha and Sujatha (2009) characterizing *Jatropha* species in India with nuclear and organelle specific primers revealed high inter-specific genetic variation (98.5% PP). Further characterization of both natural and artificial hybrids using chloroplast specific markers revealed their maternal inheritance. In addition, investigation of genetic variation with RAPD, AFLP and *combinatorial tubulin based polymorphism* (cTBP) indicated higher potentialities of *J. curcas* improvement by interspecific breeding.

Sujatha et al. (2005) studied the extent of genetic diversity among toxic and non-toxic Mexican germplasm using RAPD and reported a GS of 96.3%. However, Sudheer et al. (2009b) comparing these two populations found 15.09% and 16.49%

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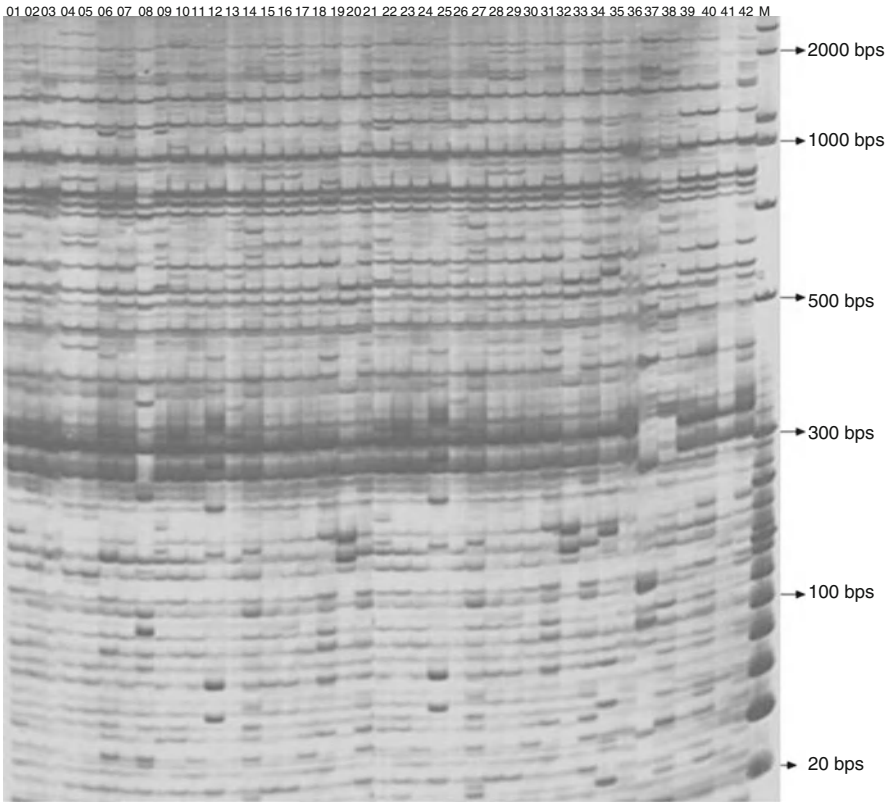

Fig. 15.3 AFLP profiles of 28 accessions of *J. curcas* L. from India (Source: Sudheer et al. 2010a). Since the genotypes are the same as those of Fig. 15.2, this figure shows that AFLP is much more informative than RAPD and that genotypic variability may not be captured by fingerprinting with RAPD

PP with RAPD and AFLP, respectively. The associated GS was 0.92 by RAPD and 0.90 by AFLP, respectively (Fig. 15.4). These authors also identified specific markers using RAPD and AFLP techniques for both varieties and efforts are continued to isolate SCAR markers for identification of non-toxic accessions of *J. curcas* (personal communication from M. Sujatha). Novel microsatellites were isolated from *J. curcas* and were characterized by RAPD and AFLP in a population showing significant deviation to the Hardy-Weinberg equilibrium, a phenomenon that may be due to a bias in accession distribution of anthropogenic nature (Sudheer et al. 2009b, 2010a). A similar observation was reported by Basha and Sujatha (2007) and Tatikonda et al. (2008). Wen et al. (2010) identified 241 novel EST-SSR and G-SSR markers in *J. curcas* that will be useful for QTL analysis.

All these results must be considered with caution since the analyses of genetic diversity in *J. curcas* were only limited to some varieties and group of populations and/or germplasm of narrow geographical area (Basha and Sujatha 2007; Sun et al. 2008; Tatikonda et al. 2008; Shen et al. 2010; Sudheer et al. 2010a).

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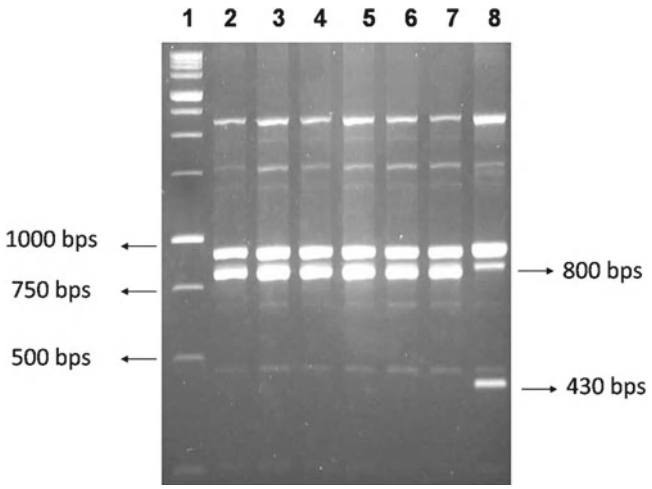

Fig. 15.4 RAPD profile with primer OPQ15 showing toxic and non-toxic specific diagnostic markers of *J. curcas*. *Lanes*: (1) 1 kb marker; (2) toxic varieties of *J. curcas*; (8) non-toxic variety of *J. curcas* (Sudheer et al. 2009b). *Arrows* show amplification products specific for toxic (*lanes* 1–7) and non-toxic (*lane* 8) varieties of *J. curcas*

The GD in *J. curcas* is far lower than normally found in other species (Jain et al. 2003; Ram et al. 2004) and the narrow GD reported by many authors is probably due to the limited number of individuals initially introduced and further distributed through vegetative propagation or by anthropogenic activity (Basha and Sujatha 2007; Sudheer et al. 2010a).

Inter- and intra-specific genetic relationship assessed by molecular markers will be useful to study the population genetic structure and to investigate the strategies of selective breeding. Molecular markers are paving the way for further characterization of species differentiation, molecular fingerprinting, physical mapping in interspecific hybrids, genetic resource management and selective breeding of economically important traits through MAS (Sudheer et al. 2011).

Markers for Toxic and Non-toxic Varieties of J. curcas

The non-toxic *J. curcas* varieties from Mexico have the dual advantage that their cultivation could provide oil for biodiesel and cake edible for livestock allowing the addition of value to the crop (Makkar et al. 1998). No significant morphological, qualitative and quantitative differences were found between toxic and non-toxic varieties except the higher levels of phorbol esters (Makkar et al. 1998; Makkar et al. 1997). Molecular markers specific to toxic and non-toxic varieties were identified using RAPD, ISSR and AFLP techniques (Basha et al. 2009b; Sudheer et al. 2009b). In addition, microsatellite markers were also tested in toxic

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and non-toxic varieties and 7 out of 12 markers showed associated PP (Sudheer et al. 2009b). The SCAR markers are specific and reproducible for the discrimination of toxic and non-toxic genotypes (Basha and Sujatha 2007) and could be used for MAS, QTL analysis and further molecular breeding investigations.

Phylogeography of J. curcas

Accessions of *J. curcas* from Mexico, Cape Verde, Madagascar, Africa, India were grouped in three different clusters by both RAPD and AFLP; however, only minor variations were observed with nrDNA ITS (Sudheer 2008) and the Indian germplasm formed only two clusters. Only one germplasm belonging to Cape Verde clustered with the Indian germplasm by both RAPD and AFLP analysis. The phylogram generated using the Indian germplasm through nrDNA ITS was not much correlated with those of RAPD and AFLP. RAPD and AFLP PPs are based on whole genome variations, while nrDNA PP is based on single locus sequence variations, which is perhaps the reason for these differences (Fig. 15.5). nrDNA ITS showed narrow diversity among the Indian accessions, but formed only a single major cluster with this germplasm. Similarly to multilocus markers, nrDNA ITS also showed maximum diversity among accessions from the Mexican germplasm. A strong genetic relationship was found between the germplasms from Mexico and Cape Verde. The phylograms based on RAPD and AFLP indicate that the African germplasm is on a separate branch. However, based on nrDNA ITS, the Cape Verde germplasm is closer to the African one. The phylogram based on nrDNA ITS showed equal relationship between accessions from India and Cape Verde or between accessions from India and Madagascar, but the bootstrap value of this last relationship was low, which challenge its validity. However, these results suggest that two distinct germplasms were introduced into India, one probably from Cape Verde via Spain and the other, possibly through an alternate route, which needs further investigations to be unveiled (Sudheer 2008).

Origin and Centre of Diversity of J. curcas

Many attempts were made to define the center of origin of *J. curcas*, but it remains controversial. The oldest fossil remains of the *Jatropha* genus were found by Berry (1929) in Peru. Dehgan (1984) and Wilbur (1954) reported that Central America might be the most probable *center of origin* of *J. curcas,* which should be, rather, regarded as a center of *diversification* if one considers the Berry's observations as correct. Aponte (1978) also reported similar conclusions. According to other sources, *J. curcas* could be native to Central America as well as to Mexico where it occurs naturally in the forests of coastal regions. Martin and Mayeux (1984) identified the state of Ceara in Brazil as a centre of origin of *J. curcas*, but without giving any proof. If this claim is consistent, it could be considered as a diversification

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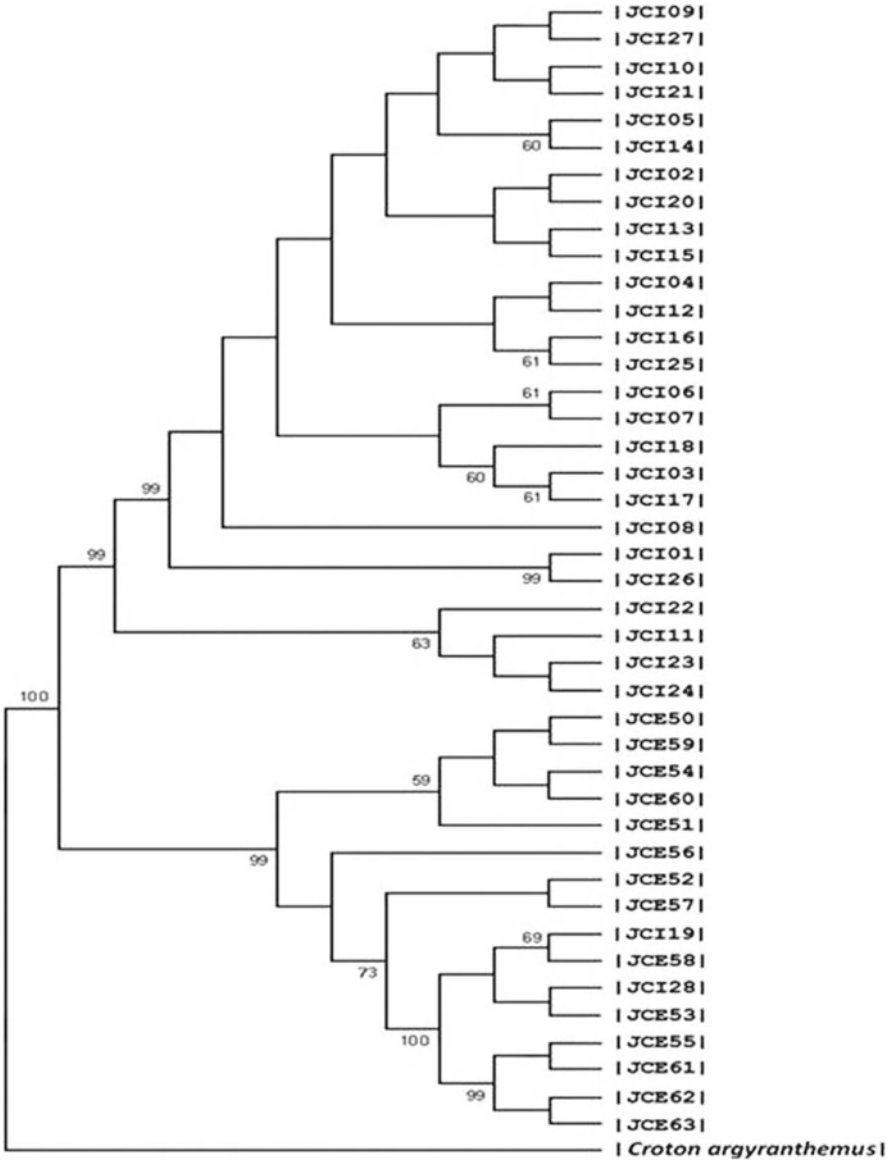

Fig. 15.5 Phylogenetic tree based on maximum parsimony using nrDNA ITS from 42 accessions of *J. curcas* (Source: Sudheer et al. 2010a)

center as that of Central America resulting from the radiation of the genus from its putative center of origin in Peru.

Herbarium specimens of the Americas were usually collected from hedges along roads and paths, live fence posts or disturbed sites ("disturbed forest"). Because of this, the Professor Dehgan's Horticultural Systematics Laboratory supports the

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argument that the species collected in the Americas were from natural vegetation. By contrast, the accessions from Africa and Asia were not collected from nature, but only from cultivated areas and it is speculated that the possible centre of origin of *J. curcas* is Mexico/Central America. However, the "true" centre of origin still has to be confirmed (Heller 1996).

The highest GD among accessions was observed in the germplasms from Mexico and Cape Verde indicating that *J. curcas* has its centre of diversity in Mexico. The nrDNA ITS from the germplasm belonging to Central America has the minimum sequence similarity scores compared to any other populations, which supports the conclusions obtained by RAPD and AFLP fingerprinting (Sudheer 2008). The phylograms showed that, unlike exotic germplasm, the clustering of Indian germplasm was random and not in correspondence to the geographical region of collection. This random clustering clearly indicates the human interference in the distribution of *J. curcas* (Basha and Sujatha 2007). The low genetic diversity of *J. curcas* in India may be due to its recent introduction and its fast distribution due to its economic relevance.

Proposed Dispersal Route of J. curcas

The migratory route of *J. curcas* has not yet been completely described, but some attempts were made based on the preliminary data available. Heller (1996) mentioned that this species was probably distributed by Portuguese seafarers via Cape Verde Islands and former Portuguese Guinea (now Guinea Bissau) to other countries in Africa and Asia. No facts are available in the literature before 1800 as to when the physic nut was introduced into Cape Verde (Serra 1950). Freitas (1906) said that *J. curcas* was already known several years prior to 1810, as written in the memories "*Memória ou descrição físico-política das ilhas de Cabo Verde*" by Antônio Pusich, governor of the archipelago at that time (http://www.barbosa.acthus.com/relatos_antigos.html). It is Burkill (1966) who speculated that the Portuguese brought *J. curcas* to Asia. However the tentative reports on fossil remnants of genus *Jatropha* observed in the Peru and Belem (Becker and Makkar 2008) may suggests the most probable origin may be the Peru or Belem and Central America may be the diversification rather than origin. Nevertheless, the proof for the proposed migration till the date does not exist. The molecular data analysis of our studies provided some information to deduce the putative migratory route of *J. curcas* from its proposed center of origin to India (Heller 1996).

The phylogram analysis based on RAPD and AFLP data showed that the Indian germplasm is very much close to that of Cape Verde (Sudheer 2008). Phylograms based on nrDNA ITS sequence also support the migration to India through Cape Verde. On a larger view, it is clear that African samples showed close relation with the Madagascar germplasm. Thus, the distribution of *J. curcas* suggests that it was introduced to Cape Verde from its center of origin, then it might have spread to Spain, Portugal and to other neighboring countries of western Africa. According to

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Fig. 15.6 Migratory route of *J. curcas* as revealed by RAPD, AFLP and nrDNA ITS (Source: Sudheer 2008)

Burkill (1996), Portuguese might have introduced *J. curcas* into India. The subsequent spread to Philippines was not reported in the study due to lack of samples, but the most probable route is from Asia rather than from Africa or Madagascar (Fig. 15.6).

DNA Sequence Repository and Genome Sequencing

As shown in the introduction, *J. curcas* has been very recently identified as a promising species for biofuel production and its genome exploitation has just started. For example, Tong et al. (2006) isolated full length cDNA of stearoyl-acyl carrier protein desaturase an important enzyme for fatty acid synthesis in seedlings of *J. curcas* and the gene was functionally expressed in *Escherichia coli*. Zhang et al. (2008) reported a novel gene of betaine aldehyde dehydrogenase, JcBD1, from *J. curcas* and the protein functionally expressed in *E. coli* conferred resistance to abiotic stresses to this bacteria. Ying et al. (2007) showed that the expression of Aquaporin (*Jc*PIP2) from *J. curcas* seedlings is increased under drought stress. Qin et al. (2011) reported that a cDNA clone encoding the small GTP-binding protein arf1 isolated from *J. curcas* endosperm has a significant homology to the *ADP- ribosylation factor* (ARF) in plants, animals and microbes. The gene expression was observed in flowers, root, stem and leaves and the accumulation of arf1 transcripts was different under various environmental stresses. Gua et al. (2011) isolated the full length genes of *Acetyl-CoA carboxylase* (ACCase) and reported that these genes are temporally and spatially expressed in leaves and endosperm of *J.curcas* under the control of plant development and environmental factors.

A library of 12,084 ESTs with average read length of 576 bp has been constructed from the roots of developing seedlings of *J. curcas* submitted to salt stress

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(Eswaran et al. 2010; Natarajan et al. 2010). The redundancy elimination released 2,258 contigs of 7,333 ESTs and 4,751 singletons. The functional annotation of these sequences using BLASTX resulted in 7,009 unigenes whose 3,982 unigenes showed significant similarity to known genes while 2,836 unigenes remained *unknown*, *hypothetical* or *putative*. The rest (191) showed no similarities with any reported genes. The functional classification of these genes revealed that they belong to a broad range of cellular, molecular and biological functions. Among the 7,009 unigenes, 6,233 unigenes were identified to be potentially full-length including the genes involved in fatty acid biosynthesis, desaturation of fatty acids and hydrolysis of fatty acids from acyl-ACP. Costa et al. (2010) generated 13,249 ESTs from seedlings of *J. curcas* and identified ESTs coding for proteins that may be involved in the toxicity of *J. curcas* seeds. They also reported a very high number of ESTs containing transposable elements.

Triacylglycerol (TAG) biosynthesis is of great interest and some of the genes involved in that process were cloned from *J. curcas* (Tong et al. 2006; Ye et al. 2009). Sato et al. (2011) annotated the genes for fatty acid and TAG biosynthesis. Gomes et al. (2010) sequenced a total of 2,200 clones; from which they obtained a set of 931 non redundant sequences from fruits of *J. curcas* at different maturity stages and reported that the expression profile of some enzymes involved in the fatty acid biosynthesis is higher in fruits as compared to leaves, which could serve to tag QTLs for oil yield.

Life Technologies Corporation, USA (Nasdaq: LIFE), a provider of innovative life science solutions and SG Biofuels, Inc., a bioenergy crop company, have announced the completion of the genome sequence to 100x coverage, using the SOLiD 4.0 System by Life Technologies. *Synthetic Genomics Inc.* (SGI) and *Asiatic Centre for Genome Technology* (ACGT) also announced the completion of *Jatropha* genome project. Finally, Sato et al. (2011) published the genome sequence and made it available in international databases (DDBJ, GenBank, EMBL).

Gene annotation helps in discovering genetic variation, which in turn helps in the process of providing information on factors controlling oil synthesis, maximizing biotic and abiotic stress tolerance and selecting for low-curcin variants. The sequence that is available to the scientific community through the Web page at http://www.kazusa.or.jp/jatropha/ will significantly accelerate the identification of genes involved in key traits and promotes progress in the development of *J. curcas* as a high yielding, low-cost source of oil for next generation biofuel.

Conclusions

Because of increasing worldwide demand for energy, concerns about security of crude oil supply, short supply of food grains and concerns over global climate change, the species *J. curcas* is seen as a very promising option for the production of biofuel from degraded areas, generating rural employment, increasing environmental quality and providing energy without compromising food production.

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However, even if scientific data were gathered at an unprecedent speed, in practice the implementation of this concept is comparatively slow. The major constraints limiting the large scale profitable cultivation are low and variable yield due to unpredictable germplasm behavior and undefined agricultural practices for optimizing yield.

J. curcas is still in its infancy with respect to the selective breeding of its genetic background for seed number, oil yield and agricultural features. Model systems have played a crucial role for understanding biological processes at genetic, molecular and plant systems levels. *Arabidopsis thaliana*, the first higher plant for which a complete genome sequence has been available and arguably the best studied plant system will be determinant for the implementation of genomics-based breeding strategies in *J. curcas*. The gene annotation of the *J. curcas* genome will also help in discovering genetic variation using marker assisted breeding and provide information on factors controlling oil synthesis, maximizing yield, managing biotic and abiotic stress tolerance and producing zero toxin cultivars. Varietal improvement in *Jatropha* should emphasize on enhancing and stabilizing productivity in various production systems and improving the quality of oil and seed meal for alternative use. The small genome size, chromosome number, eases of vegetative manipulation and transformation are favorable features for the entry of the new crop *J. curcas* in the biotechnology era. A comprehensive programme considering every biotechnological means of genetic improvement is required for this species to make this happen.

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Chapter 16
Studies on *Jatropha curcas* L. and Its Improvement Through Induced Mutagenesis

S.K. Datta and R.K. Pandey

Introduction

Systematic breeding efforts are most essential for genetic improvement and development of high yielding strains of *J. curcas*. Increasing the genetic variability is the first step for any crop improvement programme and particularly important for crops like *J. curcas* which is reported to have limited genetic variation. A number of strategies including conventional breeding, interspecific hybridization, mutation breeding and genetic engineering are available for development of new plant varieties. Breeders mostly use two methods for improvement of any crop plant i.e. conventional breeding and induced mutagenesis. Both the methods are well standardized. Plant breeding has significantly increased agricultural productivity through development of high-yielding crop varieties (Evenson and Gollin 2003). Through cross-breeding technique, the main attempt of the plant breeder is to combine the beneficial characters from different sources into one genotype. From such pooled genotypes sometimes it is possible to select directly a particular genotype which is superior to the parent cultivars. Mutation breeding on the other hand, is an established method for plant improvement. By this method, plant genes are altered by treating seeds or other plant parts with chemical or physical mutagens. The concept of induced mutagenesis for crop improvement dates back to the beginning of the twentieth century. High potential for bringing in the desired genetic improvement through induced mutations was realized after systematic studies by Gustafsson and Nybom (1949)

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and Mackey (1956). Lot of information has been generated on optimal treatment doses, treatment condition, mutation frequency and mutation spectra for several crop plants. Mutation technique by using both physical and chemical mutagens has successfully produced quite a large number of new promising varieties in different crop plants (Sigurbjornsson and Micke 1969; Micke et al. 1990; Broertjes and Van Harten 1988; Datta 1988, 2001, Datta and Basu 1988). Mutation technique is considered as the most successful tool for vegetatively propagated plants (Datta 1997).

Creation of genetic variability is prerequisite for development of new variety. Success of any technique depends upon the nature of the plant. Heterozygous nature of any plant variety offers high mutation frequency and also helps selection of parental varieties with desirable characters for cross breeding. Mutagenesis studies are rather limited in *J. curcas* but the need for widening the genetic base through mutagenesis has been realized. Both chemical and physical mutagenesis experiments were done for isolation of mutants. Mutation breeding using fast neutrons resulted in isolation of dwarf and early flowering mutants from the M_3 generation but the inheritance or productive potential of these mutants was not studied (Sakaguchi and Somabhi 1987). Use of 10 Gy irradiation resulted in identification of mutant plants with early maturity, high 100-seed weight and better branch growth (Dwimahyani and Ishak 2004a). Mutation studies carried out at the National Botanical Research Institute (NBRI), India have resulted in variations in cotyledons of *J. curcas* (Pandey and Datta 1995). Studies of Dakshanamoorthy et al. (2010) on mutation induction through gamma rays (5–25 Krad) and EMS (1–4%) showed the superiority of physical mutagen over the chemical mutagen and low concentrations of γ rays (5 Krad) resulted in early flowering and good bearing mutants. Further, Dakshanamoorthy et al. (2009) subjected the mutants to molecular analysis using RAPD markers wherein DNA polymorphism was associated with mutants showing differences in morphological traits. Zhang (2008) and Yang et al. (2012) have reported the selection and identification of 10 promising seed sources, 1 new variety and 10 mutants of *J. curcas* based on the performance of the mutants in the field in terms of their biological characteristics, economic traits and agronomic attributes. Yang et al. (2010) reported the occurrence of natural variants in wild and hybridization experiments with high yield (~3 $t\,ha^{-1}$ in 4-year old trees) and increased oil content (6% increase).

As no recommended strains of *J. curcas* are available, it was important to understand different characters of the experimental material. Therefore, in the present study a separate experiment was conducted with control material and the data was collected on cultivation, growth pattern, branching habit, spacing, pruning operation, total yield, biosynthesis of oil and fatty acid composition for 3 years. This helped in comparative assessment of characters of control and induced variants. In the present chapter an attempt has been made to give the salient features of the plant comprising all economic characters and also reports on the sensitivity of *J. curcas* to gamma rays and colchicine for genetic improvement. An attempt has also been made to highlight the important basic aspects and technical advancement of mutagenesis technology which may be helpful for large scale mutagenesis work.

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Mutation Experiments

Dry seeds of *J. curcas* were exposed to 6, 12 and 18 Krad gamma rays (Cobalt-60, radiation source) and sown in beds laid out in a randomized block design. Equal number of unpredicted seeds were sown which served as control. The number of seeds per treatment was 50 and plant to plant distance was maintained at 2 m.

Seeds were treated for 6 h in 0 (H_2O – control), 0.25%, 0.5% and 1.0% aqueous solution of colchicine. Following the treatment, seeds were thoroughly washed in running water and sown in randomized block design beds.

Experiments were also conducted by combined treatment of gamma rays (Krad) and colchicine (% aq. soln.). Dry seeds were treated in the following sequence combinations – 12 Krad + 0.5%; 0.5% + 12 Krad; 12 Krad + 1.0%; 1.0% + 12 Krad; 18 Krad + 0.5%; 0.5% + 18 Krad; 18 Krad + 1.0% and 1.0% + 18 Krad.

Data were recorded on germination, growth, morphological, flowering and seed characters of treated population *vis-à-vis* control (untreated). The total oil content per seed was estimated according to Datta (1976). Mean and standard error (SEm) of each character was determined and significant level was determined using 't' test. The seeds were removed from the fruits and the kernel separated from seed coats for total lipid extraction using methanol: chloroform (2:1 v/v) in a Soxhlet apparatus (Blich and Dyer 1959). The methanol: chloroform layer after separation was concentrated. The residue was taken up in petroleum ether and allowed to settle. The petroleum ether layer was decanted and distilled off to get the oil (Datta 1995). The oil thus obtained was subjected to saponification using N/2 alcoholic KOH at 65°C and the fatty acid methyl esters were prepared after methanolysis of fatty acids (Dasgupta et al. 1981). The fatty acid methyl esters were analysed by gas liquid chromatography (Varian Aerograph Vista-6000) on DBI capillary column at 140–220°C, at the rate of 30°C per minute. Standard fatty acid methyl esters obtained from Sigma Chemicals (USA) were employed as standards.

Fatty Acid Composition of Curcas Oil

Before starting the genetic improvement programme through induced mutagenesis, an attempt has been made to study the biosynthesis of fatty acids and its composition at different seed developmental stages. Such studies are expected to reveal the biogenic pathways for the production of important fatty acids in the seed. There was successive disappearance of lauric (12:0) and myristic (14:0) acids up to seed maturity. There was complete absence of linoleic acid (18:2) in early stages but it developed after 12 days of fruit setting and gradually increased with seed maturity. Composition of the component fatty acids of the oil at different developmental stages after fruit setting in *J. curcas* is presented in Table 16.1.

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Table 16.1 Variation in fatty acid profile in seeds of *Jatropha curcas* at different developmental stages

Days	Total oil (%)	Lauric 12:0	Myristic 14:0	Palmitic 16:0	Stearic 18:0	Oleic 18:1	Linoleic 18:2	Arachidic 20:0	Saturated	Unsaturated
5	1.0	37.2	31.0	14.7	2.0	15.1	0	0	84.9	15.1
12	1.6	10.6	16.6	39.9	7.1	25.8	0	0	74.2	25.8
26	4.0	18.1	10.0	19.1	6.4	36.3	8.3	1.8	55.4	44.6
33	8.7	3.9	17.3	29.4	1.2	36.9	10.1	1.2	53.0	47.0
40	11.8	0	15.4	30.8	1.4	39.9	11.1	1.4	49.0	51.0
47	19.1	0	0	28.1	0.3	55.6	14.8	1.2	29.5	70.5
54	40.6	0	0	23.4	0.7	60.8	13.7	1.4	25.5	74.5
61	46.4	0	0	24.3	1.8	55.1	15.9	2.8	28.9	71.1
68 (mature)	47.0	0	0	31.4	0	50.1	16.8	1.7	33.1	66.9

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Understanding the biosynthetic pathway of fatty acid composition at various developmental stages is necessary as background knowledge (Pandey et al. 1993; Datta and Pandey 1996). This will help to manipulate the important enzymes which play an important role in determining the ratio of saturated fatty acids to unsaturated fatty acids in plants (Lindqvist et al. 1996). Banerji et al. (1985) reported 2.3% stearic acid in mature seed. Raina and Gaikwad (1987) reported 5.25% and that the fatty acid composition of the curcas oil was found to be affected by the stage of maturity and season of harvesting. The present study of the fatty acid composition of developing seeds of *J. curcas* supports the pathway for fatty acid synthesis given by Austin et al. (1985).

Effects of Mutagens

All the mutagen treated plants were carefully maintained for observation in subsequent generations. The main emphasis was to observe the effects of mutagens on different morphological parameters in the first M_1 (gamma-ray treated), C_1 (colchicine treated) and combined treated populations. In the second and third year, focus was on variants selected for fruit and oil characters on individual plant basis.

Seed germination in the first generation was not affected after gamma irradiation. Germination percentage was more due to stimulation after treatment with 6 Krad and 18 Krad gamma rays and 0.25% colchicine. Germination time was almost the same in all treatments except in 6 Krad where it was significantly ($P<0.001$) earlier. Colchicine at 0.25% enhanced seed germination whereas it reduced after treatment with 0.5% (41.3%) and 1.0% (46.7%) over control (64.0%). Germination time was significantly ($P<0.01$) delayed after treatment with higher concentrations of colchicine. Both increase and decrease in germination was observed after combined treatment. Maximum germination was recorded (100%) after treatment with 18 Krad + 0.50%. Germination time was delayed in all combined treatments and it was significant ($P<0.02$ to $P<0.001$) in some treatments. Significant ($P<0.001$) reduction in height of seedlings was recorded in 15-days-old seedlings after treatment with 12 Krad and 18 Krad. Seedling height was not affected after colchicine treatment. Branch and leaf number were counted in all the treatment combinations after 30, 60 and 90 days. Branch number was increased after 90 days in all the treatments except in 12 Krad and 18 Krad treatments while in some combined treated population it was reduced significantly ($P<0.02$ to $P<0.001$). Leaf number was reduced in all treatments at 90 days, in some cases significantly ($P<0.05$ to $P<0.001$), except in 12 Krad + 1.0% and 1.0% + 12 Krad where it was increased. Leaf size in most of the treatments was significantly ($P<0.05$ to $P<0.001$) reduced. Different types of abnormalities in leaves were observed in the treated populations. The leaf abnormalities included changes in shape, size, margin, apex, fission and fusion of leaves. Leaf abnormalities were recorded at 30 and 60 days after germination. Leaf abnormalities increased after treatment on 30 days. Leaf abnormalities increased and/or decreased

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significantly on 60 days. Growth pattern of γ-ray and colchicine treated (separate and combined) populations were also recorded in subsequent generations.

In the second year, individual plant analysis showed wide variability in different treatment combinations. On the basis of improved performance, a number of plants (variants) were selected from the treated population over the best in the control in the M2 generation. The variants showed increased branch number, increased number of capsules, seeds per plant, seeds per capsule, seed weight, seed size and oil content. All the selected lines have been carefully maintained to study their true breeding nature and performance in further generations (Table 16.2). Some of the selections showed true breeding nature of their desired character even after 1 year of the growth.

From the analysis of data it is found that there are possibilities to isolate different types of mutants from the present experiments. Both tall and dwarf, more branches, high fruit and oil yielding and high biomass yielding mutants can be established (Datta and Pandey 1991; 1992a, b, c; Pandey 1993; Pandey and Datta 1995).

Mutagens and genetic variations

The effects of mutagens (γ-rays and colchicine) on *J. curcas* in the 1st and subsequent generations are in conformity with the results obtained in a large number of plants by many investigators. In the present experiments, colchicine alone and combined treatment (γ-rays and colchicine) induced desirable variations. Colchicine has been used for a long time as a polyploidizing agent. It has been used successfully to produce polyploids for cytogenetic research and for breeding programme in many plant species. As a polyploidizing agent alone, colchicine is of worldwide importance and has opened new vistas in experimental agriculture. Polyploidy has been induced in several important ornamental plants besides agricultural crops. Colchicine in the present experiment successfully induced desirable variations for some economic characters.

Very few reports are available on the application of induced mutagenesis for improvement of *J. curcas*. Most of the work is concerned on the sensitivity of *J. curcas* to mutagens (Yang et al. 2007). Sakaguchi and Somabhi (1987) carried out mutation breeding studies using fast neutrons and isolated dwarf or early flowering mutants from the M_3 generation, but the potential productivity of these variants under intensive cultivation conditions was not proved. Datta et al. (1998) studied the performance of mutagen (gamma rays and colchicine) treated plants of *J. curcas* in saline soil. Dwimahyani and Ishak (2004a, b) treated cuttings of *J. curcas*, collected from different places around Jakarta, with 10, 15, 20 and 25 Gy of gamma rays. The 20 and 25 Gy doses damaged the growth of the plants while the 10 Gy dose induced genetic variability towards early maturity, increased 100-seed weight (30% over control) and increased branch number (Figs. 16.1–16.5). Qing et al. (2007) studied radio-sensitivity of seeds of *J. curcas* of 10 provenances to

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Table 16.2 Selection of variants in M_2 from colchicine and combined treated population on the basis of branch number, 10 seed weight and oil content

Characters of selected lines				
Selection	Treatment	No. of branches	10 seed weight (g)	Oil content (%)
Selection from mother line (control)				
Sel. 1		09	4.41	38.02
Sel. 2		10	4.88	39.82
Sel. 3		16	4.11	37.92
	Colchicine (%)			
Sel. 4	0.50	32	4.73	40.54
Sel. 5	0.50	20	4.51	45.00
Sel. 6	0.50	37	5.38	40.00
Sel. 7	0.25	17	5.46	40.99
Sel. 8	0.50	16	5.87	40.04
Sel. 9	0.50	12	5.46	42.86
Sel. 10	1.0	14	5.38	44.72
Sel. 11	**Combined (1.00 + krad)**	19	6.15	40.60
Sel. 12	0.25	17	5.14	44.86
Sel. 13	0.25	13	5.38	48.32
Sel. 14	0.25	13	5.89	44.48
Sel. 15	0.50	16	5.47	49.17
Sel. 16	0.50	17	5.38	48.48
Sel. 17	0.50	11	4.17	44.16
Sel. 18	0.50	19	5.17	45.45
Sel. 19	0.50	14	4.19	44.55

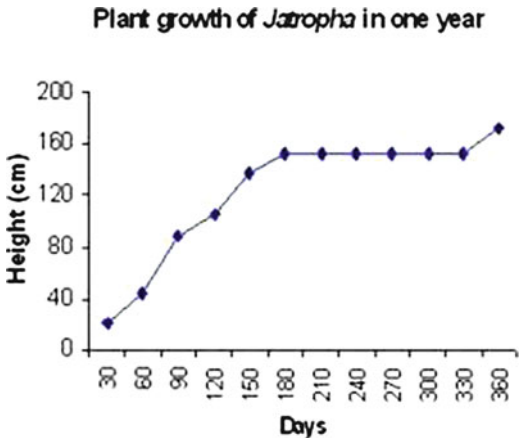

Fig. 16.1 Showing periodical growth (height) of plant in 1 year

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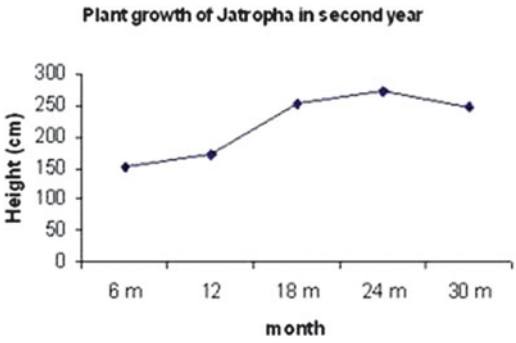

Fig. 16.2 Showing periodical growth (height) of plant in second year

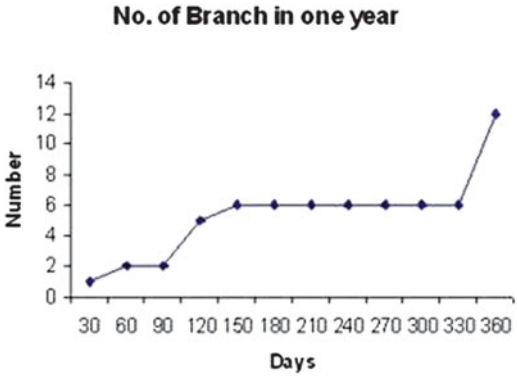

Fig. 16.3 Showing total number of branch per plant in 1 year

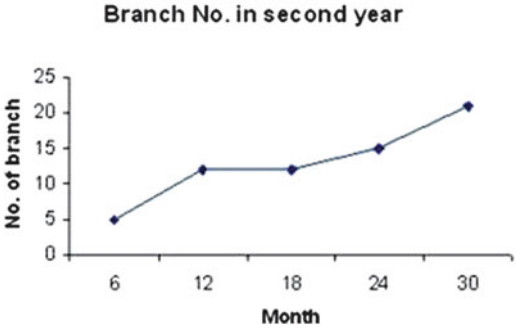

Fig. 16.4 Showing total number of branch per plant in second year

gamma radiation. LD_{50} dose was determined on the basis of germination. LD_{50} and sensitivity results provided important experimental basis for germplasm resources innovation of *J. curcas*. Dhakshanamoorthy and Selvaraj (2010) reported from their studies that RAPD marker can be used for identification of DNA polymorphism in gamma rays treated population/mutants. They suggested application of induced mutations for improvement and marker assisted selection procedure for early selection and recognition of desired mutant genotypes. Wang et al. (2009) determined the

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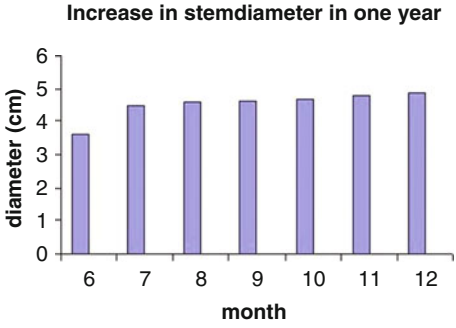

Fig. 16.5 Showing increase in stem diameter in 1 year

LD50 dose of *J. curcas* on the basis of germination after treating the seeds with 0, 100, 150, 200, 250 and 300 Gy gamma irradiation and the LD_{50} dose for Guangdong, Hainan and Indian seeds were 178 Gy, 132 Gy, and 198 Gy, respectively. They detected significant change in leaf shape in M_1 seedlings and recommended mutation breeding for *Jatropha*.

Improvement of *J. curcas* needs special mention. Knowledge on existing natural genetic variation of any crop and its availability is essential prerequisite for genetic improvement programmes. Genetic diversity of any crop is a good indicator of performance of the progeny. Success through hybridization and subsequent selection depends primarily on the selection of parents having high genetic variability for various agronomic traits. Genetic variability is very low among available strains of *J. curcas* in India (Heller 1996; Basha and Sujatha 2007; Pamidiamarri et al. 2008; Sun et al. 2008). Very little plant breeding work has been done for genetic enhancement of this crop. The genetic resources available with *J. curcas* have not been properly characterized and conserved. Genetically distinct desirable strains have not yet been identified through breeding procedure. The kind of genetic diversity present in *Jatropha* might be due to differential adaptation and probably selection criteria and selection pressure had no role in the existing diversity. Normally, every crop is a mixture of genotypes which evolved as a result of natural and man made selections over several generations under the environmental conditions in which presently it is growing. Recently, multidisciplinary work has been initiated by different researchers to enrich the knowledge on *J. curcas*. The amount of genetic diversity in *Jatropha* has not been quantified earlier. Such information is of vital importance for the breeding programmes for developing improved cultivars. Recently, extensive work has been initiated by a number of workers for classical and molecular characterization for understanding the extent of genetic diversity and relationships among different strains of *J. curcas* (Ginwal et al. 2004; Pant et al. 2006; Kaushik et al. 2006,2007; Basha and Sujatha 2007; Carvalho et al. 2008; Ranade et al. 2008; Basha et al. 2009; Ganesh et al. 2008; Pamidiamarri et al. 2008; Gupta et al. 2008; Gohil and Pandya 2008; Sun et al. 2008; Sunil et al. 2008; Sujatha et al. 2008; Montes et al. 2008; Dunping et al. 2009; Kumar et al. 2009; Leela et al. 2009; Dhakshanamoorthy et al. 2009; Dhakshanamoorthy and Selvaraj 2010; Ikbal Boora and Dhillon 2010). These studies will help assessment of wild and cultivated strains and selection of superior/elite genotypes for further improvement.

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Reports are available on genetic improvement of *J. curcas* through conventional breeding and other assessment methods (Dehgan 1984; Paramathma et al. 2006; Sujatha 2006; Parthiban et al. 2009; Basha and Sujatha 2009; Reddy et al. 2007; Abdelgadir et al. 2008; Divakara et al. 2010). Parthiban et al. (2009) attempted to develop new hybrids with more genetic diversity and new alleles through interspecific cross pollination between *J. curcas* and other *Jatropha* species.

J. curcas is monoecious i.e. male and female flowers occur in same inflorescence. The flower is protogynous and the female flower matures earlier than male flower. The number of female flowers per inflorescence is limited (approx. 1–10) which is one of the main reasons for its low productivity. Ovary attains to its full maturity (capsule) in about 60 days after fertilization. On the basis of available information, a number of traits (increased ratio of female: male flowers, increased branch number, seed yield, oil content, seed toxicity, etc.) may be useful traits for improvement of *J. curcas* (Raju and Ezradanam 2002). The male: female flower ratio trait is highly heritable (Rao et al. 2008). Modification of plant architecture may increase plant yield (Sakamoto and Matsuoka 2004).

J. curcas is propagated both by seeds and vegetative cuttings. Vegetative propagation nature is an added advantage for *J. curcas* for improvement. It is well known that the crops which are propagated vegetatively are suitable for the application of mutation breeding methods. The main advantage of mutation induction in vegetatively propagated crops is the ability to change one or a few characters of an otherwise outstanding cultivar without altering the remaining and often unique traits of the genotype (Broertjes and Van Harten 1988; Datta 1997).

It appears from the available literature and the present experiment that recently considerable data have been generated on *J. curcas* on different aspects. But high yielding variety has not yet been developed, may be due to lack of uniform basic knowledge, desirable strains, lack of technological knowledge and economic reasons. One of the main reasons with *J. curcas* is its long duration for optimum production. Reports indicate that optimum seed production in *J. curcas* starts after 5 years. Very few long term experimental data on crop improvement programmes are available on this crop. It is difficult to start any conventional breeding programme without available variability and hence, the first step is to induce variability. If we see the flow chart (Fig. 16.6), it will take a minimum of 8–10 years to develop new variety either through conventional breeding or induced mutagenesis. Unfortunately most research projects are for short duration (3 years). Research Institutions, Universities, companies should initiate long term in-house projects for improvement of *J. curcas*. Studies have proved clearly that induced mutagenesis can be exploited for the creation of new and desirable variants by inducing genetic variation in already adapted genotypes and can also enrich the germplasm of *J. curcas*. Once desirable characters are identified they may be incorporated into the cultivated varieties in a more systematic way. The 'Best available' genotype should be selected as the starting material for improvement. Desirable selected lines will be beneficial for direct use in the industry and in agriculture for future breeding programmes. The mutants themselves may not be suitable for direct release, but they do provide the necessary alleles for development of superior cultivars with desirable traits.

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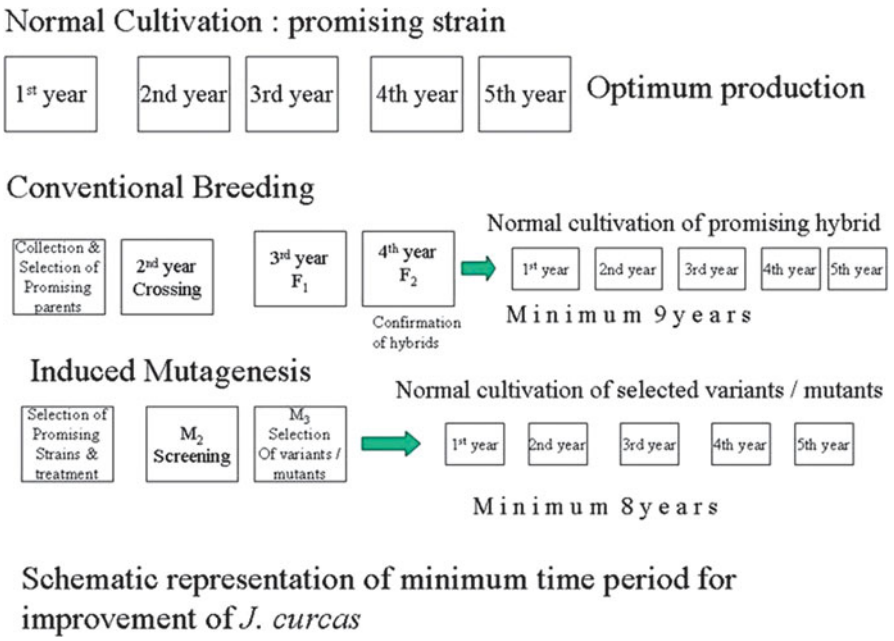

Fig. 16.6 Schematic representation of minimum time period for improvement of *J. curcas* (normal cultivation, conventional breeding and induced mutagenesis)

It is evident from the present experiments that different biological characters, which directly or indirectly contribute towards higher yield of *J. curcas,* are sensitive to mutagens and desirable variants/mutants can be induced. It is very clear that a sustainable oil seed plantation can be made feasible for commercial exploitation with proper isolation, evolution, selection and improvement of *J. curcas.*

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Chapter 17
The Use of EcoTILLING for the Genetic Improvement of *Jatropha curcas* L.

Fatemeh Maghuly, Joanna Jankowicz-Cieslak, Bradley J. Till, and Margit Laimer

Introduction

Genetic variability is a pre-requisite for any plant-breeding program. The extent and distribution of genetic variation within a species is of fundamental importance to its evolutionary potential and determines its chances of survival. The identification of taxonomic units and the determination of the uniqueness of many tree species, primitive cultivars, land races, elite breeding lines and wild relatives of crops have recently become the focus of increasing conservation concern. However, before these resources can be exploited they must be systematically evaluated to assess the genetic diversity. Genetic diversity is the basis for adaptability, stability and evolution of species or populations (Müller-Starck et al. 1992). Populations with a narrow genetic base are widely thought to be more sensitive to environmental changes or disease, leading to a decrease in productivity (Oleksyn et al. 1994; Maghuly et al. 2006a). Forest management based on natural or artificial regeneration can significantly impact the genetic diversity and population genetic structure (Rajora 1999; Maghuly et al. 2006b, c, 2007a).

Molecular markers are invaluable tools for the genotyping of accessions, the classification and management of tree germplasm collections for marker assisted breeding (MAB). *Single Nucleotide Polymorphisms* (SNPs) are a widely used class of genetic markers for population studies and genetic diversity assessment.

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SNPs are the simplest form of DNA variation among individuals. These minor changes can be either transitions or transversions and are found throughout the genome (Komar 2009). SNPs may (1) change the encoded amino acids (non-synonymous), (2) generate premature stop codons leading to premature translation termination (nonsense), (3) alter RNA splicing or (4) be deprived of any effect on protein function (synonymous) if occurring in noncoding and non regulatory regions. They may influence promoter activity (gene expression), messenger RNA (mRNA) stability, subcellular localization of mRNAs and/or proteins and affect the functionality of proteins. Therefore, identification of variations in genes and analysis of their effects may lead to a better understanding of their impact on gene function (Komar 2009). Natural SNPs can provide clues to the adaptive processes, population histories and form the basis for the intraspecific variation that is of great relevance to breeders (Gilchrist et al. 2006). A SNP database would give researchers and breeders a tool for answering questions about population structure or adaptation.

For identification of SNPs, a number of different techniques have been developed, all having their limitations (Gilchrist et al. 2006). Electrophoretic mobility assays, like single strand conformation polymorphism (SSCP) and denaturing gradient gel electrophoresis (DGGE) do not sign the location and/or the type of existing polymorphism in DNA fragments (DeFrancesco and Perkel 2001). Methods that rely on denaturation kinetics and quantitative PCR only work for small fragments of DNA (Gundry et al. 2003) and Microarray based SNP techniques are efficient only in discovering approximately 50% of the SNPs (Borevitz et al. 2003; Gilchrist and Haughn 2005; Gilchrist et al. 2006). It is possible to visualize sequence polymorphism between two DNA fragments by sequencing these fragments. However, despite advances in automation, this strategy remains prohibitively expensive and time consuming when applied to multiple loci in large numbers of individuals. On the other hand, novel sequencing approaches are being developed to characterize natural variation in populations, but the large infrastructure input and informatics load currently leave such methods out of reach of many individual laboratories. When it is not necessary to visualize the whole sequence, some indirect methods can be used that are much faster and cheaper than sequencing (Semagn et al. 2006). This means that alternative technologies are needed to identify rare allelic variations with a high-throughput and cost-effective technology.

An important tool to characterize the *in vivo* function of genes is the process of reverse genetics. Reverse genetics approaches allow the alteration of sequence or expression of specific genes to study the role of the target gene in the context of a whole organism. While several reverse genetic approaches have been developed, the *Targeting Induced Local Lesions IN Genomes* (TILLING) method has been widely developed for different plant species (Jankowicz-Cieslak et al. 2011). TILLING combines random mutagenesis with high throughput mutation discovery, provides a spectrum of stable point-alleles and is broadly applicable across most plant and animal species. A major method for mutation discovery utilized in many TILLING projects is enzymatic mismatch cleavage followed by fluorescence detection of cleaved products (Till et al. 2006a).

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The enzyme based discovery procedure used in many TILLING projects has been adapted for the discovery and cataloguing of natural polymorphisms, a method called EcoTILLING. EcoTILLING is a low cost, high-throughput reverse genetic method for haplotyping and SNPs discovery (Comai et al. 2004). The identification of naturally occurring genetic variants can provide information about gene function and can be useful for association mapping and linkage disequilibrium analysis (Gilchrist et al. 2006; Nieto et al. 2007).

Currently, more than 95% of biodiesel is produced from edible crops, particularly from rapeseed. By converting edible oils into biodiesel, food resources are actually being converted into automotive fuels. It is believed that large-scale production of biodiesel from edible oils would bring global imbalances to food supply and demand. Non-edible oilseeds such as the perennial *Jatropha curcas*, termed "second generation biofuel plants", might contribute to greening of wastelands without compromising food and fodder security (Vollmann and Laimer 2010).

However, *J. curcas* has never been domesticated and its yield is difficult to predict with accuracy. For several technical and economical reasons, the full potential of *J. curcas* is far from being realized (Fairless 2007). There is limited information available on genetics and agronomy of *J. curcas*. Moreover, at present, no major systematic exploration and evaluation of genetic resources has been published and only little and scattered knowledge is available on the basic reproductive biology of the species. There is also a lack of benchmark descriptors and information on (1) genetic variability, (2) effects of environment heritability, (3) genetic variance components, (4) genotype vs. environment interaction, (5) germplasm pathways, and (6) juvenile vs. mature correlations. Improved varieties with desirable traits for specific growing conditions are not yet available, which makes growing *J. curcas* a risky business (Vollmann and Laimer 2010). Knowledge about the degree of genetic diversity among and within natural populations in and outside the center of origin is required to gain the first ideas about where to find potentially valuable genetic material (Wen et al. 2010). In fact, the genetic map of *J. curcas* is not well developed and only few molecular markers exist that could be used to clearly distinguish worldwide accessions (Maghuly et al. 2011).

On the other hand, large-scale plantations of *J. curcas* for biofuel industry are created without consistent information about its genetic setup. For this reason, it is necessary to develop a fast and reliable technique for molecular fingerprinting. Therefore, an inexpensive, quick and high-throughput technique platform like EcoTILLING would be useful to discover SNP haplotypes in candidate genes, to study their effect in gene function and to investigate genetic diversity in the *Jatropha* genus, in general, and in *J. curcas*, in particular (Fig. 17.1). Furthermore, SNP genotyping also allows the comparison of *J. curcas* with other related species of the Euphorbiaceae. Therefore, we developed an EcoTILLING platform and validated the method through SNP discovery in 12 candidate genes across 1,200 *J. curcas* and related species from 14 different geographic regions. The methodology used for *Jatropha* spp. germplasm EcoTILLING is described in this chapter.

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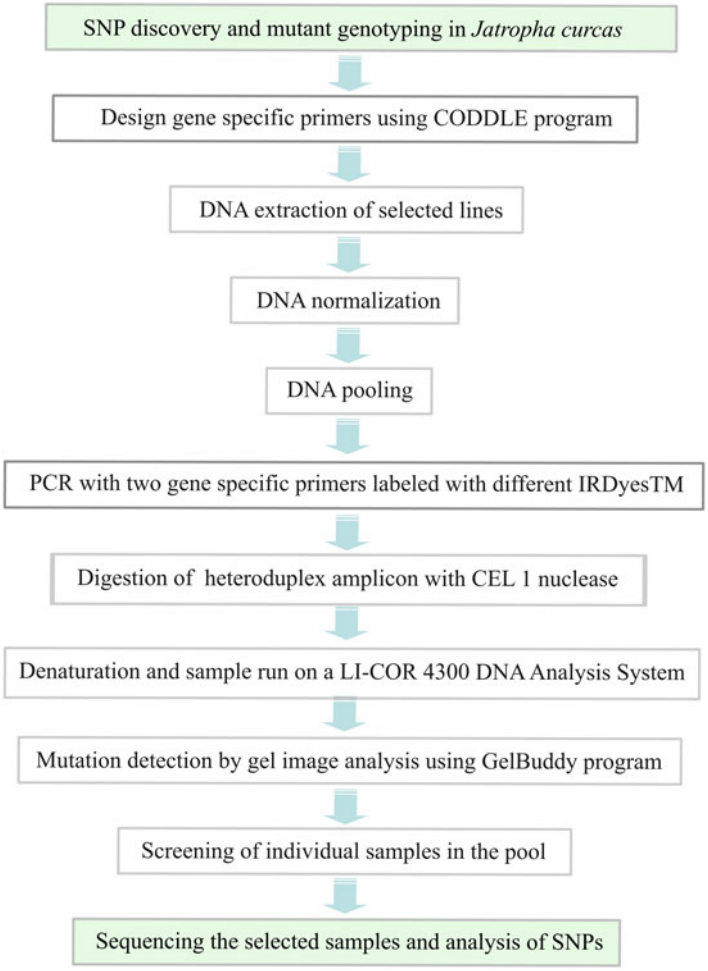

Fig. 17.1 Work flow of high-throughput EcoTILLING for the discovery of SNP haplotypes in candidate genes of *Jatropha* sp.

Collection of Suitable *Jatropha* spp. Germplasm for EcoTILLING

A live germplasm collection of 1,200 *in vivo* and *in vitro* accessions of *J. curcas* and four related species (*J. multifida*, *J. hieronymi, J. podagrica* and *J. macrocarpa*) from 14 different countries on three continents (Americas: Bolivia, Brazil, Mexico, Paraguay; Africa: Cape Verde, Ethiopia, Guinea-Bissau, Kenya, Madagascar, Mali, Senegal; Asia: China, India, Indonesia) (Fig. 17.2) were used for EcoTILLING analyses.

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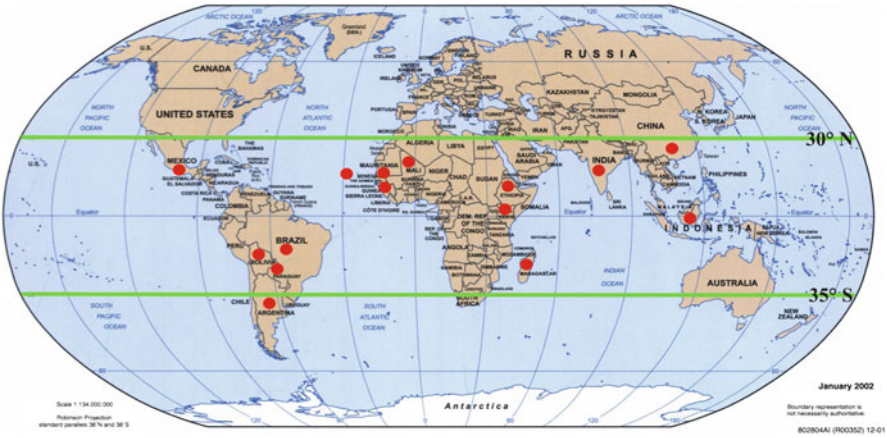

Fig. 17.2 Worldwide extension of *J. curcas* and sampling regions (*red dots*) over three continents. The level of genetic variation of *J. curcas* is higher in its centre of origin. The level of genetic variation in Africa and Asia depends on the American origin of the material that is considered. At best, worldwide emigration of *J. curcas* occurred only in few occasions given the low genetic variability outside the center of origin. This significant bottleneck is a serious limitation for the genetic improvement of *J. curcas*

Selection of Candidate Genes and Design of Gene-Specific Primers

Classically, ESTs and cDNA are used to generate primers to amplify regions of selected genes involved in toxin or oil production and stress tolerance pathways; here *J. curcas* ESTs available from GenBank (NCBI) were used.

After the identification of target genes, appropriate primers were designed using gene structures as available from public annotations to tag the coding and upstream regulatory regions. The web-based program CODDLE (http://www.proweb.org/input) was used to evaluate the probable effect of natural polymorphisms on gene function with genomic, cDNA or protein sequences as input data. Given an input sequence, the program generates models for genes with defined intron/exon position and for protein conservation. These models are then used to generate a graphic output, which shows the SNPs estimated to affect the protein function of selected genes. CODDLE allows to specifically design PCR primers to target the functional domain of interest or the most-conserved domain that is likely to be the region most sensitive to amino-acid substitutions (McCallum et al. 2000a, b; Gilchrist and Haughn 2005).

Afterwards, regions of 1,000–2,800 bp in candidate genes were selected using Primer3 (Rozen and Skaletsky 2000) with melting temperature ranging from a

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minimum of 67°C to a maximum of 73°C (Colbert et al. 2001). Primers were searched by comparison to GenBank (NCBI) and accepted when the identity score of aligned residues was ≥99%. The forward and reverse primers were labelled at the 5′ end with an infrared fluorescent dye (IRD) detectable at 700 nm (IRD700) and 800 nm (IRD 800), respectively.

In some cases, the available target coding sequence was limited by EST size and therefore also sequences from introns were selected. Among 40 primer pairs, 15 (37.5%) selected primer pairs failed to produce amplicons; three (7.5%) primer pairs amplified more than one fragment even after PCR optimization; 22 (22.5%) primer pairs amplified single fragments. Nine out of 22 primer pairs produced fragments longer than 1,500 bp, which indicated the existence of intron sequences in the selected genes. Thirteen out of 22 (32.5%) amplicons were from expected target sequences. To avoid non-specific amplification, primer pairs were chosen in order to produce a single amplicon. For example, among the amplicons evaluated by Sanger sequencing, 12 out of 22 were determined to have amplified a single product based on peak analysis. This core set of validated primers was used for *Jatropha* spp. EcoTILLING.

DNA Extraction and PCR Optimization

According to the protocol used for *Jatropha* spp. samples of genomic DNA were (1) extracted from leaves of a selected population using the DNeasy Plant mini kit (QIAGEN) following the manufacturer's protocol, (2) quantified by UV spectrophotometry and (3) evaluated by agarose gel assay with the molecular weight marker type VI (Roche, Applied Science). It is crucial to ensure that all DNA samples are equivalent in concentration to avoid sample bias when pooling experiments. Thus, DNA samples were normalized to 3 ng/μl for pooled as well as non-pooled assays. Concentrations ranging from 0.1 to 0.9 ng of template DNA were tested for PCR reaction on representative samples with each sample containing a DNA pool of eight different accessions of *Jatropha* spp. At concentrations between 0.05 and 0.03 ng, true mutations could be lost and false-positive signals begin to appear. False-positive signals possibly occur from rare random *Taq* errors happening in the early rounds of PCR (Till et al. 2006a). Using higher concentrations in the range of 0.25 or 0.3 ng resulted in the best signal-to-noise ratio of gel images. Finally, after testing different target genes, 0.3 ng was found to be the best amount of genomic DNA template for PCR reactions in *Jatropha* spp. EcoTILLING.

Establishment of Pooling Strategy

Since EcoTILLING technique was not previously established for *Jatropha*, the first challenge was to select the best pooling strategy to maximize the efficiency of recovering minor alleles in the tested population.

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The number of samples that could be pooled together was determined by testing multiple mutations or polymorphisms at different levels of sample pooling. Four different pooling strategies were used to identify homozygous and heterozygous SNP variations. Thus, variation was analyzed (1) within a single tree (heterozygous), (2) between individual trees and a reference and (3) in samples of fourfold and eightfold pooling strategies (Fig. 17.3).

In non-pooled samples, only heterozygous nucleotide changes were discovered. This is because the single-strand specific nuclease only cleaves at the site of mismatches. To uncover homozygous differences between the test and reference sample, reference DNA was added to each non-pooled sample prior to PCR. The comparison of bands identified in these mixtures to bands discovered in samples assayed alone allowed differentiating homozygous and heterozygous polymorphisms. Due to the reported low variations among *J. curcas* accessions and to the large size of *J. curcas* collection at PBU, the eightfold pooling strategy was used to determine the level of variations among 12 selected genes with the surprising result that we discovered a high level of SNPs.

To test the possibility that some polymorphisms were being missed by over-pooling of samples, the pooling was reduced to fourfold and the assays were repeated. Because the same polymorphisms by fourfold and eightfold pooling were obtained, we concluded that the pooling factor had no effect on the false negative rate in this range of variation (data not shown). This is consistent with previous evaluation of EcoTILLING according to pooling factor of DNA samples (Till et al. 2006b).

Gel Electrophoresis

Samples were amplified individually or by pools in 96 well microtiter plates using primers for the target genes. Heteroduplexes and homoduplexes were formed from the PCR products of pooled and non-pooled samples by successive heating (denaturing) and cooling (annealing). After restriction through enzymatic mismatch cleavage using a celery juice extract, amplicons were detected with a LI-COR 4,300 DNA analyzer (Biosciences) as described by Till et al. (2006a). The enzymatic restriction of PCR products with celery juice extract was performed by incubation at 45°C for 15 min. After the incubation period, restricted fragments were separated on a denaturing polyacrylamide gel attached to the LI-COR system.

Gel Image Analysis

The Tiff gel images produced by the LI-COR system were analyzed using the GelBuddy program (Zerr and Henikoff 2005) considering that the IR-Dye 700 channel showed the PCR products that carried the left end primer while the IRDye 800 channel showed the PCR products that carried the right end primer. GelBuddy allowed to detect complementary 5′ IRDye700 and 3′ IRDye800 fragments, co-migrating fragments and to identify samples of the same genotype.

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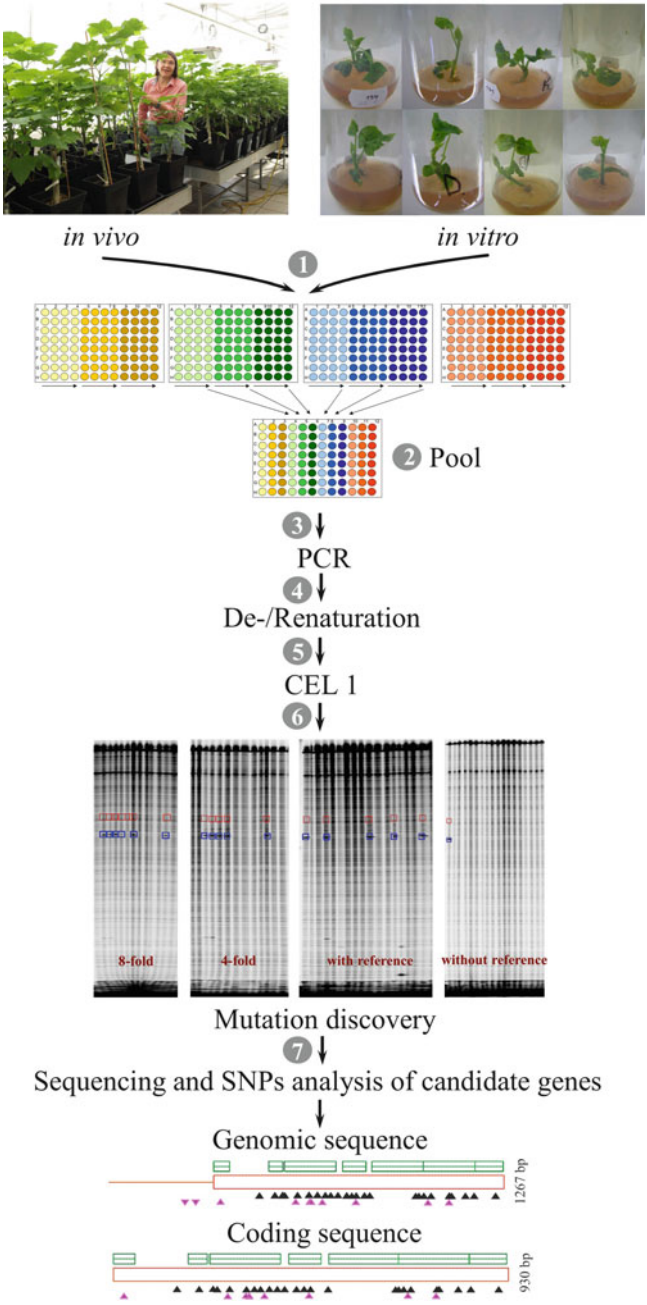

Fig. 17.3 *Jatropha* spp. EcoTILLING strategy. (*1*) Germplasm is collected and maintained as *in vivo* and *in vitro* accessions. Genomic DNA is extracted, normalized and arrayed in 96-well plates. (2) DNA samples are pooled by gathering four (fourfold) or eight (eightfold) samples together in order

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After genotyping a broader set of accessions, those with independent and new haplotype patterns of mismatches were chosen for further analyses. The amplicons for these regions were sequenced in order to validate the new SNPs and calculate their frequency (Maghuly et al. 2012, in preparation).

Identification of New SNP Haplotypes by EcoTILLING

The target genes from the reference plant were sequenced for the detection of SNPs and prediction of the expected haplotypes. The sequence analysis was performed using DNASTAR (Lasergene 8) and the potential effect of SNPs on protein function was predicted using *Sorting Intolerant from Tolerant* (SIFT) and *Project Aligned Related Sequences and Evaluate SNP* (PARSESNP) softwares (Ng and Henikoff 2003; Taylor and Greene 2003) (Fig. 17.3).

SIFT predicts an amino acid change with approximately 75% accuracy, whether or not an amino acid change is damaging a protein (Ng and Heinikoff 2003). PARSESNP allows the graphical view of all nucleotide changes discovered in a candidate gene and shows the predicted effect of changes on the translated protein using SIFT scores. A *Position Specific Scoring Matrix* (PSSN) provides information on restriction sites gained or lost due to SNPs, which can be used for developing rapid genotyping assays.

Representative alleles identified by EcoTILLING in selected *Jatropha* spp. accessions were sequenced in order to report their precise SNP position and polymorphism type.

The target gene sequences for *Jatropha* spp. EcoTILLING were chosen from GeneBank (NCBI). The primers also amplify homologous sequences in related species within the genus *Jatropha* with, however, a clear distinct pattern associated to *J. curcas* (Fig. 17.4). Interestingly, the background banding pattern is consistent within the species considered and is present in the absence of any mismatched DNA (Till et al. 2006a). The pattern likely arises from stalling of polymerase molecules during the amplification cycle corresponding to the linear accumulation of a given gene sequences. This feature alone can serve as a rapid measure of sequence relatedness in samples even in the absence of heterozygous polymorphisms and may serve as a useful means of evaluating uncharacterized germplasm.

Fig. 17.3 (continued) to increase EcoTILLING screening throughput. (*3*) The screening starts with a PCR amplification using fluorescently labelled primers. (*4*) Amplicons are denatured by heating and re-annealed by a slow cooling down, which gives amplicons the opportunity to form heteroduplexes. (*5*) These heteroduplexes are digested with a crude celery juice extract containing the single-strand specific nuclease CEL 1 that cleaves DNA at mispairing sites. The samples are purified, denatured and loaded on an absorption membrane comb and separated by electrophoresis. (*6*) The gel images are analyzed for the presence of rare SNPs with GelBuddy. (*7*) Selected amplicons are sequenced to identify SNPs and their possible effects on gene products are analyzed with PARSESNP

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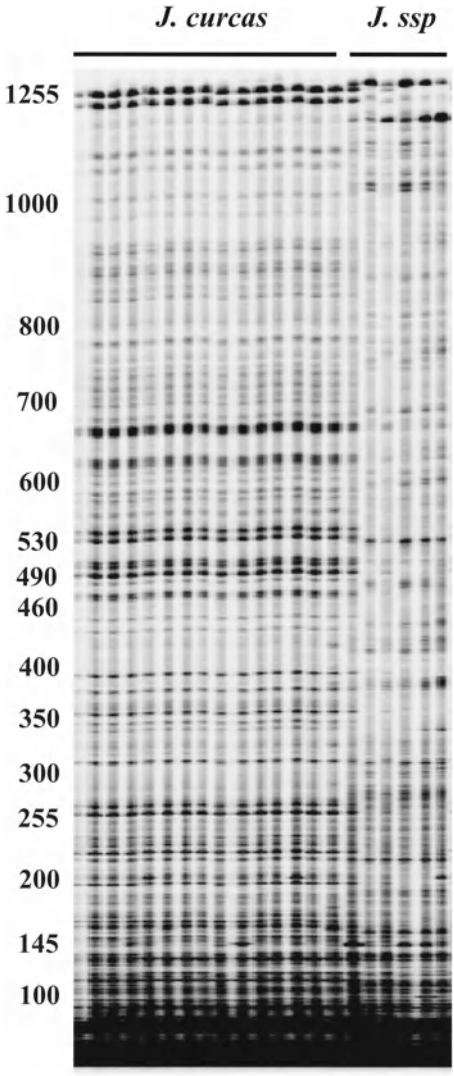

Fig. 17.4 *J. curcas* accessions and other species of the genus *Jatropha* were screened for SNPs in fourfold pooled DNAs (each lane contains DNA from four individuals). Images were collected in Tiff format and analyzed using GelBuddy. An IRDye 700 gel image of the AF gene is shown. The molecular weight of each mutant band is obtained by reference to the molecular weight scale on the *left*

The identified SNPs were isolated from the population for phenotypic analyses. By applying EcoTILLING to 12 gene fragments in our collection of 1,200 accessions and species from the *Jatropha* genus, 23 new rare SNPs were discovered in addition to common SNPs (41) that formed the large majority (Maghuly et al. 2012, in preparation). The rare non-synonymous SNPs found in *J. curcas* were, indeed, predicted by either SIFT or PARSESNP.

When using EcoTILLING in *Jatropha* spp. for functional analyses, it is important to have some knowledge about the gene copy number and/or gene family composition. Thus, an open approach to functional gene analysis is essential for gene annotation in *Jatropha* spp.

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Conclusions

The use of EcoTILLING as fast and efficient way of screening for genetic variation in plants, seems to be an efficient and cost effective strategy over sequencing for rare SNP search when the sample size is large, especially if the pooling step is optimized. EcoTILLING is also likely to be more cost-effective relative to sequencing in cases where raw polymorphism data is sufficient without base-pair determination. This is because EcoTILLING uses a mismatch-specific nuclease that cleaves amplicons at the site of a nucleotide polymorphism and similar polymorphisms in different plants can be grouped together based on the molecular weight of their corresponding bands. Furthermore, after polymorphism identification, individuals can be grouped according to their haplotypes and decided to be sequenced if judged necessary. This greatly reduces the need of sequencing since only a handful of individuals need to be evaluated to capture all the nucleotide diversity in a population. In addition, the relative position of each SNP in an amplicon is given by its banding patterns, which facilitate subsequent analysis. EcoTILLING is especially useful when working with plants that have a narrow genetic basis or looking for variation in highly conserved genes.

The EcoTILLING technology was first developed to characterize the variability of five genes within a collection of *Arabidopsis* ecotypes (Comai et al. 2004). It has also been successfully used in analyses of the natural variability of wild populations of *Populus trichocarpa* (Gilchrist et al. 2006), in the identification of allelic variation in resistance genes of barley (Mejlhede et al. 2006) and for other species genotyping (Gilchrist and Haughn 2005). EcoTILLING was adapted and set up for the first time in *J. curcas* by us (Maghuly et al. 2011, 2012 in preparation). It showed to be an effective high-throughput technique for the study of natural genetic variation in species with a low genetic variation, such as *J. curcas*.

The PBU *Jatropha* spp. collection contains a wide range of accessions including toxic, non-toxic and related species from different countries. They were chosen to examine the diversity within single trees or populations from diverse regions all over the world to obtain an estimation of the variation that exists in *J. curcase* specially in the candidate genes for economically important traits that are natural targets of selective breeding programs.

In order to increase the efficiency of EcoTILLING in *J. curcas*, a pooling strategy was designed based on the grouping of as much as eight samples without affecting accurate recovery of sequence polymorphism. Such a rather large pooling factor is possible because of the low genetic diversity between and within *J. curcas* populations. In addition, the EcoTILLING strategy allowed the identification of hetero- and homozygous SNPs as well as alleles that differ according to a reference individual.

SNP diversity in non-coding regions can be higher than in coding regions due to lower selective pressure and therefore can be more useful for diversity studies. SNP variations in coding regions allow the finding of alleles that are under selection, which suggests that SNP data combined to expression or phenotype data may release valuable information on temporal or spatial gene functions. Although SNPs in

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coding regions are less frequent than in non-coding regions, obvious changes predicted to alter protein sequences, were detected, especially in toxin genes (data not shown). Overall, the EcoTILLING method proved to be successful in detecting SNPs as well as INsertions and DELetions (INDELS) between *Jatropha* spp. However, polymorphisms were less frequent between *J. curcas* accessions in agreement with the notorious low rate of genetic diversity of *J. curcas* as it is known outside its center of origin. This lack of polymorphism was also observed in ISSR and AFLP studies (reviewed by Johnson et al. 2011; Singh et al. 2010).

The low variability found in the *J. curcas* candidate genes analyzed so far together with data on ISSR and AFLP (Maghuly et al. 2012, in preparation) suggests that genetic diversity may be limiting for traditional breeding approaches. Therefore, developing novel nucleotide diversity through mutagenesis can be also considered. Ultimately, mutagenesis can provide an allelic series of silent, mis-sense, non-sense, knockout mutations and splice site mutations, which are potentially useful in a variety of gene function, interaction studies and selective breeding (Peters et al. 2003; Till et al. 2006a). In addition, by identifying blocks of conserved sequences within relatively non-conserved non-coding regions, TILLING may help identify regulatory domains (Gilchrist and Haughn 2005).

The EcoTILLING could be adapted to discover nucleotide variation in *Jatropha* spp. (Maghuly et al. 2011). Target regions of up to 2.8 kb were screened using either individual, fourfold or eightfold pooled samples or only including a reference accession. 1,200 *Jatropha* spp. samples were screened, leading to the interrogation of ~26,000 kb for SNP detection. Because of the dual end labelling strategy, the false positive rate is low and a nucleotide change normally results in a band in the IRDye 700 and IRDye 800 channels.

While screening non-pooled samples provided a starting point for developing *Jatropha* spp. EcoTILLING, the use of pooling strategy increased throughput rate by eightfold and helped the identification of rare SNPs at the expense of rediscovering common SNPs. The presence of common SNPs in nearly all pools created a common banding pattern that blends into the background-banding pattern of the gel. Thus, the level of sample pooling can be adjusted depending on the need to accurately catalogue major versus minor alleles. The focus has been on rare alleles as phenotypic differences observed in a subset of germplasm, cannot be easily explained by common polymorphism.

EcoTILLING provides a robust high-throughput method for the identification of heterozygous SNPs and mutations with frequencies <5%. Above all, the central TILLING technology used in EcoTILLING was originally designed for the discovery of rare induced mutations (Colbert et al. 2001; Till et al. 2003).

We conclude, here, that EcoTILLING is a fast and highly cost-effective alternative to the current state-of-the-art techniques for rare polymorphism search in *Jatropha* spp.

The identified novel SNPs in *J. curcas* can be used as markers in this species. Future studies may include developing SNP markers from *J. curcas* EcoTILLING, which can be useful for marker-assisted selection (MAS) in breeding for specific traits. We expect that the information resulting from *J. curcas* SNPs will directly or

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indirectly contribute to timely topics ranging from basic studies of evolution to molecular physiology, applied plant biology, marker assisted breeding and more particularly functional genomics.

Data collected from *J. curcas* will boost research on other Euphorbiaceae and will open new perspectives for:

(a) Providing a better understanding of the basis of phenotypic diversity in the Euphorbiaceae, which comprises economically significant species, such as rubber tree, cassava and castor bean.
(b) Uncovering the mechanisms of gene regulation, signalling, disease resistance, vegetative and generative development.
(c) Reducing the time required for the analyses of large samples and discovery of SNPs.
(d) Contributing to the agronomic improvement of non-domesticated species.
(e) Understanding the influence of human impact on the genetic diversity of *Jatropha* spp.

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Chapter 18
Comparative Genomics in Euphorbiaceae

Rajinder Singh Chauhan and Archit Sood

Introduction

Euphorbiaceae is a complex heterogeneous family consisting of about 322 genera and 8,900 species worldwide (Balakrishnan and Chakrabarty 2007). The family is essentially tropical and occurs in diverse habitats from arid regions to humid tropics. As a result, the species of this family have developed various life forms from herbs, shrubs, stunted succulents to tall canopy trees. Euphorbiaceae includes several economically important species, such as cassava (*Manihot esculenta* Crantz), a primary staple food and industrial crop (Ceballos et al. 2004); rubber tree (*Hevea brasiliensis* Muell. Arg.), the main resource of natural rubber (Leitch et al. 1998); castor bean (*Ricinus communis* L.), an important oil crop; physic nut (*Jatropha curcas* L.), a oil crop with important applications in biodiesel production (Kumar and Sharma 2008) and leafy spurge (*Euphorbia esula* L.), a significant perennial weed of North American plains and prairies. Other important family members include poinsettia (*Euphorbia pulcherrima* Willd.), an important horticultural crop; annual weeds such as hop-hornbeam copperleaf and endangered species such as Akoka (*Chamaesyce spp*.) and telephus spurge (*Euphorbia telephioides* Chapm.).

The comparative genome analysis entails the extrapolation of information from one organism to another. Comparative genomics provides a strategy for utilization of sequence colinearity between two or more related species for molecular genetic studies (Windsor and Mitchell-Olds 2006). It is a powerful tool for gaining insights into genomic function and evolution. It can be applied to whole genomes or syntenic regions of different species, different subspecies or different strains of the same species. Comparative mapping and comparative sequence analysis are the major components of comparative genomics. Comparative mapping establishes

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the syntenic relationships between genomes of different species; it also assists genetic map consolidation, verification of *quantitative trait loci* (QTL), identification of candidate genes underlying QTL and a better understanding of genome evolution (Sankoff and Nadeau 2000; Kliebenstein et al. 2001). Comparative maps have been constructed among several species within a few important families of plants like Brassicaceae (Paterson et al. 2000; Barnes 2002; Hall et al. 2002), Solanaceae (Doganlar et al. 2002), Fabaceae (Boutin et al. 1995; Yan et al. 2003) and Poaceae (Feuillet and Keller 2002; Laurie and Devos 2002; Ware et al. 2002; Ware and Stein 2003). For several members of the Poaceae (former Gramineae), molecular markers have been used to develop comparative chromosome maps (Moore et al. 1995; Devos and Gale 1997) and to study genes of agronomic importance across species (Snape and Laurie 1998). The comparative maps of rice and maize provide a basis for interpreting molecular, genetic and breeding information between these two important species; it has also contributed to the establishment of a framework for ultimately connecting the genetics of all grass species (Ahn and Tanksley 1993). Most of the initial work on comparative mapping among grasses has relied on *restriction fragment length polymorphism* (RFLP) probes (cDNAs or genomic clones) to establish gross gene orders and distance in specific chromosome segments. Only to a limited extent have researchers used cloned genes, *expressed sequence tags* (EST), mutant phenotype loci or QTLs in comparative genomics. Comparative genomics in the Brassicaceae has allowed to recognize the existence of 24 conserved genomic blocks (Schranz et al. 2006), which is an extension of 21 syntenic blocks identified in *Brassica napus* L. (Parkin et al. 2005). Comparative mapping studies between members of Brassicaceae (Lagercrantz and Lydiate 1996; Lagercrantz 1998; Lan et al. 2000; Babula et al. 2003), *Arabidopsis thaliana* L. and *Arabidopsis lyrata* L. (Kuittinen et al. 2004), *A. thaliana* and *Capsella rubella* Reut. (Boivin et al. 2004) and the identification of an *ancestral karyotype* (AK) (Lysak et al. 2006) have also stimulated interest in the evolutionary processes involved in the diversification of different lineages in Brassicaceae and variations in chromosome number of different species *vis-à-vis* their ploidy status. PCR-based *intron polymorphism* (IP) markers have been used for the development of a comparative map between *Brassica juncea* L., related *Brassica* species (*B. napus* and *Brassica nigra* L.) and *A. thaliana* (Panjabi et al. 2008) and syntenic relationships thus established will facilitate precision breeding, identification and positional cloning of candidate genes contributing to traits of agronomic value. Comparisons of large-scale genomic sequences of *Brassica rapa* L. and the whole euchromatic region of *A. thaliana* revealed extensive synteny between their genomes due to at least two shared genome duplication events (Mun et al. 2009). In Solanaceae, Wang et al. (2008) reported the generation and analysis of sequences for an unduplicated *conserved syntenic segment* (CSS) in the genomes of five agriculturally important members of the family, i.e., tomato, potato, eggplant, pepper and petunia. This analysis indicated that (1) the last common ancestor of these species dates of 27–36 million years ago; (2) more than one-third of short genomic segments (5–15 bp) are under selection and (3) more than two-thirds of selected bases fall in non-coding

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regions. A comparison of eggplant (*Solanum melongena* L.) and tomato genetic maps revealed conservation of large tracts of colinear markers (Doganlar et al. 2002), a common feature of genome evolution in Solanaceae and other plant families. Rutitzky et al. (2009) made a comparison of photoperiodically regulated genes between potato and tobacco that revealed conserved species-specific responses indicating that adaptations to changes in the light environment have evolved many times and represent a blend of ancient as well as recent evolutionary processes.

A significant understanding of gene synteny and diversity between members of Euphorbiaceae is lacking, therefore, it is currently difficult to design breeding programs to manipulate genetic stocks through comparative genomics. Several groups have succeeded in genome sequencing and have initiated the task of generating ESTs from leafy spurge and related species, such as cassava and castor bean. Genomics can significantly accelerate the identification of genes involved in biomass production, food quality and vegetative reproduction as well as marker-assisted selective breeding in Euphorbiaceae crops, such as cassava, castor bean, Jatropha and rubber tree.

Physic Nut

The common names of *J. curcas* include barbados nut, purging nut, physic nut and JCL (abbreviation of *Jatropha curcas* Linnaeus). Heafter, we refer to *J. curcas* as Jatropha. is endemic to tropical America. Now it is almost pantropical and has been listed as a weed in Australia, South Africa, India, Brazil, Fiji, Honduras, Panama, El Salvador, Jamaica, Puerto Rico and other parts of Caribbean. There is a growing interest in the use of Jatropha oil to alleviate the energy crisis. Jatropha oil is relatively simple to convert to biodiesel by chemical (Berchmans and Hirata 2008) or biological transesterification (Modi et al. 2007). In addition to the low production cost, Jatropha biodiesel has been reported to be non-toxic, clean and eco-friendly (Jha et al. 2007). The plant can grow at rainfall levels as low as 200 mm per annum. The high yield of oil per unit land area that can be eventually obtained with Jatropha makes it an attractive biodiesel plant (Fairless 2007). Jatropha is currently one of the most promoted oilseed crops and its seeds have an oil content of up to 35% (Yang et al. 2009). Its major fatty acids are oleic acid (34.3–45.8%; 18:1), linoleic acid (29.0–44.2%; 18:2), palmitic acid (14.1–15.3%; 16:0) and stearic acid (3.7–9.8%; 18:0) (Gubitz et al. 1999). Despite the recent attention that Jatropha has received as an oil source for biodiesel products, its potential has not yet been fully realised. Unlike other oil crops such as soybean, maize, rapeseed and sunflower, Jatropha varieties were not yet systematically improved for its agronomic traits (King et al. 2009).

Jatropha is a diploid species having a genome size of 410 Mb (Carvalho et al. 2008) arranged in $n = 11$ chromosomes with an overall GC content of 34.7%. About 42,000 genes were diagnosed as protein encoding besides transposon related genes (Sato et al. 2011) and a dbEST list of 43,349 EST entries (GenBank dbEST release 030112, March 1, 2012).

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Castor Bean

Castor bean (*Ricinus communis* L.) is an important non-edible oilseed crop. It is a tropical perennial shrub cultivated in many tropical and subtropical regions around the world; it is indigenous to southeastern Mediterranean Basin, eastern Africa as well as India and most probably originated from tropical Africa (Weiss 1971; Govaerts et al. 2000). The castor bean plant can vary greatly in growth habit and appearance. It is a fast-growing, suckering perennial shrub, which can reach the size of a small tree (around 12 m or 39 ft). It can be self and cross-pollinated and worldwide studies showed low genetic diversity within the castor bean germplasm (Allan et al. 2008; Foster et al. 2010). Castor seed is the source of castor oil, which has a wide variety of uses. The seeds contain between 40% and 60% oil that is rich in triglycerides, mainly ricinolein. Castor bean seed oil contains 90% of the unusual hydroxy-fatty acid ricinoleic acid (da Silva Ramos et al. 1984). Because of the nearly uniform content of ricinoleic acid in castor oil and the unique chemical properties that this fatty acid confers to the oil, castor bean is a highly valued oilseed crop for lubricant, cosmetic, medical, and specialty chemical applications. Furthermore, castor bean is a potential biodiesel source, due to its high seed oil content (da Silva Nde et al. 2006). It can be cultivated in unfavourable environments, making it an appealing crop in tropical developing countries. The seed contains ricin, a toxin (Knight 1979), which is also present in lower concentrations throughout the plant.

At the moment, the castor bean (2n = 20) genome accounts for 25,828 scaffolds and 54,000 contigs sequenced with a four-fold coverage and totaling 400 Mb (Chan et al. 2010). The overall GC content is 32.5% based upon the sequencing data. The number of protein encoding genes is about 31,300. There are also 62,592 EST entries of castor bean available from dbEST (GenBank release 030112, March 1, 2012).

Cassava

Cassava (*Manihot esculenta* subsp *esculenta* Crantz) is a starchy root crop and the primary staple food for over 800 million people worldwide (Lebot 2009). It is also called yuca, (Spanish), manioc (French), and mandioca (Portuguese). Cassava is a perennial woody shrub with edible tuberous root, which is cultivated for its starchy storage roots throughout tropical and subtropical regions of the world, particularly in South America, Africa, and Asia, where it is the major source of dietary energy for more than 500 million people. Cassava grows in tropics and subtropics and was introduced into Africa and Asia by Portuguese in the fifteenth century (Jennings and Hershey 1985; Allem 1994). The height of a mature cassava plant usually ranges from 1 to 2 m, although some cultivars reach 4 m. Branching height can be as low as 20 cm, while some varieties are never branching or flowering. Cassava accounts for approximately one-third of the

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total staple food production and provides over 50% of the energy for more than 200 million people in sub-Saharan Africa (IITA 1992). In Central Africa alone, cassava is estimated to provide over 1,000 Kcal per day to 30 million people (Cock 1985).

Cassava is generally considered as a diploid with 2n = 36 even if some authors have described it as a segmental allotetraploid with chromosome number x = 9. Actually, Jos and Nair (1979) conducted studies on the meiotic behaviour of several cassava genotypes and observed regular 18 bivalent chromosomes, which is typical of a diploid figure of 2n = 2x = 36 chromosomes. Its genome size is approximately 760 Mb, but only 533 Mb of the genome has been assembled (arranged in 12,977 scaffolds). The 533 Mb assembly gathers nearly all the genic regions of the genome and the missing portion is due to repetitive sequence that could not be assembled (Prochnik et al. 2012). The remaining assembly of the genome is being annotated further. The total number of protein encoding genes in cassava is about 30,666. There are 80,631 EST entries for *M. esculenta* in dbEST (GenBank dbEST release 030112, March 1, 2012).

Rubber Tree

Rubber tree is a perennial tropical crop that belongs to the genus *Hevea*. The genus *Hevea* encompasses ten species, all originating from Amazonia (Schultes 1990). They are all strongly outcrossing and monoecious. Only one species, *H. brasiliensis,* is cultivated for latex (Schultes 1990). There are many common names associated with this plant. Some of the names are: rubber tree, jebe, Para rubber, seringueira-branca, siringa, etc. Among the other nine species, only *H. benthamiana* Schott. produces latex of reasonable quality, but this species has rarely been used in breeding programmes. Rubber tree is native to South America and more specifically to the Amazon basin, it occurs in Bolivia – Beni (north); Brazil – Amapa, Amazonas, Mato Grosso, Para; Columbia – Amozonas; Peru – Huanuco, Loreto, Madre de Dios, Pasco, San Martin. Rubber tree is the source of virtually all the world's rubber production. Cutting the bark of this tree releases the latex, which is then collected, preserved and stabilized.

Rubber tree is a diploid species with 2n = 36 chromosomes. The rubber tree genome is estimated to ~2,000 Mb (MRB TARRC Press release, October 2010), making it one of the largest among cultivated crop species and perhaps the largest plant genome sequenced to date. In comparison with cultivated crops, the rubber tree genome is around 2½ times the size of apple and cassava genomes; 4–5 times larger than the genomes of black cottonwood, cacao, rice and castor bean; and 17 times larger than *Arabidopsis* genome. Around 20% of rubber tree genome contains functional genes and it was predicted that rubber tree genome may contain 43,000 genes according to the model based on initial comparisons between genome and transcriptome sequences. There are 37,745 EST entries for rubber tree in dbEST (GenBank dbEST release 030112, March 1, 2012).

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Genetic and Comparative Mapping

For genetic improvement, a genetic linkage map is an essential tool in molecular breeding (Guimarães et al. 2007). A genetic linkage map aids in genome mapping, genetic dissection of QTLs, positional cloning of important genes and is an indispensable tool for functional genomics studies (Harushima et al. 1998). Molecular genetic maps can be employed to understand the genetic basis of yield potential and identify genetic factors involved in the partitioning of photosynthesis products. Genetic maps can also be used to (1) elucidate genetic control of root quality including starch content and quality; (2) post harvest deterioration and (3) locate genes of nutrient use efficiency, flowering, disease and pest resistance. This information can be used to (1) choose parents with greatest breeding value, (2) guide breeding decisions for multiple trait population improvement, and (3) combine complementary genes hoping to achieve new combinations for yield and quality increases. Genetic linkage maps have been constructed in a number of economically important species (Hayashi et al. 2001; Ren et al. 2009; Wang et al. 2011a,b; Xia et al. 2010) using different markers, such as protein polymorphisms, *random amplification of polymorphic DNA* (RAPD), RFLP, *amplified fragment length polymorphism* (AFLP), *simple sequence repeats* (SSR) and *single nucleotide polymorphism* (SNP) in reference families (Table 18.1).

ESTs for Comparative Mapping in Euphorbiaceae

Concerning genetic mapping, ESTs may help in increasing the density of gene markers, contribute to order *bacterial artificial chromosome* (BAC) clones onto a physical map and anchor them on the genetic map. In addition, ESTs as well as RFLP or *cleaved amplified polymorphic sequences* (CAPS) markers can be used for allele mapping among populations. The BAC tagging with ESTs is a quick procedure since it does not need polymorphism information and can be done by high throughput PCR strategies. ESTs are fantastic tools to examine evolutionary relationships within Euphorbiaceae family. Some ESTs already exist for castor bean and rubber tree, which allow their comparison with those of cassava. Similarly, for mapping purposes, there is already a detailed genetic map of rubber tree (Lespinasse et al. 2000) and many ESTs from cassava cross-hybridize with rubber tree DNA. This type of approach should help to establish syntenic regions between different genomes, facilitate positional cloning of genes of interest and increase opportunities of map-based cloning.

Comparative Mapping Between Jatropha and Arabidopsis

A genetic linkage map of Jatropha has been constructed from two backcross populations with 93 progenies (Wang et al. 2011a). A total of 506 markers were mapped onto 11 linkage groups out of which 216 were SSRs and 290 SNPs selected from

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Table 18.1 Genome statistics of major species of Euphorbiaceae

Species	Chro. no. (n)	Genome size (Mb)	Sequencing status	Genes	EST (dbEST)	Genetic map	Reference
J. curcas	2n = 22 n = 11	410	Completed	42,000	43,349	Available	Sato et al. 2011
R. communis	2n = 20 n = 10	400	Completed	~31,300	62,592	Not available	Chan et al. 2010
M. esculenta	2n = 36 n = 18	760	Ongoing (533 Mb completed)	30,666	80,631	Available	Prochnik et al. 2012
H. brasiliensis	2n = 36 n = 18	~2,000	Draft completed	43,000	37,745	Available	MRB TARRC Press release, Oct 2010

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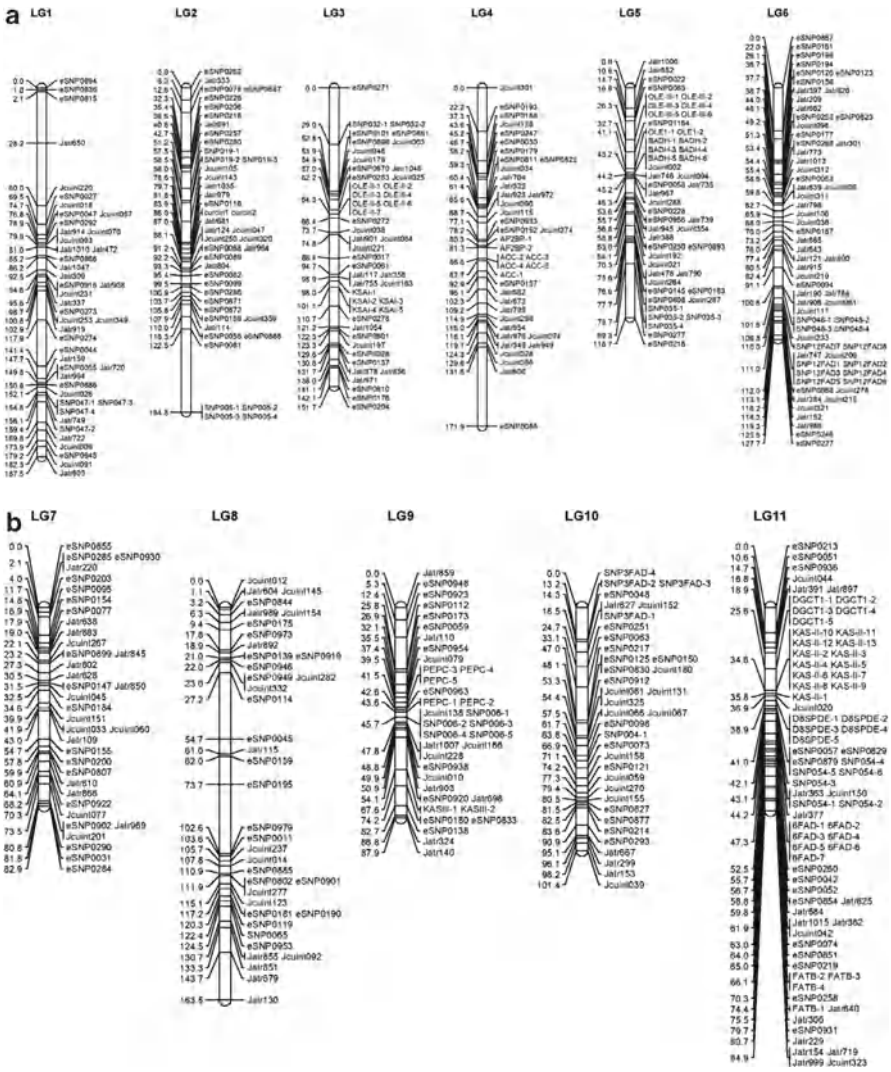

Fig. 18.1 Genetic linkage map of Jatropha. Estimates of map distances between markers are indicated in Kosambi centimorgans (Source: Wang et al. 2011a)

ESTs. SNP markers have allowed the creation of dense genetic linkage maps (Fig. 18.1a, b) and genome-wide association studies (Wang et al. 2005) because SNPs are the most abundant class of polymorphisms in genomes with an average frequency along the genome of one over 1,500 bp (Venter et al. 2001) and can be genotyped cost-effectively (Rafalski 2002). The total length of Jatropha map was 1,440.9 cM with an average marker space of 2.8 cM ranging from 1.2 to 4.3 cM. The 506 DNA markers were located in 324 discrete positions among 11 linkage groups; therefore, the average space of discrete positions was 4.4 cM ranging from

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2.7 for linkage group 11–5.7 cM for linkage group 1. In most cases, the space between two discrete marker positions was smaller than 20 cM. Only seven regions among several linkage groups were found to have distances between two discrete marker positions larger than 20 cM. Most spaces ≥20 cM were located in the end of linkage groups.

In addition, it has been shown that (1) 96.8% (215/222) of Jatropha ESTs corresponding to mapped microsatellites and SNPs were considered to be significantly similar to castor bean genome sequences; (2) 91.0% (202/222) were similar to poplar and (3) 77.5% (172/222) were similar to Arabidopsis on the basis of conserved synteny. A comparative map between Jatropha and Arabidopsis using 192 conserved DNA markers revealed the synteny of 11 Jatropha linkage groups with their Arabidopsis counterparts (Fig. 18.2a, b).

Comparative Mapping in Cassava

The first genetic map of cassava was constructed using RFLP markers and a full-sib intra-specific cross (Fregene et al. 1997). However, a F_1 progeny is not an ideal population for genetic analysis of agronomic traits such as complex QTLs or recessive and epistatic interactions. To overcome problems with genetic analysis in a F_1 cross of non-inbred parents, microsatellite (SSR) markers were used to construct a genetic map from an F_2 population (Okogbenin et al. 2006). The genetic map from a F_2 population is much more informative than from a F_1 population and is suitable to identify markers associated with agronomic traits as well as for comparative analysis with other species. The genetic map has been constructed using MAPMAKER/EXP, version 3.0 (Lander et al. 1987). One hundred and twenty two (122) polymorphic SSR markers spanning 1,236.7 cM, distributed on 22 linkage groups with an average marker distance of 17.92 cM were mapped according to polymorphic marker disjunction in the F_2 cross (Okogbenin et al. 2006). The average marker distance was 17.92 cM with intervals between loci ranging from 5.6 to 39.8 cM. The number of linkage groups in this map (22) exceeded the haploid number of chromosomes for cassava (n = 18), indicating that the map is not saturated.

The comparison of F_1 and F_2 maps revealed that the F_1 map spans 931.6 cM with 168 markers while the F_2 map spans 1,236.7 cM with 100 markers (Okogbenin et al. 2006). Forty seven SSR markers have been found common to both F_1 and F_2 maps. Consistent differences between both maps were detected for some markers due to the lower statistical confidence associated to the F_1 map.

A genetic linkage map using SSR markers from ESTs has also been constructed for cassava (Kunkeaw et al. 2011) using JoinMap 3.0 (Van Ooijen and Voorrips 2001). 56 EST-SSR and 155 SSR markers were mapped on 20 linkage groups spanning 1,177.57 cM with an average distance of 5.6 cM between markers and a density of about 11 markers per linkage group (Fig. 18.3). Given that ESTs also give an idea of the expression rate associated to genes, EST based linkage map may be useful for the identification of genes, gene-rich regions and QTLs.

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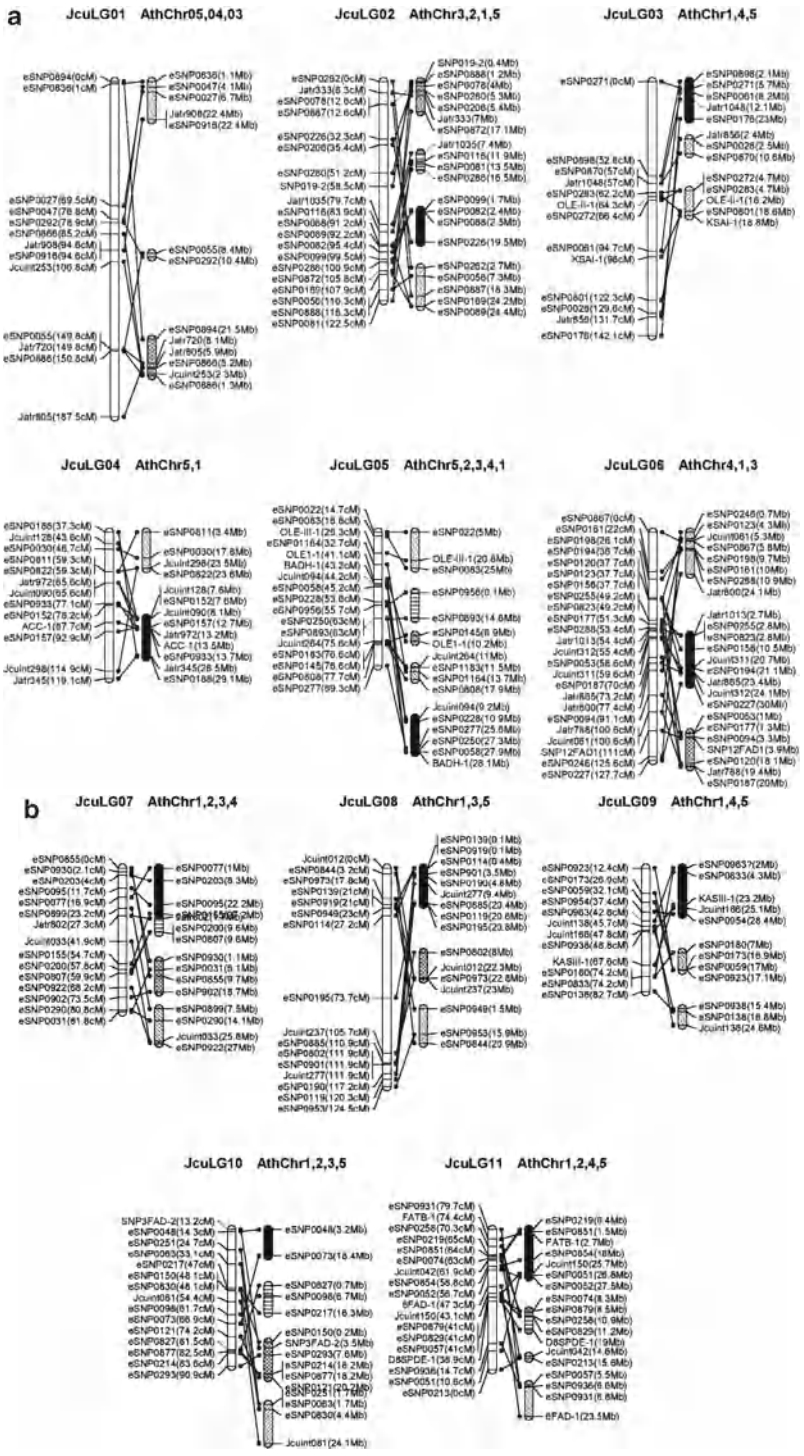

Fig. 18.2 Comparative map between Jatropha (Jcu) and Arabidopsis (Ath). Syntenic regions between Jcu and Ath chromosomes are indicated with *lines* connecting orthologous markers (Source: Wang et al. 2011a)

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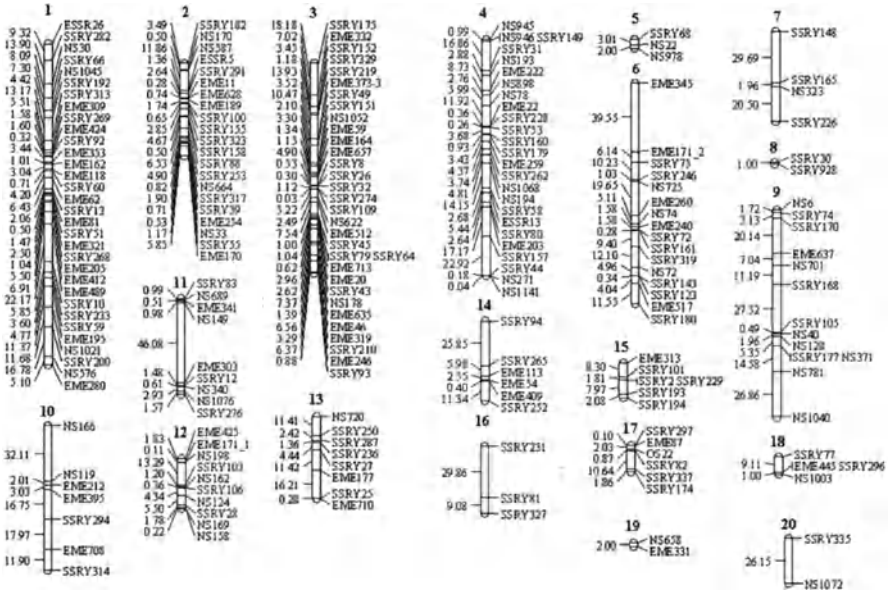

Fig. 18.3 EST-SSR genetic linkage map of cassava derived from a cross between Huay Bong 60 and Hanatee cultivars. Map distances shown on the *left* are indicated in Kosambi map units (cM) (Source: Kunkeaw et al. 2011)

Genetic Map of Rubber Tree

The genetic linkage map available for rubber tree is based on various molecular markers, such as RFLP, AFLP, SSR and isozymes. By contrast, the genetic map of Jatropha relies only on microsatellites (SSRs) and SNPs, whereas that of cassava is based on RFLPs and SSRs. The first genetic map of rubber tree (Lespinasse et al. 2000) was based on RFLP, AFLP, microsatellite, and isozymes and has been constructed with MAPMAKER and JoinMap (Fig. 18.4a, b). This map is based on a F_1 progeny (n = 106) resulting from an interspecific cross between two clones, i.e., PB260 (rubber tree) and RO38 (an interspecific hybrid of rubber tree and *H. benthamiana*). The synthetic map is derived from the maps corresponding to female and the male parents according to the pseudo-testcross strategy. A total of 717 loci constituted the synthetic map, including 301 RFLPs, 388 AFLPs, 18 microsatellites and 10 isozymes. These markers were assembled into 18 linkage groups and, thus, reflect the haploid chromosome number. The map is 2,144 cM with an average marker density of 1 per 3 cM. As expected, significantly less meiotic recombinations were found in the interspecific hybrid male parent than in the female parent when comparing both parental maps (Lespinasse et al. 2000).

Another genetic linkage map based on SSR markers has been developed for rubber tree by Souza et al. (2011). The map consists of 225 markers (59 SSR genomic

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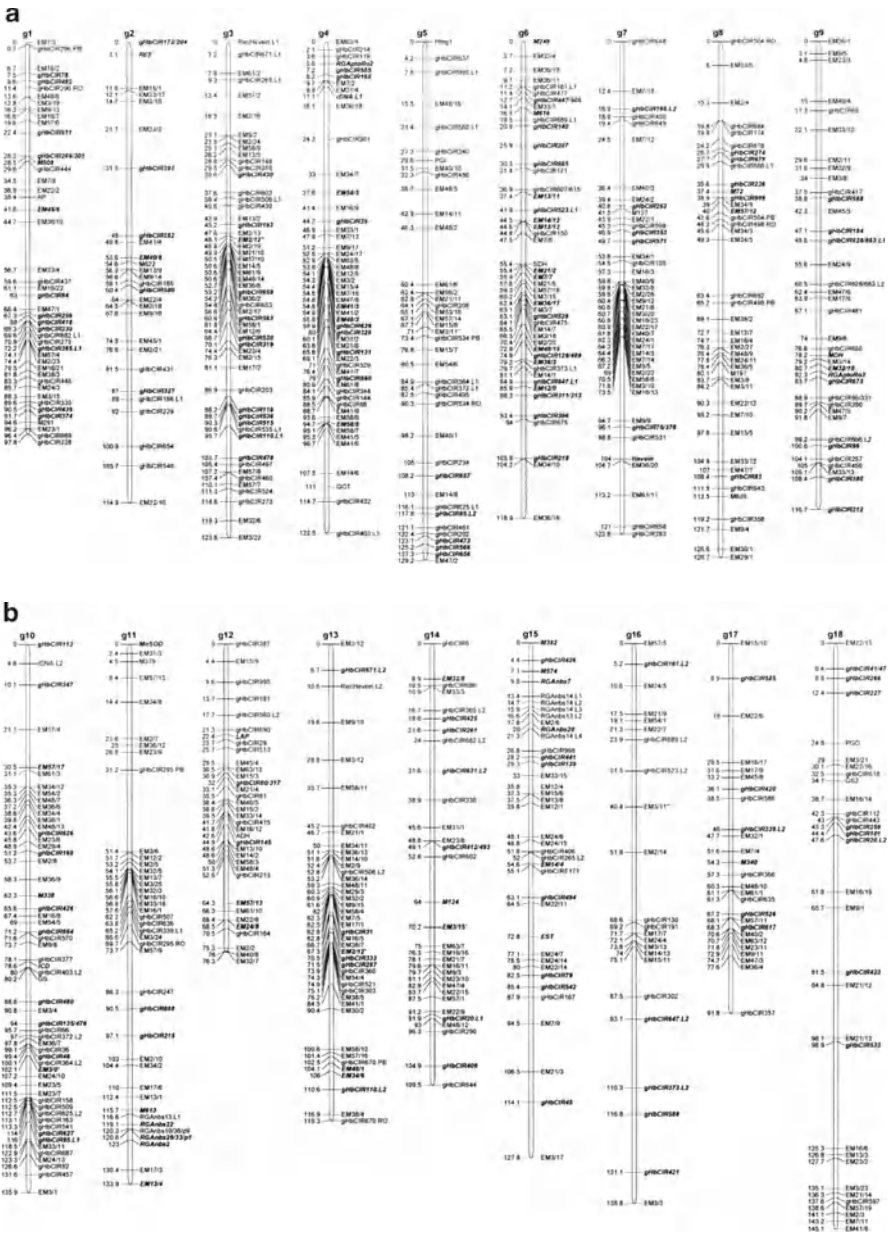

Fig. 18.4 F_1 synthetic map of rubber tree based on 717 markers distributed in 18 linkage groups. The marker set includes 301 RFLPs, 388 AFLPs, 18 microsatellites and 10 isozymes. *gHbCIR* is a RFLP probe, *RGA* is a RFLP probe of R gene, *EM* is AFLP, *M* is microsatellite. *Lx* suffix is for duplicate loci, *PB* and *RO* suffix are parental provenance (PB260 and RO38, respectively) for markers present in both parents, but not considered as bridges. Bridge markers are indicated in *bold* and *italic* (Source: Lespinasse et al. 2000)

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Table 18.2 Current status of genetic maps in Euphorbiaceae

Plant species	Genetic map	Molecular markers	Linkage groups	Total distance/ length (cM)	Reference
J. curcas	Available	SSRs, SNPs (from ESTs)	11	1,440.90	Wang et al. 2011a
R. communis	–	–	–	–	–
M. esculenta	Available	RFLPs	22	931.60	Fregene et al. 1997
		SSRs	22	1,236.70	Okogbenin et al. 2006
		SSRs (from ESTs)	20	1,177.57	Kunkeaw et al. 2011
H. brasiliensis	Available	RFLPs, AFLPs, SSRs, isozyme markers	18	2,144.00	Lespinasse et al. 2000
		SSRs, EST-SSRs	23	2,471.2	Souza et al. 2011

loci, i.e., gSSR and 166 EST-SSR) that are distributed in 23 linkage groups (LG) and cover 2,471.2 cM with an average genetic distance of 11 cM between adjacent markers.

In contrast to *Jatropha*, cassava and rubber tree that have genetic maps based on different types of molecular markers, while no genetic map is yet available for castor bean (Table 18.2).

Conservation of Genes in Euphorbiaceae and Microsyntenic Relationships

The amino acid sequences from the predicted coding sequences of Jatropha were compared with those of *A. thaliana*, *Oryza sativa* L., *Populus trichocarpa* Torr., *Vitis vinifera* L., *Lotus japonicus* (Regel) Larsen, and *Glycine max* L., as well as protein sequences in the TrEMBL protein database (Sato et al. 2011). Predicted genes in the genomes of castor bean and cassava were used as references for Euphorbiaceae protein-encoding genes. Similarity searches indicated that 4% of the predicted protein-encoding genes were found only in the Euphorbiaceae and that specific motifs were associated to the genes. The most common motifs found in these genes were the protein kinase-like domain. Furthermore, 1,176 of the genes predicted in Jatropha genome assembly had matching sequences only in the cDNA database suggesting that these genes are specific to this species.

The syntenic relationship between the Jatropha and the other plant genomes was investigated by analysing the conservation of relative gene positions using the genomic scaffolds of Jatropha (Sato et al. 2011). Of the 1,556 scaffolds with five or more predicted genes, the positions of three or more genes was conserved in 829 scaffolds (53%) compared to the position of homologous genes in the castor bean genomic sequences. It showed that a significant degree of synteny can be expected within the Euphorbiaceae family.

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Comparative Genomics of Genes from the Pathway of Fatty Acid Biosynthesis

The pathway of fatty acid biosynthesis is highly conserved in plants, but the fatty acid composition of seed oil varies considerably between species and within species. Fatty acid variations occur both in chain length and degrees of unsaturation. Consequently, the fuel properties of biodiesel depend on the fatty acid composition of the oil from which it is derived. The availability of whole genome sequences, ESTs and coding sequences from different oilseed species provide an opportunity to investigate what differences in genes can justify the variation in fatty acid contents and composition in their oils. Such information is expected to allow the identification of specific gene signatures that could assist the genetic improvement of crop plants either through marker-assisted breeding or by metabolic engineering (Rubin et al. 2000). Previous studies suggest that comparative analysis of genes involved in biosynthesis and accumulation of oil is a suitable strategy to investigate the molecular basis of variation in fatty acid content and composition in oils of different oilseed species.

Thus, we compared the genes of fatty acid biosynthesis of four oilseed species, i.e., Arabidopsis, *Brassica rapa*, soybean and castor bean because of the availability of genome sequences and several ESTs collections for these species (Sharma and Chauhan 2012). Moreover, soybean and *B. rapa* are the largest source of plant oil in the world whereas castor bean contains the unusual fatty acid ricinoleate that has chemical properties useful for industrial applications. We look to answer questions related to (1) whether there are common variations in genes, which contribute to increased seed oil content; (2) the identification of candidate genes for fatty acid, *triacylglycerol* (TAG) biosynthesis and proteins from oil-body (oleosomes), and (3) the comparative structure of these candidate genes (Sharma and Chauhan 2012). Thus, we retrieved 68 protein sequences among 32 gene families from the comprehensive lipid gene catalog of Arabidopsis (Beisson et al. 2003) and identified the functional domains associated to each gene family. We used 68 protein sequences from Arabidopsis as queries to retrieve fatty acid biosynthesis genes in *B. rapa*, soybean and castor bean databases. We retrieved 261 genes with 68 from Arabidopsis, 62 from *B. rapa*, 55 from castor bean and 76 from soybean. These genes correspond to six different categories, i.e., ACCase, desaturase, elongase, thioesterase, TAG synthesis and oil-body proteins. Further analysis revealed that the exon-intron structure of fatty acid biosynthesis genes of castor bean and soybean homologs shared larger structure similarity compared to those of Arabidopsis that is insertion, deletion and intron size variations were found in castor bean and soybean genes with reference to Arabidopsis. The genes of fatty acid biosynthesis from *B. rapa* were not included to this analysis because in most cases, their genomic structure was not available in GeneBank. The sequence and structure variations found in genes from fatty acid biosynthesis can be tested for their functional consequences on the content and composition of oil in Jatropha.

Overall, the comparative gene structure of genes from fatty acid biosynthesis provided an insight to improve oil quality for biodiesel by engineering of *FAD5*,

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FAD6 and *FatB* genes to enhance the content of saturated fatty acids. The variations in *FAD2*, *FAD3*, Stearoyl desaturase, *DGAT-1* and *DGAT-2* could be helpful to enhance the oil content in oilseeds. The detailed comparative genomics of fatty acid biosynthesis genes in oilseeds provided insights to undertake identification and utilization for the development of candidate gene markers in *Jatropha*. Thus, the sequence and structure variations identified in genes of fatty acid biosynthesis can be tested for their functional consequences for oil accumulation and composition in *Jatropha*. Oil genes showed evolutionary relatedness, but no synteny in gene order and position.

Conservation and Divergence of MicroRNAs in Euphorbiaceae

MicroRNAs (miRNAs) are non-coding RNAs of ~21 nucleotides. miRNAs are post-transcriptional regulators that bind to complementary sequences on target messenger RNA transcripts (mRNAs), usually resulting in translational repression. miRNAs act as key regulators of approximately all essential biological processes. A genomic scale analysis was performed by comparing miRNAs from miRBase (http://www.mirbase.org/) with the genome sequence of castor bean. The bioinformatics method that was applied to validate 85 conserved miRNA of castor bean proved to be successful with 86.6% success rate when applied to Arabidopsis (Zeng et al. 2009). Among the 85 miRNAs predicted in castor bean, 58 (68.2%) were experimentally verified during normal seedling development of castor bean, cassava, rubber tree or Jatropha (Zeng et al. 2009). A substantial number of putative miRNAs previously identified and characterized in model plants were conserved in members of Euphorbiaceae. Despite conservation across the four species of Euphorbiaceae, these miRNAs also exhibited diverse expression patterns in development and abiotic stress responses. Various target cleavage sites also existed that seemed to have a tendency to correlate with developmental and abiotic stress conditions, providing information of condition-specific cleavage. Overall, wide conservation of many miRNAs and diverse functions in Euphorbiaceae members during seedling growth and in response to abiotic stresses were revealed.

Comparison of Ribosome Inactivating Proteins (Rips) Among Jatropha and Castor Bean

The toxin known as curcin in Jatropha is categorised as a lectin and described as being similar to ricin from castor bean with the implication that it has similar toxicity. These are however inaccurate descriptions since curcin and ricin are both ribosome inactivating proteins (RIPs), which block protein synthesis by depurinating rRNA. Curcin is a type-I RIP (Barbieri et al. 1993; Juan et al. 2003a; Qin et al. 2005) whereas ricin is a type-II RIP. Type II RIPs contain both a catalytic A-chain

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and a carbohydrate binding lectin B-chain, which are encoded by the same gene (Hartley and Lord 2004). This lectin domain is not present in type-I RIPs such as curcin. Toxicity in type-II RIPs such as ricin is partly attributed to the ability of the lectin domain to adhere to cell surfaces and mediate the entry of the RIP into the cell (Olsnes et al. 1974). Because the lectin domain is lacking in type-I RIP, the LD_{50} values of type-I RIPs are typically over 1,000-fold higher than those for type-II RIPs (Barbieri et al. 1993). Additionally, type-I RIPs are present in many edible plant materials including cereal grains such as wheat and barley (Motto and Lupotto 2004), beetroot, spinach leaves and asparagus (Barbieri et al. 2006).

Amino acid sequence comparison of curcin and ricin reveals that both have a relatively high level of similarity since their percentage of identity was found to be 54% (Juan et al. 2003b). The similar residues among both proteins are consistent with RIP activity, which is supposed to constitute the active domain of curcin. It was found that both proteins were even more conserved at level of tertiary structure (3D alignment) than primary structure (sequence alignment). This similarity strengthens that there could be a strong preservation of three dimensional structure in these proteins with similar catalytic functions, especially with respect to critical amino acid residues in the conserved region of the active site. The conserved amino acid residues of curcin are located around the putative active site of ricin, which indicates that curcin possesses a *N*-glycosidase activity.

Transferability of SSRs Between Different Genomes of Euphorbiaceae

It has been reported that EST-derived SSRs and gSSRs show a considerable degree of transferability to related species (Cordeiro et al. 2001; Thiel et al. 2003; Gupta et al. 2003; Bory et al. 2008). A high level of transferability was effectively observed from cassava to Jatropha; the level of transferability was higher for EST-SSRs (44.63%) than for gSSRs (29.67%) (Wen et al. 2010). The higher levels of transferability of EST-SSRs than of gSSRs reflect the larger conservation rate of coding sequences compared to non-coding genomic DNA (gSSRs). It demonstrates the potential value of EST-SSR markers for genetic map development, genetic diversity assessment and marker-assisted selection (MAS) breeding in Jatropha. According to this conclusion, comparative mapping should be benefitted by the comparative analysis of genes involved in traits of economic importance among species of Euphorbiaceae.

Cross-Generic Transferability of SSR Markers

The SSR transferability among cassava and rubber tree was investigated by Whankaew et al. (2011). A set of 248 SSR markers (199 and 49 SSR markers of

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cassava and rubber tree, respectively) were used to detect 57 alleles in cassava and 120 in rubber tree, which suggests that cassava and rubber tree are closely related. Actually, the rate of transferability between cassava and rubber tree is larger than between cassava or rubber tree and Jatropha. These findings provide opportunities for comparative genomics and genome evolution studies in these valuable crops as well as recycling of markers for the documentation of relative species.

SSR Transferability Between Castor Bean and Jatropha

Genomic resources of castor bean have been successfully used for the development and utilization of SSR markers in Jatropha. High transferability (70%) of castor bean SSRs to Jatropha and other *Jatropha* species have shown higher levels of sequence identity between these plant species (Sharma and Chauhan 2011). High levels of structural and functional synteny have also been observed for other loci between castor bean and Jatropha, such as for genes involved in fatty acid biosynthesis. Whole genome analysis of castor bean allowed the identification of 580,986 SSRs with a frequency of 1 per 680 bp, on average. Genomic distribution of SSRs revealed that 27% were present in the non-genic region whereas 73% were present in putative genic regions with 26% in 5′ UTRs, 25% in introns, 16% in 3′ UTRs and 6% in exons.

Comparative Genomics of NBS-LRR Genes

Plants have developed resistance to a variety of pathogens and pests due to presence of disease resistance (R) genes encoding proteins that detect these unwanted organisms. The molecular research in the recent past on these R-genes and downstream signal transduction mechanisms has promoted their use for disease control. The majority of R-genes in plants belong to the *nucleotide binding site-leucine rich repeat* (NBS-LRR) class (Tarr and Alexander 2009; Guo et al. 2011). Recent sequencing of castor bean and Jatropha genomes have provided a plethora of R-genes including NBS-LRR genes, which were categorized into TNLs (TIR class of NBS-LRR) and CNLs (CC class of NBS-LRR) by reference to the conserved domains *Toll/Interleukin-1 Receptor* (TIR) and *N-terminal coiled-coil* (CC), respectively. However, differences were found between the number of R-genes, RPW8 domain/superfamily, members of the superfamily *disease resistance-responsive* (*dirigent* proteins) and protein kinase domain between Jatropha and castor bean genomes (Chauhan et al., unpublished data). The castor bean genome has also been used for cloning of *resistance gene analogues* (RGAs) in *J. integerrima*, which can be of practical importance in breeding for disease resistance in Jatropha. Genome synteny in plant is opening a new approach to gene evolutionary relationships and diversification among species (Table 18.3).

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Table 18.3 Comparative distribution of NBS-LRR genes between castor bean and Jatropha (Chauhan et al. unpublished data)

Characteristics	*R. communis*	*J. curcas*
Genome size	~400 Mb	~410 Mb
Number of disease resistance genes	121	91
Unique domain/superfamily present	Dirigent, protein kinases	RPW8
Occurrence of NBS domain	~0.4%	~0.3%

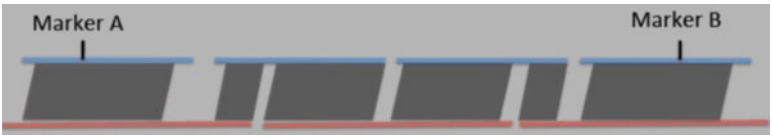

Fig. 18.5 Exploiting synteny with castor bean (*upper bars*: cassava, *lower bars*: castor bean) (Source: Steve Rounsley, personal communication; Prochnik et al. 2012)

Genome Duplication and Synteny

In addition to the syntenic relationships just described, the comparative analysis of cassava and castor bean genomes revealed genome duplication in cassava relative to castor bean (Prochnik et al. 2012). The synteny between cassava and castor bean has been exploited to targeted regions involved in *cassava brown streak disease* (CBSD) resistance. Problems associated to the investigation of syntenic relationship occurs when scaffolds are (1) too short, (2) end at same place in both species, (3) are interrupted by repetitive sequence and (4) confused by genome duplication. Extended synteny was found by aligning the 29 largest cassava scaffolds (>1Mb) with the castor bean genome (Fig. 18.5).

Comparative Genomics of Cassava and Rubber Tree, Similarity of Rubber Tree ESTs to Cassava and Other Related Plant Proteins

The efficiency of gene discovery in the rubber tree transcriptome was investigated by searching the *isotigs* (set of contig that result from the alternative expression of the same gene) and singletons for homology using BLAST against other plant reference sequences, such as cassava (Euphorbiaceae), castor bean (Euphorbiaceae), Arabidopsis (Brassicaceae) and rice (Poaceae) (Triwitayakorn et al. 2011). The majority of rubber tree unigenes matched proteins from cassava (1,02,936 or 48.1%), followed by castor bean (97,089 or 45.4%), Arabidopsis (84,643 or 39.5%) and rice (77,805 or 36.3%) (Fig. 18.6). Rubber tree, cassava and castor bean all belong to the Euphorbiaceae family, therefore, a large number of rubber tree isotigs and singletons matched proteins from cassava and castor bean (Feng et al. 2009; Tangphatsornruang et al. 2008; Raji et al. 2009).

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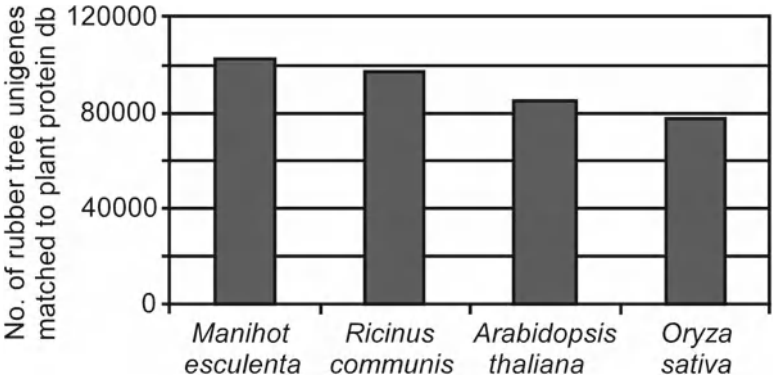

Fig. 18.6 Homology results of rubber tree isotigs and singletons that matched to proteins in the plant reference databases (*M. esculenta*, *R. communis*, *A. thaliana* and *O. sativa*) using BLASTx (Source: Triwitayakorn et al. 2011)

Conclusions

The availability of complete genome sequences has significantly altered our view on the complexity of genome organization, genome evolution, gene function and regulation in plants. The whole-genome sequences as well as other genomic resources (e.g., EST libraries, high throughput resequencing technologies) has allowed us to extend the comparative genomics to encompass the evolution of genome structure and function. Comparative genomics will be crucial for translating the basic knowledge obtained with model species into applied technology for crop species. Comparative genomic techniques will not be useful solely as a means of positional cloning or gene-finding in related species. Comparative genomics has shown both remarkable conservation and change among genomes and has proven to be an invaluable approach to understand biology not only from the point of view of patterns and processes of genome evolution, but also from gene functionality. Euphorbiaceae includes species fulfilling important economic functions, such as food (cassava, *Phyllanthus emblica*), oil (*Jatropha,* castor bean), medicine (*Jatropha gossypiifolia, Phyllanthus amarus*, etc.), rubber (Rubber tree*, Manihot glaziovii*) and ornamentals (*Euphorbia pulcherrima, Codiaeum variegatum*). Comparative analysis makes possible to learn more about the different characteristics of each organism and to link phenotypic with genotypic properties. Until now, only a little work has been done on the aspect of comparative genomics among the different members of Euphorbiaceae and it is necessary to proceed with comparative analysis among species of this family. Castor bean was the first species of Euphorbiaceae to have its genome sequenced, but its genetic map is not yet available. Thus, research should focus on developing a genetic map for castor bean and to compare it with those of the other species of Euphorbiaceae in order to accelerate breeding efficiency and

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recover maximum economical return. In addition, knowledge gained from other families like Poaceae and Solanaceae should be explored to gain insights in basic plant genome evolution and domestication.

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Chapter 19
Proteomic Perspectives on Understanding and Improving *Jatropha curcas* L.

Manish Raorane, Siam Popluechai, Angharad M.R. Gatehouse, and Ajay Kohli

Introduction

An agricultural revolution is 'brewing' to feed a projected world population of 10 billion or more by 2050. This population growth will be accompanied by consumption of proportionately more energy. Petroleum based energy sources have been sufficient until now, however, that will certainly not be the case in the long-term. The limited sources, rising prices and negative environmental impacts of fossil fuels pose significant environmental and socio-economic challenges. Globally, major national and international initiatives are underway to identify, revive, discover and recommend renewable sources of energy. One such renewable source is esterified vegetable oil i.e. biodiesel. Current and future demands for biodiesel are encouraging the diversion of food and feed crops into fuel. Using edible crops for fuel will

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drastically alter the land use pattern because food supplies need to be maintained to adequately feed the projected increase in population. Whilst there are growing concerns over the use of edible crops such as rape, soybean and palm for fuel oil, the use of non-edible seed oils or the use of direct bioconversions from waste (Demirbas 2007, 2008a, b) is now being considered as a major alternative.

In principle any oil rich plant seed can be used as a source of biodiesel. Azam et al. (2005) conducted an extensive study that compared 75 non-traditional oilseed plant species containing at least 30% w/w seed oil. The *fatty acid methyl esters* (FAME) compositions, *iodine value* (IV) and *cetane number* (CN) were compared in these plants to assess their suitability as a feedstock for biodiesel production. This analysis revealed that nearly one-third of the plants analyzed contained suitable seed oils. The results made a strong case against using edible oils for biodiesel. In fact the seed oil of 26 potentially useful species actually meets the diesel standards of the USA, Germany and the European Standards Organization (ESO, Azam et al. 2005). The list of alternative oilseed plants can be narrowed further to a few useful plants according to their oil productivity per hectare, potential economically useful by-products, growth habit (tree or shrub), habitat (arid, semi-arid, and tropical) and cultivation requirements such as fertilizers, water and the need for plant protection agents. Taking all of these criteria into consideration *J. curcas* was recommended as one of the most suitable sources of non-edible oilseeds for biodiesel feedstock (Azam et al. 2005; Chettri et al. 2008) in terms of yield of oil per unit area.

The conclusions of an international conference on *J. curcas* in 2007 stated that 'the positive claims on *J. curcas* are numerous, but only a few of them can be scientifically substantiated. The claims that have led to the popularity of the crop are based on the incorrect combination of positive characteristics which are not necessarily present in all *J. curcas* accessions and have certainly not been proven beyond doubt in combination with its oil production' (Jongschaap et al. 2007). From the data presented by Azam et al. (2005), it was clear that the two plants most suitable for biodiesel production were *J. curcas* and *Pongamia pinnata*. The presence of toxins like phorbol esters, curcins, trypsin inhibitors, saponins, phytate, lecithin in *J. curcas* and fluranoflavones, fluranoflavanols, chromenoflavones, flavones and furanodiketones as toxins in *P. pinnata* make these crops non edible. Of the two, *P. pinnata* is a tree and not readily amenable to pruning whereas *J. curcas* is a shrub that can be kept in train with a pruning regime that has been standardized to balance vegetative versus reproductive growth for maximum yields (Jongschaap et al. 2007). Therefore, the ease of harvesting the fruit from *J. curcas* in comparison to *P. pinnata* makes the former a more acceptable source of seed oil when grown as a large-scale plantation crop. Recently, Brittaine and Lutaladio (2010) summarized the advantages of *J. curcas* as a source of seed oil for biodiesel (Table 19.1). In terms of oil quantity, seeds of soybean contain 20% oil, seeds of rapeseed and *Jatropha* contain 40% oil and that of *Pongamia* contain 33% oil. Furthermore, Shuit et al. (2010) demonstrated a single step reactive extraction procedure for *J. curcas* which led to maximum conversion of 99.8% oil to biodiesel.

Biodiesel production from non-food feedstock is thus gaining interest. China recently announced an area the size of England for production of *Jatropha* and other

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Table 19.1 Strengths of *J. curcas* as a source of seed oil for biodiesel

Jatropha has the potential, through varietal improvement and good farming practices, for a high level of oil production per unit area in the subhumid tropical and subtropical environments
Jatropha grows and is potentially productive in semi-arid on degraded and saline soils
Jatropha can be used for halting and reversing land degradation
Jatropha grows fast, as compared to many tree-borne oilseeds
Jatropha trees remain small, enabling ease of management
Jatropha has periodic leaf shedding which facilitates nutrient recycling and dry season irrigated intercropping with short-term crops
Jatropha leaves are unpalatable to grazing livestock, making it a good barrier hedge to protect crops
Jatropha oil has physical and chemical properties that make it highly suitable for processing into biodiesel
Jatropha oil can be used directly in suitable diesel engines, lamps and cooking stoves
Jatropha by-products have potential value, such as using seed cake as fertilizer, animal feed (non-toxic varieties) or biogas, and using fruit shells and seed husks for biogas and combustion
Jatropha oil has markets other than for fuel, such as the production of soap, medicines and pesticides
Jatropha seed are storable and processing can be delayed, which makes production suited to remote areas
Jatropha has attracted investment, mainly from the private sector, into plant breeding, which increases the likelihood of developing *Jatropha* varieties with improved and stable oil yields

Adapted from Brittaine and Lutaladio 2010

important biofuel feed stocks. India has also decided to use 60 million hectares of non-arable land for *Jatropha* (Biodiesel-2020' 2009). In India, it is estimated that 15 billion litres of *Jatropha* biodiesel could be produced by cultivating the crop on 11 million hectares of wasteland (Mandal 2005). Brazil and Africa are also investing in *Jatropha* plantations in order to increase biofuel production.

J. curcas, also known as 'physic nut', 'pignut', 'vomit nut' or 'fig nut' is a perennial, monoecious shrub. Heller (1996) has provided a detailed morphological description of the plant. Recently, a number of physical and mechanical properties of the fruits, nuts and kernels have been described in terms of their importance in harvesting, handling and processing the oil for biodiesel production (Sirisomboom et al. 2007). The popularity of *J. curcas*, primarily based on its perceived/claimed benefits, is currently driving research with respect to co-product utilization (Kohli et al. 2009) and life-cycle sustainability assessment for rural development potential (Achten et al. 2007). If most 'traditional' claims can be validated then the improved elite varieties of *J. curcas* may live up to projected promises. However, neither *J. curcas* nor any other potentially useful non-edible oilseed plant is currently grown on a commercial scale and therefore do not compete as a source for an alternate fuel. In fact, such plants are generally undomesticated and have yet to be subject to genetic improvement with respect to yield quality and/or quantity. Thus, despite the enthusiasm in some countries for widespread plantation cropping, *J. curcas* is currently not commercially viable as a biodiesel feedstock without genetic improvement either through conventional breeding or molecular engineering.

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This review concentrates on proteins and proteome-based approaches used to identify some critical components of pathways useful for improving *J. curcas* as a source of seed oil for biodiesel. Initially, some background information is presented on the genetic, genomic and transcriptomic approaches being used towards the same goal. However, these aspects are considered in more detail in other chapters. In presenting the 'state of the art' with regards to protein research in *J. curcas,* studies dealing with identification of protein components of seed cake after oil extraction are not included in this review, which concentrates preferentially on proteins identified as integral components of the spatio-temporal expression continuum in *J. curcas*.

A Primer on Genetic Diversity in *J. curcas*

The number of studies on various aspects of harnessing the potential of *J. curcas* has steadily increased. These include studies on molecular characterization, most of which have concentrated on genotypic characterization in order to identify sufficient genetic distance between accessions to use them in breeding and marker assisted selection programmes. Repeatedly it was shown that the genetic diversity between global accessions of *J. curcas* was limited and unsuitable for use in breeding programmes (Basha et al. 2007; Wang et al. 2008; Sun et al. 2008; Popluechai et al. 2009; Sudheer et al. 2009; Tatikonda et al. 2009; Shen et al. 2010). Recently, there have been some reports of genetic diversity in the Indian and Chinese accessions (Gohil et al. 2008; Gupta et al. 2008). However, sufficient and useful variation was observed between accessions from specific regions of Mexico, which is believed to be the centre of origin of *J. curcas* (Pecina-Quintero et al. 2011), although Brazil (Martin and Mayeux 1984) and Central America (Heller 1996) are also considered as alternative centres of origin. Accessions form Mexico have some extremely useful agronomic traits such as low or no phorbol ester content (Nunez-Colin et al. 2009; Makkar et al. 2010) and only pistillate dioecious flowers (Pecina-Quintero et al. 2011).

The issue of toxicity through curcins and phorbol esters has hampered rapid adoption of *J. curcas* because the commercial potential of the seed cake and other by-products cannot be exploited (Kohli et al. 2009). In recent years however, non-toxic accessions (Nunez-Colin et al. 2009) and species of *Jatropha* (Makkar et al. 2010, 2011) have been discovered, as well as methods standardized to remove these toxins (Devappa and Swamylingappa 2008; Makkar et al. 2009). Yet, the unpredictable yield patterns for seed quality and quantity, varying and often low oil content, high male to female flower ratio, asynchronous and multiple flowering flushes, low seed germination frequency, plant height and its susceptibility to biotic and abiotic stresses are some of the common limiting factors to the success of *J. curcas*. Due to the difficulty in discovering the limited genetic diversity and a limited phenetic range in flowering and seed set patterns, an in depth understanding of molecular factors controlling flower and seed characters is required before this non-edible oil seed can be fully exploited as a fuel feedstock. Such an understanding is best advanced through the 'omic' studies whereby specific tissues can be studied at specific developmental time points.

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The Genomic and Transcriptomic Prelude

There have been some recent genomic and transcriptomic studies on *J. curcas* indicating that this plant is becoming a mainstream research system eliciting the use of high-throughput platforms to fast-track gene discovery for agronomic traits (Natarajan et al. 2010; Costa et al. 2010). The *J. curcas* genome has been sequenced both by the private (Genomeweb 2010), as well as by the public sector (Sato et al. 2011). Its chloroplast genome sequence was also made available (Asif et al. 2010). Reports on plant regeneration from leaf discs (Deore and Johnson 2008) or petiole (Kumar et al. 2011) and transformation of *J. curcas* through the methods of biolistics-mediated transformation (Joshi et al. 2011; Purkayastha et al. 2010) and *Agrobacterium*-mediated transformation (Kumar et al. 2010) set the stage for gene function validation in *J. curcas*. Another important tool for gene validation, that of gene silencing, has also been applied in *J. curcas* (Ye et al. 2009) further facilitating molecular understanding of genes and gene functions in this important plant.

Proteins Characterized in *J. curcas*

Despite progress in genomic and transcriptomic studies in *J. curcas*, there are limited protein-based studies and only a few studies at the proteomic level in this plant. For most protein-based studies, seeds were used as the tissue of choice, due to the importance of seeds as a source of the seed oil for biodiesel and for other innate seed products or by-products of the biodiesel enterprise. Although the *J. curcas* curcin gene was one of the first genes to be cloned from this plant (Lin et al. 2003), due to its importance as a toxin/anti-nutrient, which limits the use of this plant to its perceived potential, it was not studied at the protein level till recently (Lin et al. 2010b) when its LD50 was established in mice.

One of the first enzymes studied from *J. curcas* seeds was a lipase whose activity was detected in both dormant and germinating seeds (Abigor et al. 2002). This lipase was studied in terms of pH and temperature optima and for its hydrolytic activity on oil from various sources. Interestingly, it was noted that *J. curcas* lipase hydrolyzed fish oil at a higher rate than *Jatropha* oil, which was hydrolyzed at a higher rate than oil from palm, coconut or olive; it has the highest activity with mono-olein.

Proteins Involved in Fatty Acid Metabolism

Once again, due to the potential commercial importance of the seed oil, fatty acid metabolism enzymes have been targeted. Genes for some of these enzymes were cloned and recombinant proteins studied. The acyl-acyl carrier protein (ACP) thioesterase from *J. curcas* was cloned and its seed-specific overexpression in *Arabidopsis* resulted in both increased levels of saturated fatty acids, especially

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palmitate, and in reduced levels of unsaturated fatty acids (Wu et al. 2009). Dani et al. (2011) also studied the acyl-acyl carrier protein (ACP) thioesterase providing further insights into the relationship of *J. curcas* ACPs and those from other sources. The hetero-multimeric subunits of the *J. curcas* acetyl-CoA carboxylase (ACCase) were cloned and studied by Gu et al. (2011). ACCase catalyzes one of the first steps in the biosynthesis of long-chain fatty acids.

The beta-ketoacyl-acyl carrier protein synthase III (KAS III) is a condensing enzyme catalyzing the initial step of fatty acid biosynthesis using acetyl-CoA as primer. KAS III of *J. curcas* was cloned and its expression was studied in different tissues (Li et al. 2008). Although its expression was detected in most tissues, being the highest in roots, in the developing seeds its expression increased with time. Similarly the cloning and recombinant expression of *J. curcas* stearoyl-acyl carrier protein desaturase indicated that it was predominantly expressed in the developing fruit and was a part of a small gene family (Tong et al. 2006). Similar and additional fatty acid metabolism genes were also studied for expression differences at the transcript level between three accessions of *J. curcas* (Popluechai et al. 2009; Popluechai 2010); however, there were discrepancies observed between the amounts of transcripts and the protein product.

During investigations on the C_3/C_4 nature of photosynthesis in *J. curcas*, phosphoenol pyruvate carboxylase (JcPEPC) was investigated both at the gene and protein level (Raorane 2010). PEPC is known to contribute to fatty acid metabolism through an anaplerotic pathway (O' Leary et al. 2011). Interestingly, the JcPEPC turned out to be unique in that it contained six exons separated by five introns. Almost all plant PEPCs in C_3, C_4 and CAM plants studied to date, have been shown to possess 10 exons and 9 introns. Additionally, all known PEPCs belong to a multigene family, with three copies of PEPC in *Ricinus communis*, the plant most closely related to *J. curcas*. However, it was noted that JcPEPC existed as a single copy gene (Raorane 2010). The effect of this uniqueness of JcPEPC on any photosynthetic or anaplerotic activities has yet to be known.

Proteins Involved in Phorbol Esters Biosynthesis

Phorbol esters (PEs) are fatty acid esters of the diterpenoids. Comparative study of other model plants on genes involved in diterpenoid biosynthesis pathway led to identification of key genes regulating PE synthesis in *J. curcas*. An isoprenoid biosynthesis pathway gene, 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGR) and its protein was characterized by Lin et al. (2009). It catalyzes the first committed step in mevalonic acid synthesis which leads to carotenoid and phorbol ester synthesis. Also geranylgeranyl diphosphate synthase (Jc-GGPPs) gene was cloned and characterized from *J. curcas* (Lin et al. 2010). The GGPPs catalyze the condensation of isopentenyl diphosphate (IPP). GGPP is considered as one of the key precursors in the biosynthesis of isoprenoid compounds. Biological assay of these two genes in recombinant *E. coli* confirmed their role in diterpene biosynthesis. Whether manipulation of

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these genes leads to changes in phorbol ester content remains to be seen however, having a handle on these genes involved in the pathway is a start to dissecting the phorbol ester biosynthesis pathway in *J. curcas*.

Proteins Related to Stress Tolerance in J. curcas

The commercial importance of *J. curcas* also rests on its perceived, relatively high levels of tolerance towards abiotic and biotic stresses so that its cultivation can be undertaken on marginal land under minimal input conditions. For this reason, genes and proteins concerned with various stress tolerances in *J. curcas* were also investigated. In plants, water channel proteins and aquaporins, play a critical role in transmembrane water movements. Gene and protein level characterization of an aquaporin, *plasma membrane intrinsic protein* (PIP) from *J. curcas* (JcPIP2) revealed that it was upregulated under drought conditions in an accession known to be drought tolerant as compared to another accession known to be sensitive to water deficit (Zhang et al. 2007). The tolerant accession also showed higher root hydraulic conductivity, suggesting a relationship between water deficit tolerance and JcPIP2.

To further studies on drought tolerance, a betaine aldehyde dehydrogenase gene from *J. curcas* (JcBD1) was cloned and expressed in *E. coli*. This gene belongs to a multigene family and was shown to be expressed in roots, stems, leaves, flowers and young seeds, with the highest expression being in the leaves and stems. It was upregulated in leaves under stress conditions such as drought (30% PEG), heat (50 C), and salinity (300 mM NaCl) while the transformed *E. coli* also exhibited enhanced tolerance to higher salinity (Zhang et al. 2008). Similarly a *J. curcas* phospho-lipase, typically involved in phospholipid catabolism and responsible for the lipolytic cascade in membrane degradation during senescence and stress, was also cloned and expressed in *E. coli* and shown to be upregulated in leaves under stress imposed by 300 mM NaCl, drought (30% PEG), cold (4 C) and heat (50 C). It was also expressed in root, stem, leaf, endosperm and flower, but only weakly in seeds (Liu et al. 2010).

Two other examples of *J. curcas* genes cloned and expressed in *E. coli* and expressing in most plant tissues, but specifically upregulated under stress, were those encoding *allene oxide cyclase* (AOC) and the *ADP-ribosylation factor* (ARF1). Both these proteins impact the abiotic as well as biotic stress response in plants. AOC is a key enzyme in jasmonate biosynthesis. Jasmonates are known as potent signalling molecules during abiotic/biotic stress and induce gene expression for many proteins linked to the defense responses of plants (Gatehouse 2002; Sudha and Ravishankar 2002). Jasmonates also induce accumulation of additional secondary metabolites such as alkaloids, phenolics, coumarins, which further act in plant defense response (Sudha and Ravishankar 2002). Overexpression of JcAOC in *E. coli* conferred increased tolerance to salt and low temperature to the bacterial transformants (Liu et al. 2010). The expression of Jcarf1 was observed in stems, leaves, roots and highest in flowers. Jcarf1 transcript accumulation was

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different under various environmental stresses in seedlings and the *E. coli* expressing recombinant Jcarf1 exhibited binding activity toward GTP (Qin et al. 2011). Transcriptional profiling of plants under stress and non-stress conditions identified ARFs to be important in both abiotic and biotic stress responses (Sahi et al. 2006; Coemans et al. 2008).

Although *J. curcas* is now known to be susceptible to a number of diseases (Kohli et al. 2009), it is a relatively hardy species and in its native form not many pests and pathogens attack it unless highly favorable conditions are generated through plantation cropping. In this respect, knowledge from other plants has been used to isolate and/or characterize genes/proteins that could be responsible for the relative tolerance of *J. curcas* to pests and pathogens. A beta-1,3-glucanase was isolated from *J. curcas*, which exhibited in vitro antifungal activity against *Rhizoctonia solani* and *Gibberella zeae* (Wei et al. 2005). More recently an anti-microbial peptide (KVFLGLK, Jcpep7) was isolated from *J. curcas* using a novel method called cell membrane affinity chromatography (Xiao et al. 2011). Jcpep7 was active against *Salmonella typhimurium*, *Shigella dysenteriae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis*, and *Streptococcus pneumoniae*. The mechanism of Jcpep7 operated through breaking the cell walls and membranes of the microbes, followed by cell lysis.

Transcription Factors Characterized in J. curcas

A viable strategy to study stress tolerance in plants is to underpin transcription factors that can have a cascading effect on multiple genes to manifest stress tolerance. One stress associated transcription factor, a putative AP2/EREBP domain-containing protein called JcERF was cloned and characterized from *J. curcas* (Tang et al. 2007). The amino acid sequence of JcERF was not similar to other ERF proteins apart from the conserved DNA-binding domain. JcERF gene was rapidly upregulated under drought, salinity, mechanical wounding and ethylene but not under ABA treatment. It interacted with drought response element (DRE) and transgenic *Arabidopsis* overexpressing JcERF exhibited enhanced salt and freezing tolerance. JcERF is therefore, similar to other AP2/EREBP in some aspects, but not in others such as response to ABA. It is thus a novel transcription factor unique to *J. curcas*.

Another transcription factor recently characterized from *J. curcas* was the one finger Dof-transcription factor (JcDof1, and JcDof3; Yang et al. 2010, 2011) Yeast one hybrid studies with JcDof1 under different light conditions suggested that it was a circadian clock transcription factor responding to light signals of long day, short day and continuous light regimes. Under continuous dark, basal level expression of JcDof1 was observed. It was downregulated by red and blue light but not by far-red light. Flowering time in particular is controlled by photoperiod perception in plants and it is possible that this transcription factor has a role in affecting flowering time, which can provide one molecular tool in the tool box to engineer flowering in *J. curcas* because its asynchronous flowering undermines its commercial potential.

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Other Seed Proteins in J. curcas

Floral and seed proteins are obvious targets for study in *J. curcas*. Along with anti-nutrients, toxins and carcinogens, being an opportunistic crop in tropical areas, *J. curcas* can also be a source of seed/pollen allergens. One such allergenic seed protein, a 12 kDa, 2 S albumin was studied and confirmed to bind to IgE and induce histamine release (Maciel et al. 2009). Another proteinaceous component of *J. curcas* seeds was identified as the translationally controlled tumour protein (TCTP). It is a 168 amino acid protein whose expression was high in stem, endosperm and embryo but low in the flower (Qin et al. 2011). TCTP is found in both the animal and plant kingdom and is involved in calcium binding, regulation of apoptosis, and microtubules stabilization through binding to GTPase (Cans et al. 2003), mainly as a growth or cell division related protein. Knockout of TCTP in *Arabidopsis* led to flowers with normal pollen formation and germination but impaired pollen tube growth. TCTP silencing slowed vegetative growth, reduced leaf expansion and lateral root and root hair formation (Berkowitz et al. 2008).

Proteome Based Analysis of J. curcas

Studies on single targeted proteins, mostly starting from cloning of the corresponding gene from cDNA libraries, continue to provide an insight into qualitative and/or quantitative aspects of *J. curcas* plant and seed development. There are however, limited proteomic studies on *J. curcas*. One of the first such reports on proteome level investigations in *J. curcas* sought to address a limitation in using *J. curcas* globally as a source of seed oil for biodiesel, i.e. its inability to yield under temperate conditions. The chlorophyll fluorescence approach was used to link proteomic-mediated identification of photosynthesis-related proteins of *J. curcas* seedlings under cold stress (Liang et al. 2007). This combined approach of relating a physiological parameter to proteome data indicated that acclimation of PSII at the early-stage (0–12 h) and H_2O_2 scavenging mechanisms at the late-stage (after 24 h) of cold stress might be involved in the cold response mechanisms of *J. curcas* seedlings.

Other proteomic studies on *J. curcas* concentrated on the seed and/or endosperm. Popluechai (2010) compared the proteome of *J. curcas* seeds during the resting and germination stages to elucidate the proteomic differences between these two stages with a view to identifying proteins useful in engineering germination vigour. From the distinctly observed protein spots on 2D-gel electrophoresis, 60 spots were common to both stages, although there were differences in spot intensity between the two stages. The resting stage had 10 unique spots and 20 spots were unique to the germinating stage. Of the latter 20 spots, 7 spots were identified as patatins. Patatins are oil body-associated lipases with the ability to degrade stored triacylglycerides of oil seeds. High activity of lipid degrading enzymes has been reported during germination of many different oilseeds (Eastmond 2006). In terms of the early germination stage of *J. curcas* seeds, the high upregulation of patatins indicated that oil

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mobilization starts at an early stage of seed germination. This was confirmed when proteomic and ultrastructural analysis methods were combined in an attempt to better understand oil mobilization in germinating seeds of *J. curcas* (Yang et al. 2009). In addition to the presence of unique protein spots in germinating seeds, significant changes in abundance were noted for 50 protein spots during germination. Characterization of these spots revealed that several pathways including P-oxidation, glyoxylate cycle, glycolysis, citric acid cycle, gluconeogenesis, and pentose phosphate pathway were involved in oil mobilization.

Proteomic analysis of the seeds of three accessions of *J. curcas*, one each from India (JcI), Nigeria (JcN) and Thailand (JcT), revealed a number of common spots that were differentially expressed in comparison to each other (Raorane 2010). For example, eight spots were present at a much higher intensity in one or two accessions while JcT lacked two protein spots altogether. Identification of these eight protein spots revealed the proteins to be a chaperonin 60, a serine/theronine protein kinase, two heat shock proteins, two oleosins and two hypothetical proteins. The two protein spots missing in JcT but present in JcI and JcN were both identified as homologous to the wheat grain softness protein. RT-PCR data obtained from *J. curcas* accessions after designing primers based on wheat *gsp* sequence confirmed minimal transcript in JcT in comparison to JcI and JcN (Raorane 2010). The gsp per se had not previously been observed in dicot plants, although similar proteins called puroindolines are known in dicot plants. These are responsible for seed texture and may be useful in *J. curcas* towards understanding the relationship between seed texture and oil extractability.

The differences noted in oleosin content between different accessions was the basis for exploring the proteome content of oil bodies in two accessions of *J. curcas*, one each from India and Indonesia (Table 19.2; Popluechai et al. 2011). In addition to the most prominent three oleosins of *J. curcas*, the toxic protein curcin, for which *J. curcas* is notorious in terms of the non-edible nature of its seeds, was also identified in this set of proteins. Whether it is actually associated with the oilbodies is not certain, but it co-purifies with most oilbody associated proteins. In the Indonesian accession, the *J. curcas* homologue of *Arabidopsis* microspore oleosin (SM2) was also identified, as was a further protein similar to an *Arabidopsis* aquaporin (TIP3). Caleosin was predicted by homology with *Arabidopsis* and *Vitis vinifera* protein and a protein similar to *Coffea canephora* or *Ricinus communis* steroleosin was also present. Finally, a storage protein (60 kDa), identified from its homology with proteins from *R. communis* and *Quercus serrata* was also shown to be present in both accessions (Table 19.2). Whether this difference between the oil body proteome of *J. curcas* accessions from India and Indonesia is responsible for the superior oil content of the Indonesian accession remains to be demonstrated. This study also identified oligomers of oleosins likely associated with oil bodies but also with a possibility of existing without association with the oil bodies. Further, the study was also extended to investigate the presence of one of the oleosin *genes in J. podagrica, J. gossypiifolia, J. multifida*, *J. maheshwarii* and *J. integerrima*. A difference was noted in the single intron of the oleosin gene, but that difference did not seem to influence the oil content (Popluechai et al. 2011).

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Table 19.2 Oil body proteome of *J. curcas* accession each from Indonesia and India

Band	Identified protein	Apparent molecular mass (kDa)	Theoretical molecular mass (kDa)	NCBI accession number/*A. thaliana* hit	Peptide number	Coverage (%)
Indonesia						
1	*Jatropha* oleosin 3	18	14.40	ABW90150	3	10.9
	Jatropha oleosin 1	–	15.58	ABW90148	11	23.8
	Jatropha oleosin 2	–	16.60	ABW90149	14	31.6
2	*Jatropha* oleosin 2	19.5	16.60	ABW90149	16	40.0
3	*Arabidopsis* oleosin SM2	21	18.13	NP_188487/At3g18570	1	7.2
4	*Arabidopsis* α-TIP3	29	28.30	NP_177462/At1g73190	1	3.7
5	*Arabidopsis* ATS1	31	28.01	AAL36241/At4g26740	1	3.3
	Vitis vinifera caleosin	–	26.48	CAO71462	1	5.0
6	*Jatropha* curcin precursor	32	32.77	AAL86778	7	25.6
7	*R. communis* steroleosin	46	39.92	EEF34360	1	2.5
8	Coffee steroleosin	–	40.05	AAX49394	1	3.1
	Quercus serrata legumin	60	13.52	BAB12450	1	12.4
	R. communis protein storage	–	40.10	EEF35875	1	3.6
India						
1	*Jatropha* oleosin 3	18	14.40	ABW90150	2	10.9
	Jatropha oleosin 1	–	15.58	ABW90148	4	16.3
	Jatropha oleosin 2	–	16.60	ABW90149	3	18.7
2	*Jatropha* oleosin 2	19.5	16.60	ABW90149	3	13.5
3	*Jatropha* oleosin 2	21	16.60	ABW90149	4	18.1
4	*Jatropha* curcin precursor	32	32.77	AAL86778	6	24.6
5	*R. communis* storage protein	60	40.10	EEF35875	1	3.6

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Liu et al. (2009) also contributed an important proteomic study in *J. curcas,* which sought to differentiate the proteome of the endosperm from that of the embryo. Interestingly that study identified more catabolism related proteins in the endosperm and more anabolism related proteins in the embryo.

Future of J. curcas Proteomics

Selection of the appropriate proteomics platform is important. Liquid chromatography tandem mass spectrometry (LC-MS/MS) gave more informative results when compared to matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS; Popluechai, unpublished results). Liu et al. (2009) also mentioned that by LC-MS/MS helped in identification of protein spots previously difficult to characterize through MALDI-TOF MS. This is because LC-MS/MS platform concentrates the protein and is based on independent sequencing of peptides. However, LC-MS/MS analysis costs more than MALDI analysis. Therefore, depending on the infrastructural and financial resources, the proteomics platform may vary. However, there is little doubt that detail proteome analysis of *J. curcas* starting from flower development to seed maturity will contribute a treasure of information required to understand and engineer *J. curcas* towards realizing its potential as a source of biodiesel. Additionally, proteomic studies on post-translation modification of proteins during flower and seed development will be an important frontier, not just for *J. curcas* but for plants in general. Differential glycosylation of the same protein in different floral tissues has been recently noted. Such differences in glycosylation pattern led to variation in enzyme kinetics (Kohli, unpublished results). Arguably protein glycosylation is more important than phosphorylation because much larger glycosyl moieties get added to proteins, which can lead to major structural and functional alterations. In rice, differential glycosylation of the proteins was shown during stress and non-stress conditions (Komatsu et al. 2009). Plant proteo-glycomics is an advancing area but protein ubiquitination and SUMOylation are also important protein modifications during stress and developmental responses since they involve differential protein degradation and protein stabilization. Similarly, oligomerization-based functional diversity of proteins is well known at individual protein level whereby without up- or down-regulation of the protein per se, differential oligomerization of the same protein manifests as a novel activity. A heat shock protein in *Arabidopsis* for example, is multifunctional through differential oligomerization. A disulphide reductase and foldase chaperone activity is exhibited by the low molecular weight oligomeric form while a holdase chaperone activity is exhibited by the higher molecular weight oligomeric form of the protein (Lee et al. 2009). Interestingly, heat shock reversibly regulates the two oligomeric forms. Such knowledge must be used to generate high-throughput data on protein modification-based functionality. Such studies as of now are concentrated towards phospho-proteome analysis only. However, there are numerous other protein modifications critical to protein function. Development of high-throughput proteomic techniques to detect these modifications would be crucial to improving

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J. curcas since it is speculated that the transcriptome of a tissue at any given time represents only 60% of the proteins, which may further take on additional layers of complexity owing to specific post-translational and structural modifications undetected by standard proteomic analysis (Allison and Go 2004).

Conclusions

Recent years have witnessed significant research activity in terms of conventional breeding, genotyping and phenotyping, genome, transcriptome and proteome analysis. Such studies have brought *J. curcas* into mainstream biological research, and are shedding new light on information required to convert an uncultivated plant into a plantation crop. Without such an in depth understanding, *J. curcas* cannot be improved to meet the criteria required for a viable feedstock. Availability of the nuclear and chloroplast genome sequence and of transformation and gene silencing protocols readily facilitate the use of 'omic' technologies. Proteomic based studies are complementary to, and validate, transcriptome-mediated candidate gene identification, because proteins are the first reactive component affecting the innate and environmental stimuli perception and concomitant response. Identification of fatty acid metabolism, stress tolerance and transcription factor proteins in particular lay the foundations of a molecular tool-box for understanding and improving *J. curcas* as a source of non-edible seed oil for biodiesel.

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Part III
Jatropha Germplasm

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Chapter 20
Genetic Diversity, Molecular Markers and Marker Assisted Breeding in *Jatropha*

Mulpuri Sujatha

Introduction

World wide introduction of *J. curcas* for varied purposes met with limited success due to unreliable seed and oil yields and low economic returns. The major limitation with the currently used planting material is the narrow genetic base, low productivity and vulnerability to a wide array of biotic and abiotic stresses. Furthermore, the differences in the qualitative characters observed in the candidate plus trees seems to be less significant when the material is grown at a common site indicating strong influence of environment on growth and yield related characters of the crop. Comprehensive work on collection, characterization and evaluation of germplasm for growth, morphology, seed characteristics and yield traits is still in its infancy (Divakara et al. 2009).

In India, China, Mexico, Africa and Brazil, programs have been initiated by several developmental organizations (both public and private) for the promotion of *J. curcas* for greening of wastelands besides, meeting the fuel demand. The species has not been bred for productivity and most of the projects relied on naturally occurring wild populations, which are a result of the few initial introductions (Sujatha 2006). Improved varieties with desirable traits for specific growing conditions are not available, which makes *Jatropha* cultivation a risky business (Jongschaap et al. 2007).

Most of the *J. curcas* varieties are a result of selection made in the natural populations (Heller 1996; Henning 2006). There is virtually no information with regard to the number of introductions and the available genetic variability of *J. curcas* populations grown in most of the countries. Three distinct varieties are reported *viz.,* the Cape Verde variety that has spread all over the world, the Nicaraguan

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variety with few but larger fruits and a non-toxic Mexican variety devoid of phorbol esters (Henning 2006). Subsequently through selection, the first variety SDAUJ1 (Chatrapathi) has been identified for commercial cultivation in the semi-arid and arid regions of Gujarat and Rajasthan in India (www. icar.org). In China, two improved varieties *viz.,* CSC high oil content 63#, (Chuan R-Sc-Jc-002-2005), and CSC high toxin #1, (Chuan R-Sc-Jc-001-2005) were approved by the Sichuan Province Forest Improved Variety Certification Commission for planting in a large area with a validity of 10 years (Chen 2007; Chen et al. 2008). The Indonesian Center for Estate Crops Research and Development, Indonesia has released two superior composite seeds (IP-1 and IP-2) of *J. curcas* (Hasnam 2007; Prastowo 2007). The improved population IP-1 was released in 2006 with the yield around 4–6 t ha^{-1} and IP-2 in 2007 with estimated yield of 7–8 t ha^{-1}. A dwarf and early maturing variety, BT-88 was developed in Malaysia that produces fruits ready for harvest within 4 months of direct sowing (Tee 2007).

The key for success of any breeding programme lies in adequate genetic variability and availability of accessions with desired traits and maximum divergence. The ability to identify genetic variation is indispensable for effective management and use of genetic resources. Attempts are being made to assess the extent of variability in *J. curcas* germplasm using morphological (qualitative and quantitative) traits and molecular markers.

Morphological Diversity

Quantitative characters that could contribute to yield include plant height, canopy diameter, branch number, stem girth, collar diameter, oil content on seed basis, oil content on kernel basis, seed to kernel ratio, seed mass (single seed/100 seeds), seed yield per plant, ratio of female to male flowers, composition of seeds in terms of fatty acid profile, ash and protein. Forty clonal lines investigated for intraspecific variability in Thailand revealed no morphological differences (Sakaguchi and Somabhi 1987). Like wise, morphological differences were not significant in 58 samples characterized in China (Sun et al. 2008). Phenotypic variations were not distinct but seed characters and chemical composition were highly variable (Ginwal et al. 2005; Kaushik et al. 2007). Ginwal et al. (2005) reported variability in seed characters for germplasm from Central India. Analysis of 1,000 samples representing 12 states of India showed significant variation in oil content (25–44%) and kernel and seed coat ratio (0.36–2.12) with accessions from Uttaranchal recording maximum frequency of accessions (73%) with high oil (Kaushik et al. 2006). Significant variability has been reported in seed size, 100-seed weight (49.2–69.2 g) and oil content (28–38.8%) of 24 accessions of *J. curcas* collected from different agroclimatic zones of Haryana state, India (Kaushik et al. 2007). The phenotypic coefficient of variation was higher than the genotypic coefficient of variation indicating a strong influence of environment. Genotype-environment interaction was significant for vegetative and generative development (Heller 1996). High estimates

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of broad sense heritability were recorded for seed dimensions and seed weight indicating the heritable nature of the variability present and genetic gain was recorded for oil content revealing the additive gene action (Rao et al. 2008). Gohil and Pandya (2009) analysed diversity among nine *J. curcas* genotypes, including two non-toxic types and reported significant differences in phenotypic traits like plant height, plant canopy, collar diameter, number of primary and secondary branches, average seed weight, number of seeds and capsules, seed yield and oil content.

Martinez-Herrera et al. (2006) described differences in morphological characteristics from four different agro-climatic regions of Mexico (*viz.,* Castillo de Teayo, Pueblilio, Coatzacoalcos and Yautepec). These constitute regions with average rainfall ranging from 900 to 2,500 mm and from semi-hot to hot-humid. The accessions showed differences in crude protein (31–34.5%), lipid (55–58%), fibre (3.9–4.5%) and gross energy (31.1–31.6 Mj kg^{-1} DM). Proximate composition of 25 accessions from different agroclimatic zones showed wide variation in crude protein and crude lipid and maximum kernel oil content (64%) was recorded in a non-toxic accession from Huitzilan, Puebla state (Martinez-Herrera et al. 2007).

Pant et al. (2006) observed variation in vegetative and generative characters due to elevation. Altitude had a significant and positive effect on various oil yield components, including the number of branches per tree, number of fruits per branch, number of fruits and seeds per tree but a significant reduction was observed in kernel oil content with 43.1% at lower altitude as against 30.6% at higher elevations. Seed yield and seed oil content of *J. curcas* sampled from 167 sites in south and southwest China were significantly variable and ranged from 660 to 4,500 kg ha^{-1} and 18.8 to 47.95%, respectively (Sun et al. 2008). Rao et al. (2008) observed significant variation among 29 accessions from different regions in Andhra Pradesh State, India in terms of seed dimensions, plant growth (plant height, number of branches), flowering attributes (number of flowers, ratio of female to male flowers, days taken from flowering to fruiting and fruiting to maturity), yield characteristics, 100 seed weight (57.0–79.1 g) and oil content (29.9–37.1%). Highest variance was observed for 100 seed weight and oil content. Oil content on seed basis varied between 36.1% and 53.1% in Indonesian accessions (Hasnam 2007). Variation was recorded in seed mass (560–745 mg) and kernel oil content (42–57%) in 25 accessions from 23 field sites across Madagascar (Graham 2006). At PRI, Netherlands, fruit weight varied between 9.7 and 16.99 g and, maximum frequency ranged from 12.6 to 15.5 g (Montes Osorio et al. 2008). Fruit thickness varied between 26.9 and 31.95 mm and the maximum frequency was between 26.9 and 28.9 mm. Phenotypic variation for number of fruits was very high and varied from low number to very high number of fruits in the global germplasm. Accessions from Andhra Pradesh and Chattisgarh possessed higher amount of oil and disclosed higher amount of molecular polymorphism (Tatikonda et al. 2009). *J. curcas* exhibits environmental elasticity and few of which may have a genetic basis as well. Correlation of phenotypic characters with molecular markers showed association of molecular markers with pedicel length (Sunil et al. 2011) and reduced/nil phorbol ester content (Basha et al. 2009). Gross phenotypic variations like plant architecture were found in accessions from Latin America.

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Germplasm from different countries and regions were collected and assessed for desirable traits in China (Chen 2007). The seed oil content ranged from 15% to 40%. The collections were used to select superior varieties for direct release or were transferred into local adapted cultivars for increasing the oil content and stress enduring ability (Chen 2007). Depending on seasonal variation, climate and land topography, large differences in plant growth, canopy structure, flowering and fruiting ability, number of flowering flushes, fruit and seed set and seed characters were observed (Li et al. 2007).

The seed oil of *J. curcas* is rich in unsaturated fatty acids (oleic acid, linoleic acid) with predominance of oleic acid (Heller 1996; Akintayo 2004; Martinez-Herrera et al. 2006). Fatty acid composition of seed oil is reported to be under environmental influence (Martinez-Herrera et al. 2006; King et al. 2009). Analysis of fatty acid composition in four provenances from different agroclimatic regions showed that samples from Veracruz were found to be rich in oleic acid while those from Morelos were rich in linoleic acid (Martinez-Herrera et al. 2006). Significant variation was observed in 23 accessions from Madagascar for relative amounts of oleic and linoleic acid and oleic acid rich samples were obtained from sites close to sea level with mean annual temperatures higher than the mountainous regions (King et al. 2009). Wide variation was observed in proximate composition of seeds of 72 accessions from 13 countries (Basha et al. 2009). Levels of crude protein (18.8–34.5%), kernel oil content (45.4–64.5%) and ash content (3.2–6.7%) varied significantly but was not associated with geographical structure. Studies of Ovanda-Medina et al. (2011) on seed oil content and fatty acid composition of 135 accessions from Mexico showed wide variation among accessions for these two traits and also indicated that the traits are highly inheritable.

Genotypic variation has been observed for phorbol ester content as well. The content of phorbol esters in the non-toxic accessions varied from provenance to provenance (Makkar et al. 1998). The seeds of Quintana Roo were of better quality with higher levels of protein, lipid and ash and lower levels of phorbol esters, trypsin, lectin activities and saponins in the raw meal than those in meals from non-toxic provenance from Veracruz state (Makkar et al. 1998).

Most of the germplasm evaluation studies to assess diversity were conducted with material collected from plus trees of different agro-ecological regions, different aged plants (3–20 years) and propagated through seeds or vegetative cuttings. Comparison of yield contributing traits based on such material results in erroneous conclusions about the superiority of the identified clone as it is strongly influenced by the method of propagation (vegetative propagation or direct seeding), soil type (fertile or marginal; irrigated or rainfed; fertilized or not), climatic conditions, age of the plant and plant density (unstipulated spacing between plants), collection period, drying and storage, maturity of the trees, etc. Hence, evaluation to assess diversity should be done with germplasm subjected to common agronomic practices under similar edapho-climatic conditions and the superiority of the identified accessions should be confirmed through multilocation trials. The distribution and morphological diversity of 100 accessions from 100 sites in five districts of South east coastal zone of India was studied using geographic information system (Sunil

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et al. 2009). The grid maps (DIVA-GIS) that were generated based on distribution pattern, plant height, number of primary branches, collar length, number of fruits per cluster and oil content showed diversity with regard to flowering period and fruits per cluster and good variability and richness for oil content. The system also facilitated the identification of gaps in collection and for spotting the diversity richness. Such mapping studies ought to be extended to assessment of diversity in the germplasm from the center of origin. Molecular markers need to be developed for supporting the development of effective management strategies both for in-situ and ex-situ conservation of *J. curcas* genetic resources.

Molecular Diversity

Molecular markers refer to assays that allow the detection of specific sequence differences between two or more individuals. They have played a major role in the genetic characterization and improvement of many crop species and greatly expanded the abilities to assess biodiversity, reconstruct accurate phylogenetic relationships, generation of genetic linkage maps and in tagging and mapping of useful traits. Molecular markers are generally employed in management of genetic resources to estimate genetic diversity, identify duplicates in the collection, to devise appropriate conservation strategies for successful utilization of genetic resources, understand the population structure and resolve taxonomic relationships. Traditionally, diversity is assessed by measuring phenotypic variation but morphological characterization and expression of quantitative traits is subjected to strong environmental influence. Following the advances in molecular biology in the last two decades, a host of molecular marker systems have been developed for assessment of genetic diversity which differ with respect to technical requirements, level of polymorphism detected, reproducibility and cost. Molecular markers are reliable indicators of genetic diversity as they are less influenced by environment and scan the differences at the whole genome level. DNA markers play an important role in establishment of molecular finger prints for distinct and most divergent accessions as well.

As genomic resources are limited for *J. curcas,* RAPD and ISSR markers were employed in the initial studies as they are simple to use and do not require prior information of the genome. RAPD markers cover the entire genome revealing length polymorphisms in coding or non-coding and repeated or single copy sequences while ISSR markers generate polymorphism from sequences between two microsatellite primer sites. RAPD markers are not reproducible across laboratories and hence, difficult to obtain reliable estimates. Therefore, polymorphic RAPD markers have to be converted to more reliable sequence characterized amplicon region (SCAR) markers. Microsatellite markers or simple sequence repeats (SSRs) are, in general, crop specific and the initial developmental costs are high. Few laboratories (Temasek Life Sciences Laboratories, TLL, Singapore; Center for Novel Agricultural Products, CNAP) have initiated programmes on development of *J. curcas* specific microsatellite markers. In the recent past, EST databases

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of Jatropha and related genera have been mined to identify functional markers for use in Jatropha. The molecular diversity studies in *J. curcas* were carried out with RAPD, ISSR, AFLP, SSR (genomic, EST-SSR), tubulin based polymorphism and motif binding polymorphism (NBS profiling) techniques. Table 20.1 summarizes the different genotypes and molecular markers that have been used till date for molecular characterization of Jatropha.

RAPD

Randomly amplified polymorphic DNA (RAPD) involves PCR amplification of genomic DNA using a short oligonucleotide primer under low stringency conditions which results in multiple amplification products from loci distributed throughout the genome (Williams et al. 1990; Welsh and Mc Clelland 1991). The technique is simple, rapid, inexpensive and applicable to any genome without any prior information regarding the genome of the plant. RAPD analysis has been used for genetic diversity assessment and identification of germplasm in a number of plant species, taxonomic/phylogenetic relationships, fingerprinting, mapping and marker assisted selection (after conversion in to SCAR markers owing to their less reproducibility).

Initial studies on *J. curcas* were mostly carried out with RAPD primers. Analysis of genetic diversity in 42 germplasm lines collected from different regions in India using RAPD and ISSR markers revealed low inter-accessional variability (Basha and Sujatha 2007). Following this work, molecular markers were used for assessment of genetic variation in local populations from India, China and Brazil (Table 20.1). Studies of Ranade et al. (2008) indicated low genetic diversity in Indian accessions with the exception of accessions from the North Eastern part of India which were more distant from all other accessions. Gupta et al. (2008) have characterized 13 *J. curcas* accessions collected from three different states of India and reported 84.26% of polymorphism with 20 RAPD primers. Pamidimarri et al. (2010) have reported low genetic diversity among 28 diverse germplasm accessions collected from different geographical regions of India. In this study, the overall percentage of polymorphism (PP) was found to be 50.7% with 180 RAPD primers. Ikbal Boora and Dhillon (2010), Subramanyam et al. (2009) and Rafii et al. (2012) reported wide range of genetic variability in jatropha native germplasm available in the respective countries while Ranade et al. (2008) and Jubera et al. (2009) reported lower levels of genetic diversity among the native germplasm accessions of India indicating the need for widening the genetic base of *J. curcas* through introduction of accessions with broader geographical background. Assessment of genetic variation by RAPD, AFLP and combinatorial tubulin based polymorphism (cTBP) in 38 accessions from 13 countries on three continents (Asia, Africa, America) revealed narrow genetic diversity (Siam et al. 2009). Likewise, studies carried out on molecular characterization of germplasm from China and Brazil indicated the existence of low genetic diversity barring few provenances (Sun et al. 2008; Grativol et al. 2011; Rosado et al. 2010; Shen et al. 2010).

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Table 20.1 Molecular characterization studies for assessment of genetic diversity in *Jatropha*

Germplasm	Markers employed	Result	Reference
One accession each of Indian toxic and Mexican non-toxic varieties	120 RAPD primers	Reference fingerprints established for distinguishing the non-toxic variety from the toxic Indian cultivar	Sujatha et al. (2005)
42 *J. curcas* accessions from different locations of India along with a non-toxic genotype from Mexico	400 RAPD and 100 ISSR markers	Modest level of genetic variation in Indian and wide variation between Indian and Mexican genotype	Basha and Sujatha (2007)
9 populations from 5 provinces of China	10 ISSR markers	High level of genetic diversity	He et al. (2007)
J. curcas accessions from Southern Yunnan, China	ISSR	–	Xiang et al. (2007)
5 *J. curcas* accessions from Tamil Nadu and 7 *Jatroph*a species native to India	26 RAPD primers	High genetic variability among the eight species (80.2% polymorphism)	Ganesh Ram et al. (2008)
56 Chinese and 2 Malaysian *J. curcas* accessions	17 SSRs and 7 AFLP primer combinations	Very low genetic variability (14.3% polymorphism)	Sun et al. (2008)
7 *Jatropha* species native to India	33 RAPD and 27 AFLP primer combinations	High genetic variability among the species (97.7% by RAPD and 97.2% by AFLP)	Pamidimarri et al. (2008a)
7 *J. curcas* accessions (6 toxic + 1 non-toxic)	52 RAPD, 56 AFLP and 7 SSR markers	All the markers are effective in differentiating both the toxic and non-toxic accessions	Pamidimarri et al. (2008b)
7 *Jatropha* species along with a natural hybrid occurring in India	2 ITS sequences encoding the 18, 5.8 and 26s nuclear ribosomal RNA subunits	Usefulness of the nrDNA ITS sequences in phylogenic analysis of genus *Jatropha*	Pamidimarri et al. (2008c)
18 *J. curcas* accessions from different regions	7 RAPD and 4 DAMD primers	Usefulness of SPAR method for diversity assessment in *Jatropha* has been demonstrated	Ranade et al. (2008)
13 *J. curcas* genotypes from different parts of India	20 RAPD and 14 ISSR primers	Importance of both the markers in *J. curcas* genetic diversity assessment	Gupta et al. (2008)
3 *J. curcas* accessions and 8 *Jatropha* species	9 ISSR primers	Clustering of *J. curcas* accessions	Senthil Kumar et al. (2009)

(continued)

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Table 20.1 (continued)

Germplasm	Markers employed	Result	Reference
8 *Jatropha* species along with a natural hybrid occurring in India	200 RAPD, 100 ISSR and 50 organelle specific primers	High genetic variation (98.5% polymorphism) among species used in the study	Basha and Sujatha (2009)
72 *J. curcas* accessions collected from 13 countries	100 RAPD, 100 ISSR and 17 SSR markers	Rich diversity among Mexican genotypes and narrow genetic variation among accessions from different regions of the world	Basha et al. (2009)
48 *J. curcas* accessions from 6 different states of India	7 AFLP primer combinations	High genetic variability (88% polymorphism)	Tatikonda et al. (2009)
28 accessions from distinct geographical regions of India	52 RAPD and 18 AFLP primer combinations	Low genetic diversity among the accessions used	Pamidimarri et al. (2010)
5 *J. curcas* accessions from Coimbatore and 7 *Jatroph*a species native to India	19 morphological and 21 ISSR markers	Importance of ISSR markers in genetic diversity assessment of *Jatropha* species	Vijayanand et al. (2009)
38 *J. curcas* accessions from 13 countries and 6 *Jatropha* species	10 RAPD, 32 AFLP primer pairs and 2 combinatorial tubulin based polymorphism (cTBP)	Narrow genetic diversity in accessions from Thailand, Nigeria and India	Siam et al. (2009)
26 accessions from Rajasthan, India	26 RAPD primers	–	Kumar et al. (2009)
120 accessions from 3 regions in China	ISSR primers	69% polymorphic	Ou et al. (2009)
45 accessions from distinct geographical regions of China	36 EST-SSR and 20 G-SSR markers from cassava	Intergroup genetic diversity was higher than the intragroup diversity index	Wen et al. (2010)
224 accessions including 219 from China and 5 from Myanmar	15 ISSR primers	High genetic diversity in Chinese germplasm	Cai et al. (2010)
40 genotypes from 5 states in India	44 RAPD primers	Wide genetic base	Ikbal Boora and Dhillon (2010)
30 accessions of *J. curcas* and 2 accessions each of 3 species	27 ISSR markers	*J. curcas* accessions from Mexico were genetically diverse and the 3 species formed separate clusters	Tanya et al. (2011)
6 species of *Jatropha* and *J. tanjorensis*	31 SSR markers	Assessed the cross species transferability of *J. curcas* microsatellite markers	Pamidimarri et al. (2011)
25 accessions of *J. curcas,* 5 *Jatropha* species and castor	51 EST derived SSR markers	Low to moderate level of informativeness within the EST-SSRs and 57.0–95.6% transferability among *Jatropha* species	Yadav et al. (2011)

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38 *J. curcas* accessions including 37 from China and 1 from Indonesia	9 AFLP primer combinations	Low genetic diversity	Shen et al. (2010)
17 accessions from Tamil Nadu including one accession from Zimbabwe	13 ISSR primers and their combinations	Moderate genetic diversity	Umamaheshwari et al. (2010)
192 *J. curcas* accessions from Brazil	96 RAPD and 6 SSR primers	Low genetic diversity	Rosado et al. (2010)
γ-irradiated material of *Jatropha curcas*	23 RAPD primers	Genetic variation in mutants	Dakshnanamoorthy et al. (2010)
332 accessions from 8 states in Brazil	7 ISSR primers	High level of genetic differentiation	Grativol et al. (2011)
10 accessions from three states in India	10 RAPD primers	High genetic variation	Subramanyam et al. (2010)
192 accessions from Brazil	96 RAPD and 6 SSR primers	Limited genetic diversity	Rosado et al. (2010)
88 accessions from Chiapas, Mexico	6 AFLP primer combinations	High level of polymorphism and several rare fragments in a pistillate accession	Pecina-Quintero et al. (2011)
48 accessions from Malaysia	8 RAPD primers	High genetic variation	Rafii et al. (2012)
63 populations from 10 countries in Asia, Africa, Mexico	4 AFLP primer combinations	High genetic variation in populations from Mexico	Shen et al. (2012)

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ISSR

Inter simple sequence repeats (ISSR) use primers designed based on microsatellites to amplify the inter-simple sequence repeat sequences. Various microsatellites anchored at the 3′ end are used for amplifying genomic DNA, which in turn increases their specificity. These are mostly dominant markers, though occasionally a few of them exhibit co-dominance. An unlimited number of primers can be synthesized for various combinations of di-, tri-, tetra- and pentanucleotides [(4)3 = 64, (4)4 = 256], etc. with an anchor made up of a few bases and can be exploited for a broad range of applications.

ISSR primers were used for characterization of germplasm from India, China and Brazil. Basha and Sujatha (2007) characterized 42 *J. curcas* accessions collected from different crop growing regions of India along with a non-toxic genotype from Mexico and reported molecular polymorphism of 33.5% with 100 ISSR primers. In this study, two polymorphic ISSR markers one each specific to Indian toxic and a non-toxic Mexican genotypes were converted to SCAR markers. Gupta et al. (2008) characterized 13 accessions from three different states of India using 25 ISSR markers and reported 76.54% of polymorphism among the accessions used. Similarly, 72 *J. curcas* accessions representing 13 countries were characterized using 100 ISSR primers which disclosed distinction of non-toxic Mexico accessions from all other genotypes collected from rest of the world (Basha et al. 2009). Studies of Tanya et al. (2011) based on ISSR markers showed that the Mexican accessions were genetically diverse and separated accessions from Mexico into separate cluster. Umamaheshwari et al. (2010) reported moderate genetic diversity in 17 accessions from Tamil Nadu.

He et al. (2007) using ISSR markers reported very high level of genetic diversity among eight populations from five provinces of China *viz.,* Guangxi, Guizhou, Hainan, Sichuan and Yunnan. Similar results were reported by Ou et al. (2009). Cai et al. (2010) reported high genetic diversity in Chinese Jatropha germplasm comprising of 219 accessions when characterised with ISSR primers and were divided into two distinct groups *viz.,* tropical or island group and the mainland group. Based on stepwise clustering with random sampling (SCR) strategy, a core was constructed which included 46 accessions which accounted for 90% of the polymorphism of the initial population. The constructed core can effectively maintain the genetic architecture of the initial collection besides reducing the cost of conservation of the *Jatropha* germplasm.

Recently, Grativol et al. (2011) characterized 332 accessions from eight states in Brazil with seven ISSR primers which disclosed 91% polymorphism in the Brazilian germplasm. The study indicated that the accessions from Natal region were most diverse with a high value of genetic diversity. Although it is the same set of ISSR primers (University of British Columbia set #9) that were employed in all the studies, results in terms of the number of polymorphic bands, primers disclosing polymorphism and the PIC varied with the germplasm used in different laboratories.

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SCARs

Sequence characterized amplified regions (SCAR) markers were introduced by Michelmore et al. (1991) and Martin et al. (1991) wherein the RAPD/ISSR marker termini are sequenced and longer primers are designed (22–24 bases long) for specific amplification of a particular locus. These are better reproducible than RAPD and ISSR markers. SCARs are usually dominant markers; however, some of them can be converted into co-dominant markers by digesting them with restriction enzymes. In Jatropha, Basha and Sujatha (2007) developed two diagnostic SCAR markers (ISPJ-1 and ISPJ-2) for differentiating the Indian and Mexican genotypes. Subsequently, Basha et al. (2009) developed three SCAR markers (RSPJ-1, RSPJ-2 and ISPJ-3) to differentiate non-toxic Mexican genotypes from the toxic genotypes collected from the rest of the world.

AFLP

Amplified fragment length polymorphism (AFLP) technique combines the components of RFLP and PCR. It involves restriction of DNA using a combination of rare and frequent cutters followed by ligation of oligonucleotide adapters, of defined sequences including the respective restriction enzyme sites, and selective amplification of sets of restriction fragments, using specifically designed primers. AFLP technique is reliable since stringent reaction conditions are used for primer annealing. It is extremely useful in detection of polymorphism between closely related genotypes (Breyne et al. 1999). AFLPs are extremely useful as tools for DNA fingerprinting, studying diversity, germplasm characterization, tagging, mapping and marker assisted introgression programs.

AFLP profiles of *J. curcas* collected from different agro-ecological regions of India revealed a narrow genetic base (DBT 2007). Likewise, Pamidimarri et al. (2008b, 2010) using AFLP markers reported low genetic variability among toxic *J. curcas* accessions from India and wide variability between toxic Indian and a non-toxic Mexican accession. In this study, the similarity between toxic and non-toxic genotypes was found to be as high as 83.5%. AFLP analysis of 48 accessions from six different states of India with seven AFLP primer combinations showed accession clustering in accordance to their geographical location (Tatikonda et al. 2009). The seven AFLP primer combinations generated a total of 770 fragments with an average of 110 fragments per primer combination. A total of 680 (88%) fragments showed polymorphism in the germplasm analyzed, of which 59 (8.7%) fragments were unique (accession specific) and 108 (15.9%) fragments were rare (present in less than 10% accessions). Higher genetic diversity was documented in the accessions from Andhra Pradesh and Chhattisgarh.

Sun et al. (2008) characterized 58 accessions (56 from China and two from Malaysia) using SSR and AFLP markers. The study also revealed low genetic diver-

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sity in the Chinese germplasm but the studies showed distinctness of accessions in Guizhou from the other samples. AFLP polymorphism proved to be a better marker system when compared with SSRs as it discriminated all the accessions while SSRs could differentiate only the Chinese from the Malaysian accessions. Sun et al. (2008) opines that the source of *J. curcas* in China may be the same as that in India and the germplasm in Southeast Asian countries could probably have a common ancestry. The observation was supported by the study of Shen et al. (2010) who reported low genetic diversity and a lack of variation pattern among the populations in China when 38 accessions were subjected to molecular analysis with nine AFLP primer combinations. Further, studies of Shen et al. (2012) on characterization of 63 populations from 10 countries in Asia, Africa and Mexico using AFLP markers confirmed the genetic distinctness of Mexican populations. AFLP analysis of 88 accessions from the state of Chiapas, Mexico confirmed the existence of broad gene pool in the germplasm from this region (Pecina-Quintero et al. 2011). The study also showed several rare fragments in an accession that was 100% pistillate.

SSRs or Microsatellites

Microsatellites, which are commonly referred to as *simple sequence repeats* (SSRs), are tandemly arranged repeats of short DNA motifs (1–6 bp in length) that frequently exhibit variation in the number of repeats at a locus. Because of their abundance and inherent potential for variation, SSRs have become a valuable source of genetic markers (Temnykh et al. 2001). Microsatellites are ubiquitous in eukaryotic genomes and exhibit highly variable numbers of repeats at a locus and polymorphism in the repeat motif is referred to as *simple sequence length polymorphism* (SSLP). Their abundance, multi-allelic nature, co-dominant nature, high reproducibility, locus specificity and extensive genome coverage make them valuable as genetic markers (Weber and May 1989; Graham et al. 2004). These markers have become quite useful in various aspects of molecular genetic studies, including assessment of genetic diversity, fingerprinting, ecological genetic studies, marker-assisted selection and genetic linkage mapping.

Very few reports are available on the development of genome-derived SSR markers in *J. curcas*. Sun et al. (2008) developed 17 genomic SSRs, out of which only one was polymorphic (5.87% polymorphism) among the 58 accessions of *J. curcas* collected across China. In a study carried out by Pamidimarri et al. (2010), 17 genomic SSRs were reported out of which 12 were polymorphic (70%) across 32 accessions of *J. curcas.*

Pamidimarri et al. (2008b) using 12 SSR markers reported maximum genetic variation between six Indian toxic accessions and a non-toxic Mexican accession. Similarly, diversity analysis of 72 *J. curcas* accessions representing 13 countries using a different set of 17 microsatellite markers revealed variation in accessions from Mexico and Salvador, while accessions from other countries failed to be distinguished (Basha et al. 2009).

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EST-SSRs

Expressed sequence tag SSRs (EST-SSRs) are those microsatellites developed from sequencing of EST libraries/cDNA clones rather than from genomic DNA libraries. Different softwares are available to select SSR rich EST sequences. Unique sequences flanking the microsatellites are used for primer designing such that the microsatellite in between is amplified. SSR markers in Jatropha are relatively limited because their *de novo* development is expensive, laborious and time consuming. But with the availability of enormous EST sequence data from the public databases and with the advent of bioinformatics tools, it has become cost effective and a fast approach to scan for microsatellite markers. Since EST-SSRs identify variability in the transcribed regions of the genome, the development of gene-based maps may lead to rapid identification of functional candidate genes and increase the efficiency of marker-assisted selection and aid in creation of physical and genetic maps based on synteny (Varshney et al. 2005). About 2–5% of the ESTs in different plant species are reported to contain SSRs suitable for marker development (Kantety et al. 2002). The EST-derived SSRs exhibit lower levels of polymorphism than the genomic sequence-based SSRs. However, they possess advantages like easy access, presence in gene-rich regions, and high level of transferability to related species (Scott et al. 2000; Thiel et al. 2003) which enable these to serve as anchor markers for comparative mapping and evolutionary studies (Varshney et al. 2005).

Since only a few SSR markers are available in *Jatropha*, efforts were made to design EST-SSR markers from the EST sequences available in the database. Yadav et al. (2011) developed EST-SSRs using 5,851 transcriptome contigs sequences developed at NBRI and 13,201 expressed sequence tags (ESTs) of *J. curcas* from National Center for Biotechnology Information database. Seven hundred and two sequences containing 786 SSRs with 93.4% simple and 7.6% compound repeat motifs were identified. *Dinucleotide repeats* (DNRs) were most abundant, followed by trinucleotide and tetranucleotide repeats. AG/CT was the most common motif (50.0%) followed by AT/AT (38.8%) and AC/GT (10.0%) among the DNRs. Four hundred and six primer pairs were designed out of the 702 SSR-containing sequences of which 50 randomly selected EST-SSR markers were amplified in 25 accessions collected from different geographical regions of India. Polymorphic information content value ranged between 0.04 and 0.61 with an average of 0.25 ± 0.16, indicating low to moderate level of informativeness with EST-SSRs.

Wen et al. (2010) studied the genetic relationships between 45 *J. curcas* accessions using 36 EST-SSRs and 20 genomic-SSRs designed based on cassava sequence information. A total of 183 polymorphic alleles were detected and the accessions were classified into six groups, in which the genotypes showed a correlation with geographic origin. The estimated mean genetic diversity index was 0.5572, which suggests that *J. curcas* germplasm used in the study has a high level of genetic diversity.

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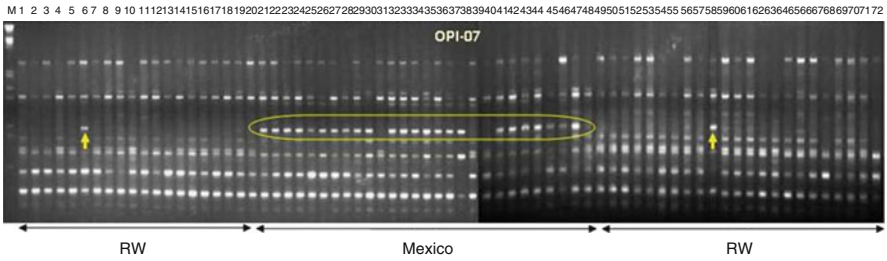

Fig. 20.1 Characterization of germplasm from Mexico and rest of the world (RW) using RAPD primer OPI-07 (Sujatha, unpublished)

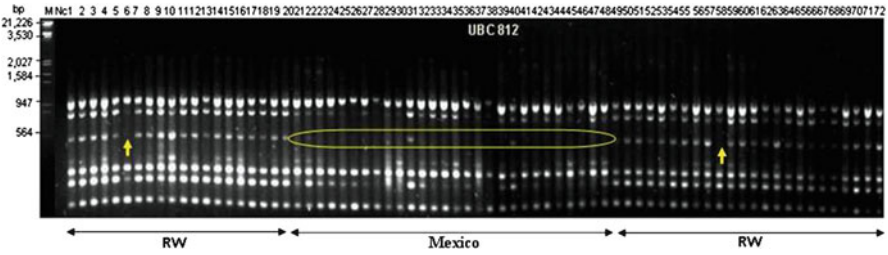

Fig. 20.2 Characterization of germplasm from Mexico and rest of the world (RW) using ISSR primer UBC-812 (Sujatha, unpublished)

Distinguishing Toxic from Non-toxic Accessions

Sujatha et al. (2005) for the first time using RAPD markers could differentiate the toxic Indian and the non-toxic (low *phorbol ester* – PE) Mexican accessions. Following this several studies showed distinct molecular profiles for the toxic and low PE lines and separate clustering of non-toxic Mexican accessions from the toxic accessions of different countries (Basha and Sujatha 2007; Basha et al. 2009; Pamidimarri et al. 2010; Siam et al. 2009).

Based on the observations of existence of low genetic variation in local populations in different countries indicating a common ancestry, the need for assessment of genetic diversity in global germplasm has been realized. Sujatha et al. (2005) demonstrated genetic distinctness between toxic Indian and non-toxic Mexican accessions using molecular markers. Studies of Basha et al. (2009), with a representative set of 72 accessions from 13 countries showed clear separation of accessions from Mexican region with those from the *rest of the world* (RW) (Figs. 20.1 and 20.2). The study indicated the possible spread of 1 or 2 toxic accessions from the Mexican region to all the countries and the existence of rich diversity in the Mexican germplasm. In this particular study, molecular data was corroborated with proximate composition data which showed the association of molecular markers with the presence/absence of PEs. In an accession from El-Salvador a unique allele

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specific to the accession was detected through SSR analysis which reiterates the need for characterization of germplasm from other Central American regions as well (Basha et al. 2009). Under the GJEP programme at PRI, Netherlands, 60 accessions were studied for genetic variation using AFLP and NBS (motif binding) markers (Montes Osorio et al. 2008; Van Loo et al. 2008). High diversity was detected in accessions from Central American region and a low diversity in accessions from Africa and India. Genotyping studies of Guatemalan accessions separated the accessions into regions with high, average and low yields which indicate the existence of large untapped genetic resources in *J. curcas* (Van Loo et al. 2008). AFLP analysis of 63 populations from Asia, Africa and Mexico also showed that the populations from Mexico are exceptional with high genetic diversity (Shen et al. 2012). It is envisaged to subject the global germplasm comprising at least 400 samples from different countries for genotyping under the GJEP programme (www.jatropha.wur.nl) which should provide more information on the allelic diversity in the centre of origin, genetic relatedness of the accessions in the Central and Meso-American regions and serve as a valuable resource for trait based gene transfer. Results at CNAP using AFLP and SSR markers indicate very little genetic variation between accessions from India, Ghana, Tanzania and Madagascar but significant variation with Mexican accessions (Graham 2006). Narrow genetic base in African and Asian regions was attributed to few introductions, the predominance of asexual mode of reproduction and/or due to the occurrence of apomixis and could probably have a common ancestor (Kohli et al. 2009; Basha et al. 2009). Analysis of global diversity thus, confirms the observation of Heller (1996) who showed the distribution and spread of *J. curcas* in the tropical belt via the Cape Verde islands. All the studies unequivocally establish the fact that the Central American and Meso-American regions harbor accessions with useful and novel genes that provide a good basis for widening the genetic base of *J. curcas.* Cluster analysis failed to show any specific relation between clusters based on geographical distance and qualitative and quantitative traits. However, in the study of Basha et al. (2009), the non-toxic Mexican accessions formed a distinct cluster and were clearly demarcated from the toxic accessions regardless of the region. This was further substantiated by the presence of rich allelic diversity which distinguishes the toxic from non-toxic accessions. Molecular markers specific to non-toxic accessions could be successfully used for characterization of lines with low phorbol ester levels when *J. curcas* is exploited for edible purposes.

Cross-Taxa Transferability

Microsatellite markers are available for a few Euphorbiaceae members and are being developed for castor (Lespinasse et al. 2000; Okogbenin et al. 2006; Chan et al. 2010). As the developmental costs for microsatellite markers are high, cross taxa utility of molecular markers from Hevea and Cassava were assessed in jatropha. Wen et al. (2010) studied the transferability of 419 *expressed sequence tag* (EST)-SSRs

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and 182 *genomic* (G)-SSR primers that had been developed for Cassava *(Manihot esculenta*). The cassava-jatropha cross-genera transferability was 44.63% with EST-SSRs and 29.67% with genomic-SSR and was estimated to be high. The higher levels of transferability of EST-SSRs than of G-SSRs reflect the conserved nature of coding sequences as compared with non-coding genomic DNA, and the fact that the mutation frequency of EST sequences is lower than that of genomic DNA sequences. These results demonstrate the potential value of EST-SSR markers for the development of genetic maps, assessment of genetic diversity, and marker-assisted selection (MAS) breeding in *J. curcas*. Recently, EST-SSR markers were developed from jatropha sequence information available in the public database which showed 57.0–95.6% transferability among five different species of jatropha (Yadav et al. 2011). EST-SSRs developed from jatropha had a transferability rate of 47.0% in castor (*Ricinus communis*) (Yadav et al. 2011) indicating their potential use in castor.

Thus the above studies clearly indicate the lack of adequate genetic diversity in *J. curcas* germplasm with the exception of the accessions from the Central American region. Genetic variation reported in the molecular studies was mainly due to inclusion of wild species (Ganesh Ram et al. 2008; Ranade et al. 2008; Senthil Kumar et al. 2009) or geographically isolated germplasm (Sujatha et al. 2005*;* Basha and Sujatha 2007; Pamidimarri et al. 2008b). Some of the studies were confined to few accessions (<10) and limited number (<10) of primers. It is important to have a cautious approach while carrying out the genotyping assays and one need to consider the minimum population size, the number of data points, the polymorphism information content of the markers being employed, besides understanding the population structure in terms of its geographic isolation and mode of reproduction to draw meaningful conclusions. Regardless of the number of accessions used, the robustness of the primer and number of marker data points, all accessions from India clustered together confirming the existence of low genetic variation in the *J. curcas* ecotypes being genotyped. Similar results were obtained with germplasm from China and Brazil (Sun et al. 2008; Grativol et al. 2011; Rosado et al. 2010; Shen et al. 2010). Diversity analysis with local germplasm thus, indicate the need for widening the genetic base of *J. curcas* through introduction of accessions with broader geographical background (Basha and Sujatha 2007; Ranade et al. 2008; Sun et al. 2008; Shen et al. 2010, 2012). Cross-pollinating species have a significantly higher genetic diversity compared to self-pollinating species. Although a predominantly out-breeding species, *J. curcas* exhibited lower genetic variation in local populations which could probably be due to its propagation through vegetative cuttings and or apomixis.

Genomics

The genome of *J. curcas* is relatively small and is estimated to be between 210 and 220 Mb (Hong 2008), 300 Mbp (Graham 2006), 416 Mb (Carvalho et al. 2008), 980 Mb (Kohli et al. 2008) which is equivalent to castor (400 Mb) and rice (430 Mb)

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but smaller than that of other species of Euphorbiaceae (1.3–28.6 pg). The 2 C value is 0.85 pg with an average base composition of 38.7% GC. The GC levels calculated for *J. curcas* genome is about the same as that reported for *Arabidopsis* and is characteristic of core dicots. The number of genes was predicted to be approximately 34,000 and the abundance of non-coding sequences (microsatellites and transposable elements) was lower as compared to that of rice. The karyotype constitutes 22 relatively small 5 metacentric and 6 submetacentric chromosomes with sizes ranging from 1.71 to 1.24 μm. The chromosomes 1–10 from the haploid complement (n = 11) can be paired (1–2, 3–4, 5–6, 7–8, 9–10) because of their identity for total size, the size of short and long arms and arm ratio. The morphometric similarities observed between chromosomes from heterologous pairs suggests that *J. curcas* is an autotetraploid species (Carvalho et al. 2008). Like in *J. curcas,* the chromosomes of four *Jatropha* species (*J. gossypiifolia, J. integerrima* – red and pink flowers*, J. multifida, J. podagrica*) were very small and ranged from 1 to 3.67 μm (Soontornchainaksaeng and Jenjittikul 2003). Most of the *Jatropha* species have 22 chromosomes with the exception of *J. heterophylla* Hyne, and few samples of *J. curcas* (Missouri Botanical Garden 2003), that have 44 chromosomes. The small genome size, few chromosomes, easy regeneration and transformability make *J. curcas* an ideal crop for genome sequencing and manipulation through biotechnological tools.

Gomes et al. (2010) characterized the cDNA library from seeds of *J. curcas* at three stages of fruit maturation before yellowing. Sequencing of about 2,200 clones led to identification of nucleotide variations among *J. curcas* accessions for genes of fatty acid, terpene, alkaloid, quinine and hormone pathways of biosynthesis which could pave way for selective breeding of *J. curcas* for oil and other economically important traits. The expressed sequence tags (EST) projects have generated a vast amount of publicly available data for members of Euphorbiaceae *viz., Jatropha* (46,944), castor (64,457), *Hevea* (39,918), *Euphorbia* (59,724) and Cassava (83,028) which serve as a valuable resource for mining the simple sequence repeats and genes with known function through comparative mapping. At Temasek Life Sciences (TLL), Singapore, the ESTs were mined to isolate 1,000 microsatellites and 100 single nucleotide polymorphism (SNPs) and characterization of genes controlling agronomic traits like flower development, seed number, seed size, fatty acid content and composition is under investigation (Yue 2008). The Centre for Novel Agricultural Products (CNAP) in collaboration with FOFIFA, Madagascar and University of Chapingo, Mexico has established tools for genotyping and metabolite profiling (oil content, fatty acid profile and phorbol ester analysis) (Graham 2006; King et al. 2007). The project aims at obtaining extensive sequence data from developing seeds of toxic and non-toxic varieties for identification of genes involved in phorbol ester biosynthesis as well as target genes for genetic improvement through classical mutagenesis. Under the 454 sequencing project at CNAP, cDNA from developing seeds of toxic and non-toxic varieties was sequenced accounting for 94 Mbp from which SNPs and SSRs were detected. The sequence data will enable identification of molecular markers to aid in marker assisted breeding programme. Transcriptomic studies of Costa et al. (2010) in jatropha generated 13,249 expressed sequence tags

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(ESTs) from developing (7,320) and germinating seeds (5,929) cDNA libraries. The lengths of the ESTs after trimming ranged from 100 to 848 bp, with an average size of 561.5 bp. They identified ESTs coding for enzymes involved in synthesis (99.71) and degradation (250) of fatty acids and synthesis (11.53) and degradation (88.21) of try acyl glycerols which influence oil quality. Transcripts coding for curcins, 2 S albumins and enzymes involved in synthesis of terpenoids which are associated with phorbol ester biosynthesis pathways were also identified.

Life Technologies Corporation (Nasdaq, Life) in collaboration with SG biofuels have announced the completion of sequencing of *J. curcas* genome to 100 x coverage which would accelerate the identification of key traits for obtaining superior yields with the broader objective of development of most profitable and sustainable biofuel feedstock (http://www.sgbiofuels.com/). The whole genome of *J. curcas* has been sequenced which serves as a valuable resource for acceleration of basic and applied research including genetic improvement through marker-assisted breeding and transgenic approaches (Sato et al. 2011). The draft genome sequence of castor is also available which could be used for sysnteny analysis and comparative genomics (Chan et al. 2010).

The chloroplast genome of *J. curcas* has been sequenced (Asif et al. 2010). The cpDNA is a circular molecule of 163,856 bp in length and codes for 110 distinct genes (78 protein coding, four rRNA and 28 distinct tRNA). Genome organisation and arrangement are similar to other land plant chloroplasts, with the exception of the loss of *rps16* and *infA* genes. The phylogenetic analyses showed that Jatropha forms strong relationship with *Manihot* and also shares many similarities with *Populus*, including the loss of both the *infA* and the *rps16* genes. The availability of the *Jatropha* chloroplast genome sequence can facilitate the design of vectors for efficient transformation of *Jatropha*, besides using the sequence information for unraveling the phylogeny.

Characterization of *Jatropha* Species

Wild relatives have been exploited in several crops as important reservoirs of genetic variability for various characters. In fact, major crop improvements relied on the exploitation of abundant gene pools from the wild relatives (Feldman and Sears 1981; Kalloo 1992). The transfer of desirable genes from wild relatives to the cultigens is largely dependent upon the extent of cross compatibility between them (Stalker 1980). Consequently, the elucidation of fertility-sterility relationships in the interspecific crosses is essential for the genetic manipulation of crop species.

Determination of genetic relationships among species is equally critical for the management of genetic resources and success of interspecific hybridization. In *Jatropha*, taxonomic classification and infrageneric relationships were based on leaf epidermal morphology (Dehgan 1980), petiolar anatomy (Dehgan 1982); crossability relationships (Dehgan 1984) and phenetic and cladistic analysis based on

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morphological characters (Dehgan and Webster 1979; Dehgan and Schutzman 1994). Anatomical features of the petiole are singularly not sufficient in delineating evolutionary phylogenetic sequences but strengthen other anatomical, morphological and experimental approaches in solving taxonomic problems. Likewise, morphological studies of epidermis and other traits are insufficient by themselves as taxonomic evidences. Electrophoretic patterns of seed and leaf proteins of *Jatropha* species found in India were determined to assess similarity index between the species (Sathaiah and Reddy 1985; Sujatha 1996). Molecular markers reveal more quickly and accurately, genetic differences far exceeding those obtainable using morphological or biochemical methods without the obscurance of environment. Nuclear and plastid DNA analysis represent an important tool for phylogenetic and diversity analysis of plants. Knowledge, access and exploitation of available genetic diversity in domesticated and wild relatives are essential for broadening the genetic base of cultivars to increase crop stability and performance.

Biochemical Markers

Biochemical markers like isozymes reveal polymorphism at protein level and have been used for characterization of plant genetic resources, establish relationships at lower taxonomic levels as well as to detect geographic origin. In *Jatropha*, isozyme markers have been used to study the genetic relatedness of species and confirmation of hybridity in interspecific crosses. Sathaiah and Reddy (1985) used isozymes for characterization of four *Jatropha* species. The study revealed distinct protein profiles for each of the four *Jatropha* species used in their study and maximum similarity (45.0%) was reported between *J. curcas* and *J. gossypiifolia*. Similarly, leaf protein profiles of total protein, protein subunits and esterase isozymes showed maximum similarity (50.9%) between *J. multifida* and *J. podagrica* belonging to the section peltatae (Sujatha 1996). Studies of Prabakaran and Sujatha (1999) based on cytological and biochemical (peroxidase isozyme studies) characters confirmed *J. tanjorensis* as a natural hybrid between *J. curcas* and *J. gossypiifolia* and not a distinct species as described by Ellis and Saroja (1961).

Characterization with Nuclear Markers

Molecular characterization of *Jatropha* species was confined to taxa naturalized in India (Table 20.1). The Indian species represent different sections and subsections and the morphological differentiation was associated with high molecular polymorphism (Figs. 20.3 and 20.4). Sujatha and Prabakaran (1997) have collected different species of *Jatropha* native to India and evaluated them for their beneficial characteristics, such as drought resistance, photoperiod insensitivity, resistance to wilt and tolerance to lepidopteran insect pests. Krishnan and Paramathma (2009)

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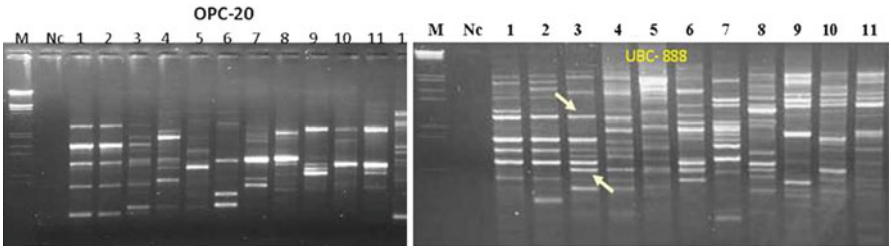

Fig. 20.3 Molecular characterization of *Jatropha* species using RAPD (OPC-20) and ISSR (UBC-888) primers. (1) *J. curcas* (non-toxic), (2) *J. curcas* (toxic), (3) *J. tanjorensis,* (4) *J. gossypiifolia*, (5) *J. glandulifera*, (6) *J. podagrica*, (7) *J. multifida*, (8) *J. integerrima*, (9) *J. villosa* var. *ramnadensis*, (10) *J. maheshwarii*, (11) *J. villosa*, and (12) *Ricinus communis*

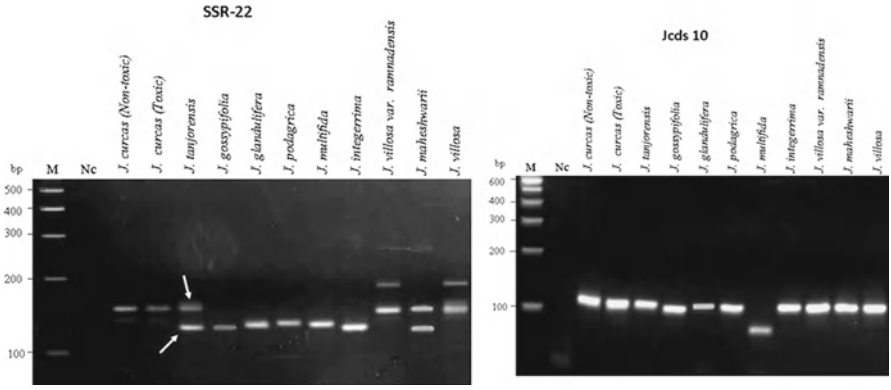

Fig. 20.4 Molecular characterization of *Jatropha* species using microsatellite primers

have reported important morphological features and their desirable traits for 12 *Jatropha* species occurring in India. Similarly, Basha and Sujatha (2009) have also reported desirable attributes for eight *Jatropha* species and a natural interspecific hybrid occurring in India. Vijayanand et al. (2009) based on 19 morphological traits characterized 7 *Jatropha* species native to India along with five *J. curcas* accessions and concluded that leaf petiole length, leaf length, leaf breadth, branch length, stem shape and seed coat color could be used as characters to distinguish the germplasm entries.

Molecular characterization of eight *Jatropha* species along with a natural hybrid occurring in India using 200 RAPD markers revealed high interspecific genetic variation (98.4% polymorphism) corroborating with the morphological differentiation of the species used (Basha and Sujatha 2009). In this study, the RAPD, ISSR and SSR markers confirmed the hybridity of *J. tanjorensis* between *J. curcas* and *J. gossypiifolia* (Figs. 20.3 and 20.4). Pamidimarri et al. (2008a) have characterized six *Jatropha* species along with a natural hybrid occurring in India using 33 RAPD primers and reported 97.74% polymorphism among the species used in the study. Likewise,

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genetic diversity assessment of wild *Jatropha* species along with *J. curcas* accessions revealed high genetic variability (80.2%) among the *Jatropha* species used in the study (Ganesh Ram et al. 2008). Siam et al. (2009) demonstrated the utility of the combinatorial tubulin based polymorphism as a simple, rapid, efficient and powerful genotyping tool for characterization of *J. curcas* germplasm, species and F_1 hybrids and can be applied to any plant genome of unknown sequence. However, use of few markers (Senthil Kumar et al. 2009) or variations at single locus (Pamidimarri et al. 2008a) is insufficient to draw meaningful conclusions about the genetic relationships. Molecular studies should substantiate the classical taxonomic classifications founded on morphological, cytological and crossability success and aid in resolving ambiguities in phylogenetic relationships. Molecular studies related to taxonomic classification should take into account the pioneering work of Dehgan (1984) and consider the phylogenetic relationships, evolutionary trends, crossability relationships, employ optimal number of marker loci depending on the polymorphism and their coverage of the whole genome in obtaining reliable estimates of genetic relationships among accessions.

Characterization with Microsatellite and Organelle Specific Primers

Universal primers targeted to mononucleotide repeats present in chloroplast genomes serve as a valuable tool to study chloroplast variation (Weising and Gardner 1999). Chloroplast specific microsatellites have been used for assessment of maternal versus paternal plastid inheritance, assessment of interspecific polymorphism, the detection of hybridization and introgression and phylogeny of plant populations. Basha and Sujatha (2009) used 50 organelle specific microsatellite primers comprising of a set of 10 consensus chloroplast microsatellite primers and 40 rice mitochondrial and chloroplast specific primers for characterization of eight agronomically important *Jatropha* species along with a natural hybrid native to India. In this study, the polymorphic PCR products obtained with organelle specific primers were subjected to sequence analysis and the sequences were deposited in NCBI GenBank. Characterization of *Jatropha* species native to India using chloroplast specific microsatellite primers revealed maximum variability in the intergenic regions of ORF77-ORF82 and *rp12-rps19* regions which could be used for distinguishing *Jatropha* species and identification of haplotypes (Basha and Sujatha 2009).

There is an immediate need for characterization of *Jatropha* species endemic/native to the Central American region using molecular markers to support the taxonomic classification and facilitate interspecific gene transfer. Interspecific hybridization could be used for supporting genetic mapping studies as well. *Jatropha* species should be characterized for the phytochemical constituents for pharmaceutical and insecticidal uses as useful products from *J. maheshwarii* (Viswanathan et al. 2004), *J. podagrica* (Aiyebagbe et al. 2007), *J. gossypiifolia* (Kupchan et al. 1970), etc. are reported.

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Marker-Assisted Breeding

Jatropha is characterized by long gestation period and large plant size and hence, elimination of undesirable progenies in breeding populations through marker assisted selection reduces cost and allows breeders to select population comprised of individuals carrying desirable genes of interest. Unlike in the past, the advent of molecular markers has accelerated crop breeding programs. Perusal of literature related to *Jatropha* reveals initiation of studies by different groups on development of molecular markers and framework maps. At TLL, a linkage map with 219 microsatellites, 200 SNPs and 160 AFLP markers has been constructed using backcross populations (Yue 2008). Subsequently, the first generation linkage map of *Jatropha* based on 216 microsatellites and 290 SNPs with a total length of 1440.9 cM and average marker space of 2.8 cM has been reported (Wang et al. 2011). At CNAP, > 400 SNPs were detected that could be sufficient for a dense map and in marker assisted breeding (Graham 2006). These linkage maps would serve as framework maps for mapping economically important traits. Microsatellite and SCAR markers linked to low phorbol ester levels have been identified which could be used to fast-track the breeding programmes designed to develop low-toxic genotypes (Pamidimarri et al. 2008b; Basha et al. 2009). Marker assisted selection can be useful for traits that are difficult to measure, exhibit low heritability and/or are expressed late in development but the key for success in MAS lies in the selection of diverse parental lines for generating segregating populations reiterating the need for collection and characterization of germplasm from the centre of origin. Research at PRI, is for mapping genes controlling number of fruits, tree architecture and toxicity while at CNAP, focus is on development of low-toxic genotypes.

Conclusions

In the light of the foregoing discussion, it is evident that morphological variation in the germplasm being augmented in different countries is rather limited. There was no geographical structuring of the germplasm in the studies with the exception of the studies of Basha et al. (2009) which disclosed clear separation of the non-toxic Mexican accessions from the rest of the world. Molecular analysis indicates a common ancestry for germplasm in the African and Asian regions and existence of rich diversity in Central American and Mexican regions (Montes Osorio et al. 2008; Van loo et al. 2008; Basha et al. 2009). Studies carried out in India and China indicated a very high probability of the movement of germplasm with the same origin within and across provinces. Basha and Sujatha (2007) indicated that the intrapopulation variability was on par with the interpopulation variability. Personal queries to different researchers indicate the existence of proliferating and bushy types in Mexico (Martinez, Pers. Comm), plants with altered architecture (Montes Osorio et al. 2008), 100% pistillate plants (Pecina-Quintero et al. 2011) and plants

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with large and very small lamina (author's personal collection). SSR analysis showed a unique allele in the accession with large leaves from EL Salvador (Basha et al. 2009). Thus, assessment of *J. curcas* germplasm using morphological and molecular markers indicate

- Low phenotypic and genotypic diversity in local populations in Asian regions and close clustering of accessions from Africa and Asia indicating a common genetic base.
- Phenotypic diversity in most cases was not associated with genotypic diversity indicating strong influence of environment.
- Preliminary studies at global germplasm analysis using molecular markers confirmed the availability of rich allelic diversity in South American, Mexican and Meso-American regions.
- Variations are reported for low and high number of fruits, tree architecture, toxicity (in terms of phorbol ester levels), seed mass and seed oil content.

Although molecular markers unravel variation, molecular measures of genetic diversity have a very limited ability to predict quantitative genetic variability (Sun et al. 2008). Hence, morphological characterization and estimates of molecular diversity need to be combined to identify divergent material for future breeding programme.

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Chapter 21
Interspecific Hybridization in the Genus *Jatropha*

Mulpuri Sujatha, Bir Bahadur, and T. Papi Reddy

Introduction

The genus *Jatropha* is morphologically diverse and geographically widespread encompassing 175 species of herbaceous perennials, shrubs, woody trees, rhizomatous sub-shrubs, succulents, facultative annuals and geophytes, each having a narrow geographical range in the seasonally dry tropics (Dehgan 1984). The genus is classified into two sub-genera *viz*., *Curcas, Jatropha*, ten sections and ten sub-sections to accommodate the old and new world species (Dehgan and Schutzman 1994). The two sub-genera can be distinguished by several morphological characteristics (Dehgan 1980, 1982, 1984; Dehgan and Webster 1979). The subgenus *Curcas* comprises all Mexican, one Costa Rican, two African and one Indian species, while the subgenus *Jatropha* includes all South American, African (except two species), Antillean, all Indian (except one species) and two North American species (Dehgan 1984). *Jatropha* species are naturalized throughout South American and Meso-American regions in Mexico, Cuba, Peru, Bolivia, Costa Rica, Paraguay, Jamaica, Brazil, El Salvador, Gautemala, Argentina, Dominican Republic, Columbia, Nicaragua and parts of North America in the states of Arizona and Texas (Heller 1996). *J. villosa* and related species have their origin in India (Ramamurthy 1967), while *J. mahafalensis* is a species of Nicaragua.

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Several *Jatropha* species are cultivated for their ornamental leaves and flowers, while some are grown in the tropics for various other economic uses. Leaves in various species are heterogenous with regard to size and architecture. Leaf size varies from 2 to 3 mm in extreme xeric habitats to 20 cm or more under mesic conditions. Leaf lamina are simple, palmately 3, 5 or 7-lobed, and divided into a maximum of 11 segments (*J. multifida*). The majority of the species are long petioled, while a few taxa are sub-sessile. Evolutionary trends in the genus tend towards xerophytic adaptation with changes in the growth habit from arborescent perennial growth habit to facultative annual habit; series of reductions in the reproductive structures of stamens, style branches, number of flowers, number of locules, seeds, reduced number of vascular bundles, gradual shift from hermaphrodite to monoecy to gynodioecy and dioecy and increase in chromosome numbers from diploidy to tetraploidy (section *Mozinna*; 2n = 44) are observed. These changes exhibit a morphological continuum from south to north with the southern taxa possessing the more primitive and the northern species the most advanced features and increasing aridity (Dehgan and Schutzman 1994). *J. curcas* and its allied taxa grow in tropical-mesic forest regions, whereas the taxa with reduced vascular bundles are most advanced and occur in dry, warm deserts (Dehgan and Webster 1979).

Wild relatives have been exploited in several crops as important reservoirs of genetic variability for various characters. Morphologically, the genus *Jatropha* closely resembles *Ricinus (*Castor*)* and has attracted interest because it possesses various beneficial traits not found in castor (Sathaiah and Reddy 1985; Reddy et al. 1987; Sujatha 1996). Attempts have been made to exploit *Jatropha* in the improvement of castor (Reddy et al. 1987; Sujatha 1996). Investigations based on similarity indices of seed protein profiles of castor and four *Jatropha* species indicated *J. gossypiifolia* as the closest to *Ricinus* (Sathaiah and Reddy 1985). However, studies of Sujatha (1996) based on crossability relationships, pollen-pistil interactions, characteristics of reproductive structures and leaf-protein profiles, revealed closeness of castor to *J. integerrima*. Successful introgression of some of the desirable genes of *Jatropha* into castor remained virtually untapped owing to lack of compatibility in diverse castor-*Jatropha* combinations (Sathaiah and Reddy 1985; Reddy et al. 1987; Sujatha 1996). Failure of intergeneric crosses was mainly due to incongruity caused by mismatching partners with differences in the chromosome numbers of castor (2n = 20) and *Jatropha* (2n = 22). Molecular phylogenetic analysis of Euphorbiaceae comprising 85 species of 83 genera, using plastid and nuclear DNA sequences (*rbcL, atpB, matK*, and 18 S rDNA) along with ovule and seed characters, revealed the closeness of the genus *Jatropha* to Cassava and *Hevea* rather than to *Ricinus* (Tokuoka 2007). Cross-taxa transferability of EST-derived SSR markers in *J. curcas* to castor was 47.0% (Yadav et al. 2010), while from Cassava to *J. curcas* it was 44.6% (Wen et al. 2010).

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Interspecific Hybridization

Interspecific hybridization played a vital role in the genetic improvement of diverse crop plants. The genus *Jatropha* could also be benefited through introgressive breeding and hence, there is a need for collection, assembly, conservation, characterization, evaluation and utilization of *Jatropha* species in broadening the genetic base of *J. curcas* for various agronomical attributes.

Jatropha has been domesticated in several African and Asian countries and as such there is an immediate need to broaden the genetic base of *J. curcas* germplasm. Among the various crop breeding approaches, interspecific hybridization is an important option for genetic enhancement of *J. curcas* (Sujatha 2006; Basha and Sujatha 2009; Parthiban et al. 2009a). However, the present status of crop improvement work in *J. curcas* is very limited and interspecific hybridization has been attempted between different species of *Jatropha* with limited success (Dehgan 1984; Sujatha and Prabakaran 1997; Sujatha 2006). In order to alter its status from wild perennial form to a cultivable crop, it is essential to introgress desired genes for characters like higher seed yield and oil content, early maturity, reduced plant height, higher female to male flower ratio, resistance to pest and diseases, drought tolerance and improved fuel properties from wild *Jatropha* species to *J. curcas* through interspecific hybridization (Sujatha 2006).

Traits That Could Be Modified Through Interspecific Hybridization

Yield

Yield can be enhanced through selection of superior germplasm, exploitation of heterosis and increasing the number of flowering flushes. Characterization of Jatropha species indicates the possibility of obtaining pistillate plants. Evolutionary trends of the inflorescence in the genus *Jatropha* indicate the feasibility for reduction of co-florescence to a single pistillate flower in the section *Collenucia* (Chiov). In the subgenus *Curcas*, inflorescences were drastically reduced to a few terminal or lateral flowers together with a gradual change from monoecy to dioecy (Dehgan 1984). Interestingly, the monoecious *J. curcas* crosses readily with the dioecious species *J. cordata* and *J. cinerea*. Most of the Jatropha species, with the exception of *J. curcas*, produce flowering flushes continuously throughout the year but with variations in flowering density depending on the season and the species. This attribute can be transferred to *J. curcas* to enhance its seed/oil yield potential.

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Oil Content

Jatrophas are rich sources of hydrocarbons, and *J. multifida* with big round seeds possesses higher oil content (50%) as compared to *J. curcas* (23–38%) (Banerji et al. 1985; Sujatha 1996). The crossability barriers between these two species are weak (Basha and Sujatha 2009), as such this cross-combination can aid in the enhancement of oil content. Seeds of *Jatropha* species, such as *J. podagrica, J. integerrima* and *J. gossypiifolia* have thin hull when compared to *J. curcas* and such types are needed for efficient recovery of the oil. Also, *J. platyphylla* with its larger seed size (2–3 folds higher than *J. curcas*) and high oil content (60% on kernel basis) could be exploited in interspecific hybridization programme for improved seed traits (Makkar et al. 2011).

Alteration of Fatty Acid Composition

Analysis of seed oil fatty acids showed the predominance of linoleic acid with a higher linoleic to oleic acid ratio in all *Jatropha* species with the exception of *J. curcas,* which is rich in oleic acid (Banerji et al. 1985; Rao and Lakshminarayana 1987; Sujatha 1996). Cetane number is one of the most important factors for biodiesel which should be 47 as per ASTM D6751 and 51 as per EN 14214 standards. Variation in the fatty acid profiles significantly influences the cetane number (King et al. 2009), and hence, interspecific derivatives with desirable cetane value could be isolated and developed.

Biofuel Properties

Determination of the energy values of oils indicated much higher energy content for *J. gossypiifolia* (42.2 MJ/kg), *J. glandulifera* (47.2 MJ/kg) and *J. multifida* (57.1 MJ/kg) than for *J. curcas* (39.8–41.8 MJ/kg) (Banerji et al. 1985; Jones and Miller 1991). *J. gossypiifolia* is reported to have 18.5% ricinoleic acid in its seed oil (Hosamani and Kotagi 2008), and physico-chemical properties of the biodiesel derived from this species is in the acceptable range for use in diesel engines (de Oliviera et al. 2009). Accordingly, this trait assumes importance when considering the potential of interspecific crosses for improvement of *J. curcas* as a fuel crop. *J. mahafalensis,* a species endemic to Madagascar and *J. nana* from Pune, were reported to have equal energetic promise as that of *J. curcas.*

Value Addition to the Cake and By-Product Utilization

Use of *J. curcas* seedcake as food or feed source is restricted as the seeds are found to be toxic to animals (Becker and Makkar 1997). The toxic and irritant

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compounds isolated include β-D-glucosides of sitosterol (Bose et al. 1961), the lectin curcin (Stripe et al. 1976), flavonoids vitexine and isovitexine (Sankara et al. 1971), the 12-deoxyl-16-hydroxy phorbol (Adolf et al. 1984) and diterpenes from roots (Chen et al. 1988). Enhanced production of *J. curcas* as a fuel source will increase the production of the toxic by-product (meal) and the immediate concern is to detoxify it. Toxicity could be reduced through traditional methods of breeding with germplasm containing low levels of toxic substances or through interspecific hybridization. A non-toxic variety of *J. curcas* has been found in the Papantla region of Veracruz State in Mexico, which is suitable for human consumption after roasting and the innocuous nature of this *J. curcas* variety was established using fish and rats as experimental models (Makkar et al. 1998). Most of the economically important *Jatropha* species, *viz., J. elliptica, J. glauca, J. gossypiifolia, J. aceroides, J. tanoresisi, J. macarantha, J. integerrima, J. glandulifera, J. podagrica* and *J. multifida,* are reported to have a cocktail of toxins including phorbol esters (Siam et al. 2009; Devappa et al. 2010). However, *J. platyphylla* an edible species from Mexico is found to be non-toxic (Makkar et al. 2011) and the seeds are free from the phorbol esters. While the phorbol esters from *J. curcas* are toxic and co-carcinogenic, the Jatrophone – a macrocyclic diterpenoid from *J. gossypiifolia* showed anti-cancerous activity and also possessed significant antileukemic activity against P-388 lymphocytic leukemia (Kupchan et al. 1970). Non-toxic edible accessions of *J. curcas* are available but the presence of other anti-nutrients poses a serious problem. Detailed investigations with regard to the presence of toxicological compounds in the sexually compatible species followed by interspecific hybridization could lead to the development of toxin-free *J. curcas. Jatropha* species should be characterized for the phytochemical constituents for pharmaceutical and insecticidal uses, as useful products have been identified in *J. maheshwarii* (Viswanathan et al. 2004), *J. podagrica* (Aiyelaagbe et al. 2007), *J. gossypiifolia* (Kupchan et al. 1970) and other species.

Improved Resistance to Pests and Diseases

J. curcas has been considered as an ideal candidate crop for biofuel purpose because it is less attacked by insect pests and diseases. However, in the recent past incidence of pests and diseases is frequently reported especially, when the crop is raised as monoculture. Screening of *Jatropha* species against foliage feeders, which attack other Euphorbiaceous members, revealed varied levels of resistance within *Jatropha* species, with *J. integerrima* conferring maximum resistance in terms of larval mortality, feeding cessation and with or without pupation (Lakshminarayana and Sujatha 2001). These studies clearly indicate the potential value of related species for genetic enhancement of resistance of *J. curcas* against various biotic stresses.

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Adaptability to Problematic Sites

J. gossypiifolia, a facultative annual, has heavy fruit bearing ability and is adapted to saline regions of Northeast Thailand and India. Apart from interspecific crosses, grafting was considered as one of the means for improving the productivity of *J. curcas* (Sakaguchi and Somabhi 1987). Graftings were done in all possible combinations of *J. curcas* with *J. gossypiifolia, J. multifida* and *J. podagrica.* Grafting combination with the stock of *J. gossypiifolia* and scions of *J. curcas* is assumed to be one of the counter measures to expand the adaptability of *J. curcas* to saline soils. The species *J. tanjorensis,* found abundantly in Tanjore, Pudukottai and Ramnad districts of Tamil Nadu (India) has been identified as a natural interspecific hybrid between these two species (Prabakaran and Sujatha 1999). Studies were primarily confined to the characterization of the putative hybrid through cytological and biochemical methods, and further crosses were not attempted owing to high pollen sterility. *J. tanjorensis* is more vigorous and less attacked by insect pests and diseases. Large number of crosses with the parental species as pollen parents may result in the development of backcross material for use in the breeding programmes aimed at the development of materials resistant to abiotic stresses.

Alteration of Ideotype

The species *J. integerrima, J. multifida* and *J. podagrica* are well known ornamental plants that are drought hardy and have continuous bearing unlike *J. curcas* which has two to four flowering flushes depending on the agro-ecological conditions. *J. curcas* produces unisexual flowers in dichasial cymes. In the inflorescence, the central branches end in female flowers while the laterals terminate in male flowers. The average male to female ratio is 29:1 (Solomon Raju and Ezradanam 2002), and depending on the environmental factors, the sexuality ratio gets modified. Under harsh environments, maleness is promoted, and barring one or two paracladia of the inflorescence, all others form male flowers only. However, such drastic influence on the sexuality has not been observed in other *Jatropha* species. *J. nana* and *J. villosa* are often found in dry stony places. Also, *J. nana* and *J. heynii* from Africa may also be exploited as sources of dwarfing genes as the crop should be of manageable height for mechanization. Availability of species with such diverse plant types and wide adaptability offers vast scope in improving the genetic architecture and agronomic attributes of *J. curcas.* The genus *Jatropha* has evolved with adaptations to arid conditions, and hence, *Jatropha* species well adapted to the Northern hemisphere could serve as a valuable source for the development of drought resistant cultivars. Thus, there is immense scope for transfer of beneficial traits from wild *Jatropha* species to *J. curcas* such as heavy bearing, photoperiod insensitivity, improved fuel characteristics, high oil content, desired oil quality, plant architecture, earliness, reduced toxicity of endosperm proteins and wider adaptability (Sujatha 2006, 2008).

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Interspecific Crosses

Pollen-Pistil Interactions

Crossability in terms of pollen-pistil interactions was carried out by Indian researchers (Reddy et al. 1987, Reddy 1988; Sujatha 1996; Parthiban et al. 2009a; Senthil Kumar et al. 2009a). Reddy et al. (1987) assessed crossability relationships in intergeneric crosses between castor and *Jatropha* species, *J. gossypiifolia, J. glandulifera, J. curcas, J. podagrica, J. panduraefolia (J. hastata), J. multifida* besides interspecific crosses including reciprocals. A maximum pollen germination of 20–30% was recorded in these crosses. In crosses with *J. curcas* as female, pollen grains of *J. podagrica, J. hastata* and *J. multifida* failed to germinate. These crosses showed strong cross-incompatibility and pollen germination was confined to the stigmatic surface which was arrested with coiled or spathulate pollen tips. Both intergeneric and interspecific crosses, with the exception of cross between castor × *J. gossypiifolia,* failed to set seeds while the seeds obtained in the intergeneric cross did not germinate.

Sujatha (1996) investigated pollen germination in 23 interspecific cross combinations of *Jatropha*. In compatible crosses, pollen tubes maintained their normal structure and integrity throughout the path from pollen germination on stigma to entry of pollen tubes into the ovule. In all the cases, pollen adherence and pollen grain germination started within 15–20 min after pollination, and by 4–8 HAP the pollen tubes entered the ovule (Fig. 21.1). The style lengths are about 0.2–0.56 cm giving a growth rate in the order of 2.5–7 mm/h. The rate of pollen germination and pollen tube growth are sufficient to accomplish fertilization within the allotted time. Depending on the cross combination for each pistil, many (>25) pollen tubes were seen penetrating the stigmatic surface and traveling through the vascular tissue of the style. The overall frequency of pollen germination ranged from 51.2% to 100%. On *J. curcas* styles, the pollen from different species of *Jatropha* germinated with cent per cent frequency. However, in the reciprocal crosses a lower frequency (66.7%) of pollen germination was recorded in the cross between *J. multifida* and *J. curcas*. Further, in crosses with *J. integerrima, J. podagrica* and *J. multifida* as pollen parents, pollen tube growth of *J. curcas* and *J. gossypiifolia* were restrained by callose deposition. Likewise, pollen germination in *J. integerrima* × *J. multifida* and *J. podagrica* × *J. integerrima* crosses were hindered by callose deposition. Pollen of *J. integerrima* germinated with a high frequency (96.7–100%) on the stigmas of other *Jatropha* species tested. Correlations between seed set and callose deposition did not indicate any positive relationship between the two parameters.

In the studies carried out by Sujatha and Prabakaran (2003), pollen germination was 100% in *J. curcas* × *J. integerrima* cross while it was 96.8% in the reciprocal cross, and pollen germination was associated with formation of callose plugs with restricted pollen tube entry at the style-ovule junction. Furthermore, pollination of *J. integerrima* following stigma excision at different lengths proved unsuccessful.

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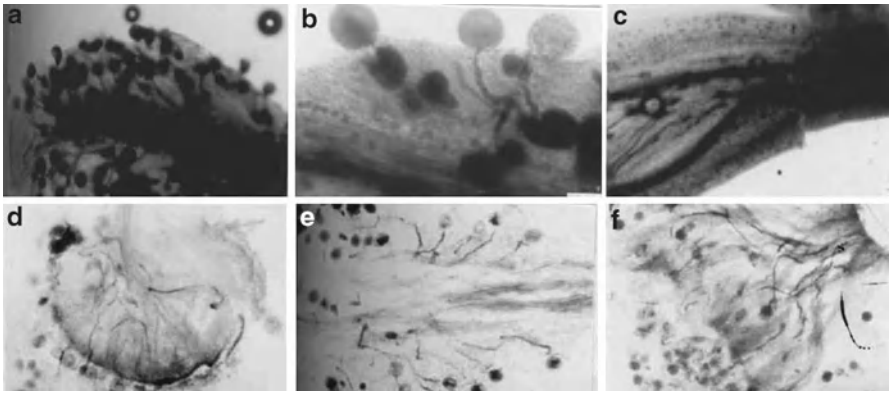

Fig. 21.1 Pollen-stigma interactions in interspecific crosses: (**a**) *J. integerrima* × *J. multifida* with 100% germination; (**b**) close up of *J. integerrima* × *J. multifida* showing callose plugs; (**c**) *J. integerrima* × *J. podagrica* showing normal pollen tubes in the stylar region; (**d**) *J. multifida* × *J. podagrica* with 100% germination; (**e**) *J. integerrima* × *J. curcas* with pollen tubes halfway in the stylar region; and (**f**) *J. gossypiifolia* × *J. integerrima* showing pollen tube entry in the ovule region (Source: Sujatha 1996)

Dehgan (1984) attempted interspecific hybridization of 20 *Jatropha* species in eight sections in all possible combinations. Unilateral compatibility was found in all the crosses except for the cross between *J. curcas* × *J. integerrima.* In the reciprocal crosses, capsule enlargement with normal embryo was observed but the endosperm aborted. Some of the intersectional hybrids were successful which could serve as bridge crosses.

Parthiban et al. (2009b) determined pollen tube growth characteristics in various interspecific crosses involving *J. curcas* as female parent. Depending on the cross-combination, pollen germination varied from 15.6% to 50% with least for pollinations with *J. multifida* and maximum for the cross with *J. integerrima*. Although pollen germination was observed, pollen tubes were restricted either at the midstylar or the ovular region, with the exception of crosses involving *J. integerrima* and *J. gossypiifolia* wherein pollen tubes entered *J. curcas* ovules with a frequency of 8 and 5%, respectively.

Senthil Kumar et al. (2009a) investigated the pre-and post-zygotic barriers through pollen-pistil interactions between *J. curcas* and other wild *Jatropha* species. In this study, failure of interspecific hybridization in *Jatropha* species was due to the poor pollen germination and inhibition of pollen tube growth between *J. curcas* and the wild species, but in case of reciprocal crosses the incompatibility existed at the ovarian level.

Shortening of the styles for facilitating interspecific crosses between *J. multifida* × *J. podagrica* showed no significant effects (Sujatha 1996). Styles of *Jatropha* species with the exception of *J. integerrima* are very short. Apparently, cutting back the styles cause mutilation injury and deprive the pollen grains a suitable matrix for their germination. Failure to obtain enhanced seed set following stigma excision indicates

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that the barriers to interspecific hybridization are not operative during pre-fertilization. As the pollen tubes were found to penetrate the micropyle, most probably, post-fertilization barriers have inhibited the growth of various interspecific hybrids. Furthermore, this observation is corraborated by the large number of early and late aborted capsules, small shrivelled seeds, seeds with aborted endosperm and failure of the surgical procedures in the improvement of capsule set. So far, only those F_1 hybrids that can be recovered from direct crosses were considered. The pollen germination studies indicated that the barriers restricting interspecific crosses are mostly post-zygotic. Furthermore, it was feasible to recover maximum number of cross combinations by resorting to embryo-rescue techniques.

Barriers to interspecific crossability were weak in crosses when *J. integerrima* was used as the pollen parent. *J. integerrima* showed cross-compatibility with several species and it formed reciprocal hybrids with *J. curcas;* as such it could be successfully exploited as a bridge species in difficult-to-cross hybrids. The hybridity of F_{1s} can be confirmed by the number of leaf lobes, leaf pigmentation and flower colour of the F_1 hybrid (Fig. 21.3a; Basha and Sujatha 2009).

Capsule Set

Dehgan (1984) obtained several F_1 hybrids from different interspecific crosses with high pollen fertility and the most successful ones were with *J. curcas* as the maternal parent (Table 21.1). Despite the production of several F_1 hybrids, only two hybrids namely *J. curcas* × *J. integerrima; J. curcas* × *J. macrorhiza* could produce seed and F_2 progeny. In both cases, seed set was low with a frequency of 5.9% and plants segregated for vegetative and floral characters. Sujatha (1996) reported capsule set in different interspecific crosses which varied from 0.41% to 9.25% depending on the cross-combination. Artificial hybrids were obtained from the crosses of *J. curcas* with *J. integerrima*, *J. gossypiifolia* (including reciprocal), *J. multifida, J. integerrima* var *rosea*; *J. multifida* with *J. integerrima; J. podagrica* with *J. integerrima* and *J. multifida.* Subsequently, interspecific hybrids of *J. curcas* were also produced with *J. multifida, J. maheshwarii, J. villosa, J. villosa* var *ramnadensis* and *J. gossypiifolia* (Basha and Sujatha 2009). Photographs of an artificial interspecific hybrid (F_1 generation) between *J. scaposa* (female parent) and *J. curcas* (pollen donor) are available (www.flickr.com/photos/47108884@N07/4612049908/) which show intermediacy for vegetative and floral characters. *J. curcas* is a Mexican species and *J. scaposa* is a Mozambique endemic but both species belong to the same section *Curcas*. The objective of interspecific hybridization is for reduction of plant size (permits mechanized harvesting), early yield, increased yield and seed oil content, besides improved adaptation to drought and heat.

Hybridization between *J. curcas* and *J. gossypiifolia* was successful in both the directions (Table 21.1). However, seeds from the cross with *J. gossypiifolia* as the maternal parent failed to germinate. Failure of seedling emergence in the reciprocal cross indicates the restrained control of the incompatibility barriers. Dehgan (1984)

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Table 21.1 Crossability relationships among *Jatropha* species

↓ →	CUR	INT	HER	MAC	CAP	UNI	CAT	MUL	AUG	POD	EXC	GOS	CUN	DIO	CAR	COR	CIN	PLA	MOR	MCV	VIL	MAH
J. curcas	S	H	X	H	H	X	H	H	X	H	X	H*	X	X	X	H	H	E	E	–	H	H
J. integerrima	H	S	E	H	H	X	–	–	–	E	X	E	–	X	–	–	–	X	–	–	–	–
J. hernandiifolia	X	E	S	–	E	X	–	–	X	X	X	E	–	X	–	–	–	–	–	–	–	–
J. macrorhiza	–	H	–	S	H	–	–	E	–	X	X	–	–	–	–	–	–	–	H	–	–	–
J. capensis	–	H	E	H	S	X	–	E	–	E	X	X	X	X	–	–	–	–	–	–	–	–
J. unicostata	X	E	X	–	X	S	X	X	–	–	X	X	–	–	–	–	–	–	–	–	–	–
J. carthartica	–	–	–	–	–	X	S	E	–	H	X	X	X	X	–	–	–	–	–	–	–	–
J. multifida	H	H	–	E	E	X	E	S	X	H	–	–	–	–	–	–	–	E	–	–	–	–
J. augistii	X	–	X	–	–	–	–	X	S	–	X	E	–	–	–	–	–	–	–	–	–	–
J. podagrica	–	H	X	X	E	–	H	H	E	S	–	X	X	X	X	–	–	–	–	–	–	–
J. excisa var *pubescens*	X	X	X	X	X	X	X	–	X	–	S	X	–	–	–	X	–	–	–	–	–	–
J. gossypiifolia	H	E	E	–	X	X	–	E	–	X	X	S	X	–	–	X	–	–	–	–	–	–
J. cuneata	X	–	–	–	X	–	X	–	–	X	–	X	S	–	–	–	–	–	–	–	–	–
J. dioica	X	X	X	–	X	–	X	–	–	X	–	–	–	S	–	–	–	X	–	–	–	–
J. cardiophylla	X	–	–	–	–	–	–	–	–	X	–	–	–	–	S	–	–	–	H	–	–	–
J. cordata	–	–	–	–	–	–	–	–	–	–	X	X	–	–	–	S	H	–	–	E	–	–
J. cinerea	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	S	–	–	–	–	–
J. platyphylla	E	X	–	–	–	–	–	E	–	–	–	–	–	X	–	–	–	S	–	–	–	–
J. moranii	E	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	S	–	–	–
J. mcvaughii	–	–	–	–	–	–	–	–	–	–	–	–	–	–	E	–	–	–	–	S	–	–
J. villosa	H	H	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	S	–
J. maheshwarii	H	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	S

Source: Dehgan (1984); Sujatha and Prabakaran (2003); Basha and Sujatha (2009)

S self, *H* hybrid, *X* cross failure and no fruit or seed enlargement, *E* fruit and seed enlargement, endosperm lacking; – not attempted, * natural hybrid

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failed to obtain seed from this cross combination which was attributed to the greater phylogenetic distance between the two taxa. However, the leaf protein profiles of *J. gossypiifolia* indicated its closest affinity with *J. curcas* (Sujatha 1996). Sathaiah and Reddy (1985) also reported a higher similarity index for these two species. Existence of *J. tanjorensis,* a natural hybrid between *J. gossypiifolia* and *J. curcas* (Prabakaran and Sujatha 1999; Basha and Sujatha 2009) indicates the possibility of obtaining hybrids through artificial hybridization as well. Hybridization between *J. curcas* and *J. gossypiifolia* showed a very high degree of incompatibility due to post-fertilization barriers (Sujatha 1996). Examination of flower buds of *J. tanjorensis* revealed meiotic abnormalities, irregular sporads and sterile pollen grains with high degree of polymorphism, which indicated that it is a cross derivative (Prabakaran and Sujatha 1999). Peroxidase isozyme analysis of *Jatropha* species native to the region confirmed *J. tanjorensis* as a hybrid between *J. curcas* and *J. gossypiifolia* (Prabakaran and Sujatha 1999). *J. tanjorensis* showed luxuriant growth habit and was intermediate for phenotypic characters, *viz.,* stem pigmentation, leaf shape and margin, flower colour, etc, of *J. curcas* and *J. gossypiifolia,* the two economically important species of the genus.

Interspecific gene transfer is generally limited by crossability barriers, ploidy differences and genetic distance of taxa. Mc Vaugh (1945) and Wilbur (1954) considered *J. curcas* as the most plesiomorphic or primitive member of the genus because of its ability to interbreed with species from the subgenera, its palmately lobed leaves, arborecsent growth habit and occasional hermaphrodite flowers. Neither geographical isolation nor extensive morphological diversification, particularly, with respect to growth habit, have produced strong barriers to interspecific compatibility and inter-and intra-sectional hybrids could be produced with *J. curcas*. The primitiveness of the taxon *J. curcas* was justified by Dehgan and Webster (1979) on morphological grounds and was supported by the results of Dehgan and Schutzman (1994). Dehgan (1980) enumerated the primitive features of *Jatropha* and suggested its possible ancestral position in the Euphorbiaceae. Numerous morphologically distinguishable taxa deserving specific rank have evolved in the genus *Jatropha* (Dehgan 1984). Successful hybridization between members of different sub-sections indicates that the various taxa in *Jatropha* are phylogenetically related and neither geographical isolation nor extensive morphological diversification, especially with respect to growth habit, has produced strong barriers to interspecific compatibility. Rupert et al. (1970) reported that allelic mutations rather than chromosomal rearrangements could be primarily responsible for speciation.

Based on crossability studies and pollen stainability in F_1 hybrids, Dehgan (1984) suggested closeness of *J. curcas* with species of Polymorphae (*J. integerrima, J. macrorhiza*) followed by Tuberosae (*J. capensis*) and Peltatae (*J. multifida*), while the greatest phylogenetic distance was observed with members of section Jatropha (*J. gossypiifolia, J. excisa* var *pubescens*). Reciprocal differences in the interspecific crosses could be due to the plasmon-genome interactions which cause abnormal development and subsequent abortion of the young ovules. The pollen-stigma interactions in *Jatropha* crosses are a clear indication of an active pollen-stigma recognition-rejection phenomenon. According to Dehgan

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(1984), related species are separated by incongruity or preferential fertilization rather than incompatibility, while phylogenetically distant taxa are separated by actual genetic incompatibility barriers.

Interspecific Hybrids and Confirmation of Hybridity

Natural Hybrids

In the genus *Jatropha,* occurrence of natural hybrid complexes are reported such as, *J. curcas-canascens* complex from Mexico (Dehgan and Webster 1978), *J. integerrima-hastata* complex from Cuba and West Indian islands (Pax 1910) and *J. curcas-gossypiifolia* (*J. tanjorensis)* from India (Prabakaran and Sujatha 1999). Germplasm exhibiting gross morphological differences should be subjected to pollen studies and accessions showing pollen abnormality or poor seed set should be investigated in detail before drawing conclusions about the distinctness. Putative hybrid plants should be characterized using morphological and molecular markers for confirmation of their hybridity. One such study using phenotypic and genotypic markers has confirmed *J. tanjorensis* as a natural interspecific hybrid between *J. curcas* and *J. gossypiifolia* (Fig. 21.2). CCMP sequence analysis (Basha and Sujatha 2009) and nr DNA ITS sequence analysis (Sudheer Pamidimarri et al. 2009a) showed closeness of *J. tanjorensis* with *J. gossypiifolia*. Highest cross species amplification of SSR markers derived from *J. curcas* with *J. tanjorensis,* indicates its relatedness with *J. curcas* (Sudheer Pamidimarri et al. 2011). The cTBP analysis also confirmed the hybridity of *J. tanjorensis* between *J. curcas* × *J. gossypiifolia* (Siam et al. 2009).

Artificially Produced Hybrids

Dehgan (1984) attempted interspecific hybridization of 20 species in eight of the ten sections and the study was confined to identification of crossability barriers and morphological characterization of the F_1 hybrids. Most of the crosses showed unilateral compatibility with preferential fertilization and viable hybrids were obtained in crosses involving *J. curcas* as the ovule parent. The interspecific hybrids showed morphological intermediacy for leaf pigmentation, number of leaf lobes, flower colour and earliness (Fig. 21.3). All F_1 hybrids except *J. curcas* × *J. multifida* were more vigorous than their parental species and flowered earlier. Reciprocal crosses are possible with *J. integerrima* and interspecific hybrids between *J. curcas* and *J. integerrima* could be developed (Rupert et al. 1970; Dehgan 1984; Sujatha and Prabakaran 2003). Pollen fertility in the F_1 hybrids varied between 42–69% (Basha and Sujatha 2009) and 12–66.2% (Dehgan 1984). Although the F_1 hybrids were

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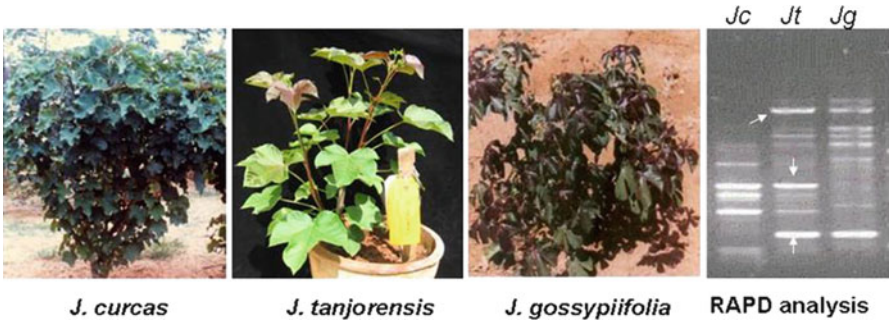

Fig. 21.2 Confirmation of hybridity of *J. tanjorensis* based on morphological and molecular markers

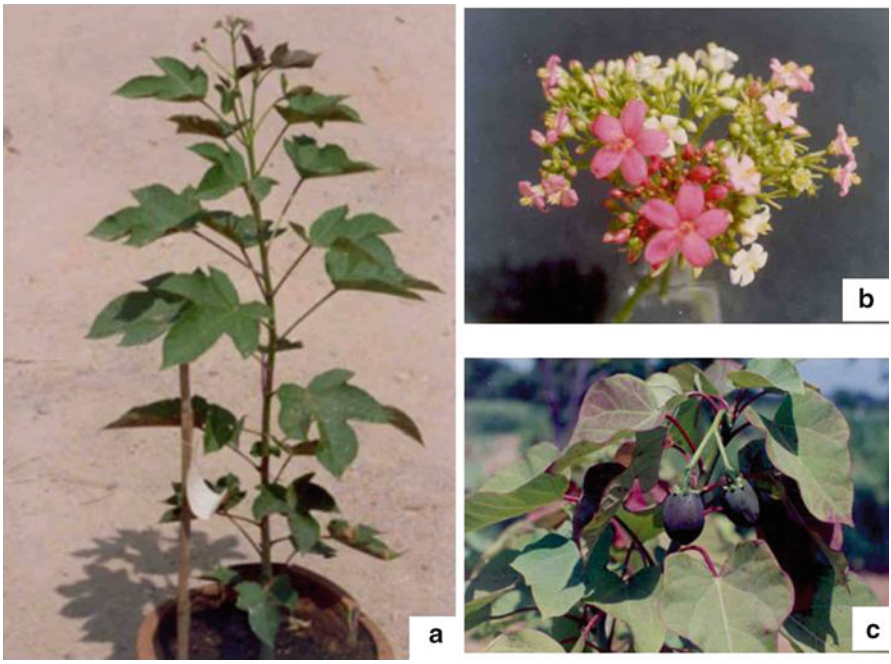

Fig. 21.3 Interspecific hybrid of the cross between *J. curcas* × *J. integerrima;* (**a**) F_1 plant, (**b**) flower colour variations in F_1 plants; and (**c**) *Purple* coloured fruit in the F_1 hybrid

partially sterile, backcrosses resulted in improved pollen fertility, seed set, seed size, qualitative and quantitative traits including the levels of phorbol esters and oil quality (Sujatha and Prabakaran 2003; Siam et al. 2009*;* Basha and Sujatha 2009; Parthiban et al. 2009a, 2009b). Parthiban et al. (2009a) reported successful hybridization between *J. curcas* and *J. integerrima* with good seed set, while the reciprocal crosses and crosses with other Jatropha species failed to produce seeds. Similarly,

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Dhillon et al. (2009) attempted reciprocal crosses between *J. curcas* and *J. integerrima* and reported that interspecific hybrids were successful only when *J. curcas* was used as the female parent. In this study, the F_1 hybrids exhibited wide range of variation for vegetative and reproductive traits, suggesting considerable heterogeneity between the parental plants.

Interspecific hybrids derived from the cross between *J. curcas* and *J. integerrima* have been widely studied (Rupert et al. 1970; Dehgan 1984; Sujatha and Prabakaran 2003; Siam et al. 2009; Dhillon et al. 2009; Parthiban et al. 2009a, 2009b). At Temasek Lifesciences Limited (TLL), Singapore, interspecific hybrids and segregating populations were developed from the cross between *J. curcas* × *J. integerrima* for genetic mapping and introgression of desirable traits (Hong 2008). Interspecific hybridization between these two species was carried out with the objective of combining the desirable traits of the two species *viz.,* high oil content, oil quality, resistance to biotic and abiotic stresses and non-shattering capsules. Seed set in crosses of *J. curcas* × *J. integerrima* involving *J. curcas* as the ovule parent was 7.6% (Sujatha and Prabakaran 2003) and 5.4% (Dhillon et al. 2009). The F_1 hybrids showed morphological intermediacy for several traits and produced flowers with three distinct colours, *viz.,* white, dark pink and light pink unlike the parents with green and crimson red colours and flowered earlier (5–8 months) when compared to the parents (Fig. 21.3; Sujatha and Prabakaran 2003). However, in the studies of Rupert et al. (1970), only one flower colour intermediate between petal colour of the parents was reported. Back crossing and selfing of the hybrids resulted in transgressive segregants exhibiting variations in several qualitative and quantitative traits including flower colour that varied in intensities from dark pink through green to white enhancing the ornamental value of the genus (Sujatha and Prabakaran 2003). The interspecific derivatives of this cross (F_1, F_2, BC_1F_1, BC_1F_2) being developed at the Directorate of Oilseeds Research, Hyderabad has been spared to several researchers. Backcrossing and generation advancement of the hybrids between *J. curcas* × *J. integerrima* resulted in novel plant materials with high fruit yield, low toxicity, continuous flowering, bushy growth with more number of branches, etc. (Basha and Sujatha 2009; Parthiban et al. 2009a, b; Siam et al. 2009). Basha and Sujatha (2009) demonstrated the possibility of obtaining hybrids with *J. curcas* as pollen parent crossed to *J. multifida, J. maheshwarii, J. gossypiifolia* and *J. villosa* (Fig. 21.4).

Hybridity of crosses between *J. curcas* × *J. integerrima* was confirmed through RAPD analysis (Sujatha and Prabakaran 2003). Siam et al. (2009) used a simple, rapid and powerful technique of combinatorial tubulin based polymorphism (cTBP) for confirmation of hybridity of the interspecific hybrids of *J. curcas* × *J. integerrima; J. curcas* × *J. maheshwarii;* and *J. curcas* × *J. multifida.*

Diversity Analysis and Phylogenetic Relationships

In *Jatropha*, taxonomic classification and infrageneric relationships were based on leaf epidermal morphology (Dehgan 1980); petiolar anatomy (Dehgan 1982); crossability relationships (Dehgan 1984); and phenetic and cladistic analysis based on

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Fig. 21.4 Interspecific hybrids of Jatropha. (**a**) *J. curcas* × *J. multifida* showing seven leaf lobes in the hybrid which is intermediate of *J. curcas* (five) and *J. multifida* (nine); (**b**) *J. maheshwarii* × *J. curcas* (*middle*) with parents on either side

morphological characters (Dehgan and Webster 1979; Dehgan and Schutzman 1994). Anatomical features of the petiole are singularly not sufficient in delineating evolutionary phylogenetic sequences but strengthen other anatomical, morphological and experimental approaches in solving taxonomic problems. Likewise, morphological studies of epidermis and other traits are insufficient by themselves as taxonomic evidences. Electrophoretic patterns of seed and leaf proteins of *Jatropha* species found in India were determined to assess similarity index between *Jatropha* species (Sathaiah and Reddy 1985; Sujatha 1996). Molecular markers reveal more quickly and accurately, genetic differences far exceeding those obtainable using morphological or biochemical methods without the influence of environment. Molecular markers can be used successfully for characterization of species, confirmation of hybridity in interspecific hybrids, determination of crossability success, establishment of phylogenetic relationships, marker-assisted selection and construction of framework linkage maps. Nuclear and plastid DNA analysis represent an important tool for phylogenetic and diversity analysis of plants.

During the past 2–3 years, studies on molecular characterization of *Jatropha* species has been carried out but were confined to taxa naturalized in India (Table 21.2). The *Jatropha* species found in India represent different sections and subsections and the morphological diversity was associated with high molecular polymorphism.

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Table 21.2 Molecular characterization of *Jatropha* species and interspecific hybrids

Sl. no.	Species	Markers used	Polymorphsim percentage	Reference
1	*J. curcas* × *J. integerrima* hybrid	RAPD markers	Confirmation of hybridity	Sujatha and Prabakaran (2003)
2	*J. curcas, J. ramnadensis, J. glandulifera, J. gossypiifolia, J. integerrima, J. podagrica, J. tanjorensis, J. villosa*	18 RAPD primers	80.2	Ganesh Ram et al. (2008)
3	*J. curcas, J. glandulifera, J. gossypiifolia, J. integerrima, J. multifida, J. podagrica, J. tanjorensis, J. maheshwarii, J. villosa*	9 ISSR primers	98.14	Senthil Kumar et al. (2009b)
4	*J. curcas, J. glandulifera, J. gossypiifolia, J. integerrima, J. multifida, J. podagrica, J. tanjorensis*	nr DNA ITS sequence	–	Sudheer Pamidimarri et al. (2009a)
5	*J. curcas, J. glandulifera, J. gossypiifolia, J. integerrima, J. multifida, J. podagrica, J. tanjorensis*	33 RAPD, 27 AFLP primer combinations	68.48 RAPD, 71.33 AFLP	Sudheer Pamidimarri et al. (2009b)
6	*J. curcas, J. glandulifera, J. gossypiifolia, J. integerrima, J. multifida, J. podagrica, J. tanjorensis, J. maheshwarii, J. villosa, J. ramnadensis*	RAPD, ISSR, SSR, CCMP markers	Confirmation of hybridity; putative progenitors	Basha and Sujatha (2009)
7	*J. curcas, J. glandulifera, J. gossypiifolia, J. integerrima, J. multifida, J. podagrica, J. villosa, J. rosea*	cTBP	–	Siam et al. (2009)
8	*J. curcas* × *J. integerrima, J. curcas* × *J. gossypiifolia* = *J. tanjorensis, J. curcas* × *J. multifida, J. curcas* × *J. maheshwarii* hybrids	cTBP	Confirmation of hybridity	Siam et al. (2009)
9	*J. glandulifera, J. gossypiifolia, J. integerrima, J. podagrica, J. tanjorensis, J. ramnadensis, J. villosa*	21 SSR primers	99.31	Vijayanand et al. (2009)
10	*J. curcas, J. glandulifera, J. gossypiifolia, J. integerrima, J. multifida, J. podagrica, J. tanjorensis*	31 SSR markers	38	Sudheer Pamidimarri et al. (2011)
11	*J. curcas, J. gossypiifolia, J. integerrima, J. podagrica*	27 ISSR markers	–	Tanya et al. (2011)
12	*J. curcas, J. glandulifera, J. gossypiifolia, J. integerrima, J. multifida, J. podagrica*	21 EST-derived SSR markers	–	Yadav et al. (2010)
13	*J. curcas, J. gossypiifolia, J. integerrima, J. multifida, J. podagrica*	10 AFLP markers	–	Suwannoi et al. (2011)

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A wide range of molecular markers have been used which showed a high degree of polymorphism correlating with the extensive phenotypic diversity existing among the species. Basha and Sujatha (2009) characterized eight agronomically important *Jatropha* species native to India along with a natural hybrid. In this study, high genetic variability (98.6% polymorphism) was reported with RAPD, SSR and ISSR markers, which corroborated with the morphological differentiation of the *Jatropha* species used in the study. The species were characterized with nr DNA ITS region sequences but the size variation obtained among seven species of *Jatropha* was narrow and ranged from 647 to 657 bp (Sudheer Pamidimarri et al. 2009a). Senthil Kumar et al. (2009b) characterized nine *Jatropha* species including *J. curcas* using nine ISSR primers. Also, the cross-species amplification of EST-SSRs derived from *J. curcas* was tested among species and *J. curcas* had maximum similarity with *J. integerrima* followed by *J. glandulifera, J. gossypiifolia, J. multifida* and *J. podagrica* (Yadav et al. 2010). Characterization of *Jatropha* species using chloroplast specific microsatellite primers revealed maximum variability in the intergenic regions of ORF77-ORF82 and *rp12-rps19* regions which could be used for distinguishing *Jatropha* species and identification of haplotypes (Basha and Sujatha 2009). Suwannoi et al. (2011) studied genetic relationships among five Jatropha species along with castor using ten AFLP primers also characterized interspecific hybrids of *J. curcas* with *J. integerrima, J. podagrica* and *J. multifida*. Percentage of methylation in the hybrid derived from *J. curcas* × *J. integerrima* was lower than that of the parents and was more similar to *J. curcas* than *J. integerrima.* Meiotic analysis of the hybrids from *J. curcas* × *J. integerrima, J. curcas* × *J. podagrica* and *J. curcas* × *J. multifida* showed normal cells with occasional abnormal tetrads in some cells of hybrids.

The major limitation with these studies lies in the use of few markers (Senthil Kumar et al. 2009a) or variations at single locus (Sudheer Pamidimarri et al. 2009a) which are insufficient to draw meaningful conclusions about the genetic relationships. Thus, molecular studies using RAPD, AFLP and nr DNA ITS sequence polymorphism failed to support that *J. tanjorensis* is a natural hybrid between *J. curcas* × *J. gossypiifolia* (Sudheer Pamidimarri et al. 2009a, b; Vijayanand et al. 2009). Studies of Ganesh Ram et al. (2008) on characterization of six *Jatropha* species found in India showed clustering of all species together with the exception of *J. glandulifera* indicating its genetic distinctness from other species. However, the study with few data points (18 primers) resulted in several ambiguities in the establishment of genetic relationships among *Jatropha* species. *J. tanjorensis* is a spontaneous hybrid between *J. curcas* and *J. gossypiifolia* (Prabakaran and Sujatha 1999) but it failed to show its genetic closeness with either of its parental species. *J. gossypiifolia* of section jatropha grouped together with *J. integerrima* of section polymorphae. The study included *J. villosa* var *Ramnadensis* and *J. villosa* var *villosa* and *J. villosa* (Ramamurthy 1967). The cv. Ramnadensis clustered with *J. gossypiifolia*, while the cv. villosa formed a separate group with *J. tanjorensis*.

Consequently, the relationships among species varied with the study and the molecular markers employed, and as such there is no consensus on the genetic relationships between species. While most of the species are of the Central American

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origin, studies were restricted to species found in India and, thus, there is a need for characterization of *Jatropha* species found in the center of origin. Careful understanding of the phylogeny and use of adequate number of molecular markers are essential prerequisites for drawing valid inferences about the genetic affinities between species. Molecular analysis related to taxonomic classification should take into account the pioneering work of Dehgan (1984) and consider the phylogenetic relationships, evolutionary trends, crossability relationships, employ optimal number of marker loci depending on the polymorphism and their coverage of the whole genome in obtaining reliable estimates of genetic relationships among different species.

Conclusions

Tree shrub breeding is a time-consuming process. Nevertheless, most of the *Jatropha* species are photoperiod insensitive and bear flowers continuously, and hence selection and generation advancement can be accomplished without much time lapse. The other advantage is the high propensity of *J. curcas* and interspecific hybrids involving *J. curcas* for propagation both through seeds and vegetative cuttings, which helps in rapid multiplication and acceleration of the breeding programmes. Determination of genetic relationships among species is critical for the management of genetic resources and success of interspecific hybridization. There is an immediate need for characterization of *Jatropha* species endemic/native to the Central American region using molecular markers to support the taxonomic classification and facilitate interspecific gene transfer besides supporting genetic mapping studies. Keeping in view the primitiveness of *J. curcas,* its cross compatibility with several members of the genus and overlapping distribution of the wild species, there is a possibility of recovering several morphotypes in the tropical American region. *Jatropha* species are amenable to tissue culture manipulations, which indicate scope for widening the genetic base through parasexual hybridization and biotechnological tools. Interspecific crosses that are limited by post-fertilization barriers and viable hybrids could be recovered through adoption of embryo rescue techniques.

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Chapter 22
Genetic Affinities of *Jatropha* with Other Euphorbiaceous Taxa

Mulpuri Sujatha, Tummala Papi Reddy, V. Sathaiah, and Bir Bahadur

Introduction

The genus *Jatropha* belongs to the family Euphorbiaceae and members of the family have world wide distribution. The family includes 235 genera with about 6,500–7,000 species including monoecious herbs, perennial shrubs and trees (Govaerts et al. 2000). The economically important plants of the family include castor (*Ricinus communis* L.) – a source of the highly valued castor oil and the deadly poison ricin; rubber (*Hevea brasiliensis* Mull.-Arg) – source of natural rubber; cassava (*Manihot esculenta* Crantz) – a major staple food crop with its roots being utilized for bio-ethanol production; and *Euphorbia* species – encompassing ornamental plants. Furthermore, the family includes several other oil, timber, medicinal, dye and ornamental plants. The chromosome numbers (2n) of the family vary from 20 to 196 having 20, 22, 38 and 72 for castor, *Jatropha*, *Hevea* and *M. esculenta*, respectively (Table 22.1). The other genera with $x = 11$, as that of *Jatropha,* include *Trewia, Aleurites, Gelonium, Euphorbia* species, *Hura crepitans* and *Joanesia*. The genome size of most of the Euphorbiaceae members is small ($1\ C < 2.0$ pg). Members of the Euphorbiaceae are primarily used as ornamentals with the exception of *Hevea*, cassava, *Euphorbia*, *Jatropha* and *Ricinus* which are exploited for diverse industrial uses.

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Table 22.1 Chromosome numbers and DNA C-values of *Jatropha* and other Euphorbiaceae members

Genus	Chromosome number (2n)	Ploidy level	C-values – 1 C (pg)
Jatropha	22	2	0.43
Mercurialis	16, 32, 56, 64	2, 4, 6, 8	0.66–2.35 with mean of 1.43
Putranjiva	40	–	0.85
Emblica	98	–	1.48
Bischofia	196	–	1.85
Trewia	22	2	1.95
Gelonium	22	2	1.18
Euphorbia	20, 22, 28	2, 4	0.35–14.35 with mean of 7.19
Hevea	38	2	2.11
Manihot	72	2	0.83
Aleurites	22	2	2.40
Hura	44	4	5.03
Bridelia	26	2	1.25
Joanesia	22	2	0.80
Phyllanthus	26	2	1.00
Cicca	26	2	0.96
Ricinus	20	2	0.52

Source: http://data.kew.org/Cvalues

Wide Hybridization

Attempts were made by researchers to cross *Jatropha* species with castor owing to the morphological resemblances existing between the two genera. Repeated crosses made by researchers to hybridize *Jatropha* with castor proved unsuccessful (Sathaiah and Reddy 1985; Reddy et al. 1987; Sujatha 1996). The recent interest in exploiting castor and *Jatropha* as biofuel crops has evoked considerable interest in intergeneric hybridization to facilitate desirable gene transfer across the genera.

Characterization of castor genotypes and five *Jatropha* species for flowering behaviour, fatty acids of seed oils, reaction to *Fusarial* wilt and castor semilooper, and leaf-protein profiles revealed substantial differences between the two genera as well as within the *Jatropha* species (Sujatha 1996). The *Jatropha* species are of perennating habit and flower throughout the year although seed set is confined to the post-rainy season unlike castor where the seed yield drastically declines after the production of the seventh and eighth order inflorescences. In castor, the principal fatty acid is ricinoleic acid while in *Jatropha* it is linoleic acid (Table 22.2). The major concern with regard to the use of these two non-edible oilseed crops is the presence of toxic proteins in the seed meal which creates health problems among growers, processors and consumers. *J. curcas* contains toxic substances, such as, curcin (lectin), phorbol esters, saponins, protease inhibitors and phytates due to which the seeds, press cake and the oil cannot be used for human or animal nutrition. Ricin, a two-chained polypeptide and *Ricinus communis* agglutinin (RCA), a four-chained polypeptide are the two major toxins present in castor seeds. Screening

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Table 22.2 Fatty acid composition (%) of seed oils of *J. curcas* and castor

Fatty acids	*J. curcas*	Castor
Myristic acid	0–0.1	–
Palmitic acid	9–22	1
Stearic acid	5–8	1
Arachidic acid	0–2	–
Behemic acid	0–2	–
Palmitoleic acid	0–1	–
Oleic acid	35–51	3
Linoleic acid	27–42	4.2
Ricinoleic acid	–	89.5
Dihydroxystearic acid	–	0.7
Linolenic acid	–	0.3
Eicosanoic acid	–	0.3

of castor and *Jatropha* species against *Fusarial* wilt under artificial inoculation conditions showed complete resistance of *Jatropha* to the wilt pathogen. However, screening of *Jatropha* species against castor semilooper showed varied levels of resistance to the pest, while maximum resistance was disclosed by *J. integerrima* and *J. integerrima* var. Rosea (Lakshminarayana and Sujatha 2001). Thus, the genus *Jatropha* represents a potentially valuable source of germplasm for *Ricinus* which belongs to a monotypic genus, and as such *Jatropha* species possess very interesting characteristics, such as drought tolerance, photoperiod insensitivity, resistance to *Fusarial* wilt and castor semilooper besides desirable oil quality.

Crossability Relationships Between Jatropha and Castor

Scanning electron micrographs showed that the pollen grains of castor are triangular in shape, tricolpate with smooth exine. The size of pollen grains varied between 17 and 25 μm. The pollen grains in the genus Jatropha are globose, binucleate, inaperturate with the exine possessing massive hexagonally arranged processes (Fig. 22.1). The diameter of pollen grains of different species ranged from 30 to 50 μm. In *Jatropha*, the maximum pollen diameter was found in *J. multifida*, while it was least in *J. integerrima*. Wide differences were observed in pistil characteristics of *Ricinus* and *Jatropha* species (Sujatha 1996). The pistil lengths of *Ricinus* varied between 7.5 and 10 mm, while it ranged from 2.0 to 5.6 mm in *Jatropha*. Among the *Jatropha* species, the maximum pistil length was recorded in *J. integerrima*, while it was least in *J. gossypiifolia* and *J. multifida*. Also, the pistils revealed discernible differences in the style to stigma ratios which varied between 0.1 and 0.66.

The pollen-pistil relations between *R. communis* and *Jatropha* species were investigated by Reddy et al. (1987), Reddy (1988) and Sujatha (1996). Reddy et al. (1987) attempted intergeneric hybridization of castor with six *Jatropha* species. In

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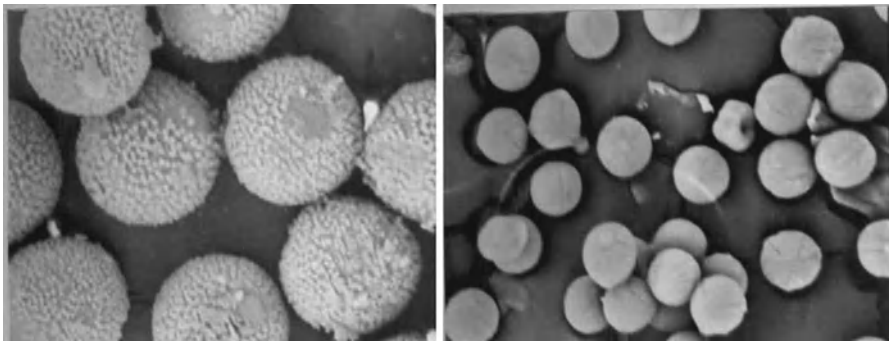

Fig. 22.1 Scanning electron micrographs of the pollen grains of *Jatropha* and *Ricinus* (Source: Sujatha 1996)

all these crosses, the *Jatropha* species showed absolute cross-incompatibility with the pollen of castor as well as in the reciprocal crosses as judged by the pollen germination failure and arrested pollen tube growth with most of the pollen tubes showing abnormalities being either coiled or with spathulate callose tips. Also, a strong barrier to crossability was observed in the cross between *Ricinus* and *J. curcas*.

Sujatha (1996) attempted comprehensive intergeneric hybridizations between castor and six species of *Jatropha* and met with limited success. Most of the crosses failed due to incompatible pollen-pistil recognition mechanism which often resulted in the failure of pollen-tube growth and formation of callose plugs (Fig. 22.2). Attempts at overcoming the pre-fertilization barriers using hormonal treatments and stigma excision proved unsuccessful. Interestingly, one of the cross combination, *viz.,* castor x *J. integerrima*, showed some seed set. *In ovulo* embryo culture resulted in the recovery of intergeneric hybrid but it failed to grow to maturity. The seed protein profiles and reproductive characteristics of *J. integerrima* differed from that of other *Jatropha* species but showed relatedness with that of castor (Sujatha 1996). Detailed investigations on the phylogenetic relationships of *Jatropha* and *Ricinus* with particular reference to *J. integerrima*-probably a missing or connecting link between the two genera-might provide valuable insights into the possibilities of transfer of beneficial genes across these two genera.

Genetic Affinities

Biochemical Parameters

The seed protein electrophoresis is an important approach for estimating the species relationships and tracing the evolution of various groups of plants as genetically related species exhibit more bands in common in their profile than

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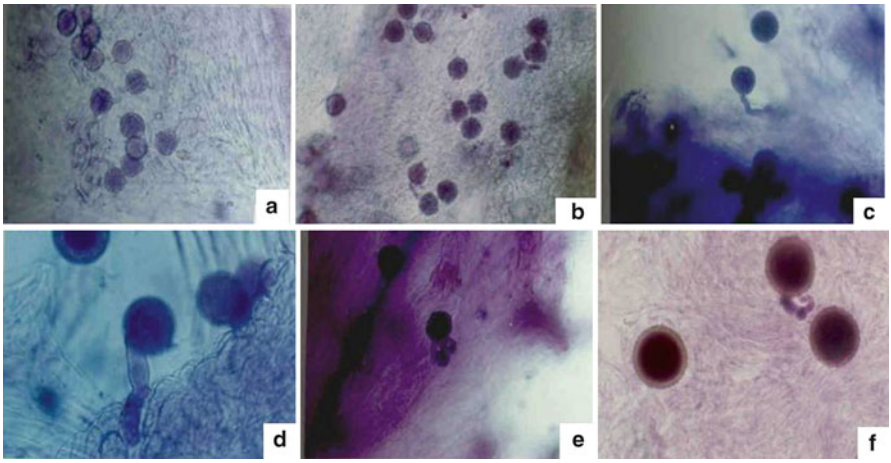

Fig. 22.2 Pollen-stigma interactions in intergeneric crosses. (**a**) Castor × castor showing 100% germination 30 min after pollination; (**b**) *J. podagrica* × castor, 24 HAP; (**c**) castor × *J. integerrima*, 24 HAP; (**d**) castor × *J. integerrima*, showing tube penetration 6 HAP; (**e**) castor × *J. podagrica*, showing coiling of pollen tubes; (**f**) castor × *J. multifida*, showing coiling of pollen tubes; (*HAP* hours after pollination) (Source: Sujatha 1996)

do remote species. The seed and leaf protein profiles of *Jatropha* and *Ricinus* were established for assaying affinities between these genera (Sathaiah 1984; Sathaiah and Reddy 1985; Sujatha 1996).

Sathaiah and Reddy (1985) reported that the castor seed protein profile had a total of 17 distinct bands. The R_f values of these electrophoretic bands ranged from 0.07 to 0.80, indicating that protein bands are present at all the regions of mobility. The seed protein profiles of four species of *Jatropha* showed a total of 14 protein bands in *J. curcas*, 13 in *J. heynei* (*J. heterophylla)*, 15 in *J. gossypiifolia* and 13 in *J. panduraefolia* and each of the *Jatropha* species exhibited a distinctive protein profile. The R_f values of these electrophoretic bands ranged from 0.04 to 0.80, indicating that bands are present at all the regions of mobility as in the case of castor. A medium mobility band at R_f 0.40, and a fast migrating anodal band at R_f 0.65 were found in all the species.

The seed protein profiles of castor and four *Jatropha* species indicated that 11 bands of *Jatropha* had their homologues in castor. Out of these, *J. gossypiifolia* had – 9, *J. heterophylla* – 1, *J. curcas* – 6 and *J. panduraefolia* – 5 homologous bands. Similarity indices between castor and *Jatropha* revealed that *J. gossypiifolia* has maximum affinity (40%) with castor. On the other hand, least affinity (20%) was observed between *J. panduraefolia* and castor (Table 22.3). The remaining species, *viz., J. curcas* and *J. heterophylla*, occupied intermediate positions in this respect. However, based on protein profiles, none of the *Jatropha* species studied could be considered as the wild progenitor of *Ricinus,* and these two genera might have evolved from a common stock now extinct.

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Table 22.3 Similarity indices (%) between the seed protein profiles of *Ricinus* and *Jatropha*

		Jatropha		
	Ricinus	*J. curcas*	*J. heynei*	*J. gossypiifolia*
J. curcas	24.0			
J. heynei	30.4	28.6		
J. gossypiifolia	40.0	45.0	47.4	
J. panduraefolia	20.0	35.0	33.3	27.3

Source: Sathaiah and Reddy (1985)

The leaf proteins of castor and five *Jatropha* species were subjected to electrophoretic analysis using total proteins, SDS-PAGE and isozymes of esterase (Sujatha 1996). The similarity indices based on leaf protein profiles of castor and different *Jatropha* species varied between 14.8% and 24.0%. Castor recorded maximum (24%) affinity with *J. integerrima* while it was least with *J. podagrica*. The other species, *viz., J. curcas, J. gossypiifolia, J. multifida* and *J. integerrima* var Rosea occupied intermediate positions further substantiating that these two genera are genetically distinct.

Genetic Affinities Using Molecular Markers

Molecular markers reveal more quickly and accurately the genetic differences far exceeding those obtained with morphological or biochemical markers as these are neutral, scan the differences in the entire genome and are less influenced by environment. Molecular profiles of castor generated with RAPD and ISSR primers were compared with those of the *Jatropha* species native to India (Fig. 22.3; Sujatha, unpublished). Molecular polymorphism showed unique fingerprints for castor and wide variations among the *Jatropha* species corroborating with the morphological diversity. Dendrogram analysis clearly separated castor from the *Jatropha* species and the overall similarity between the two genera was only about 10% (Fig. 22.4).

Cross-Taxa Transferability

EST databases have proved to be a valuable source of polymorphic microsatellites/SSRs in a number of plant species and owing to their evolutionarily conserved nature are known to show a high degree of transferability (Varshney et al. 2005). Microsatellite markers are available for a few Euphorbiaceae members and are being developed for castor (Chan et al. 2010; Lespinasse et al. 2000; Okogbenin et al. 2006). As the developmental costs for microsatellite markers are high, cross-taxa utility of molecular markers from *Hevea* and cassava were assessed in castor and *Jatropha* (Raji et al. 2009; Feng et al. 2009; Yadav et al. 2010).

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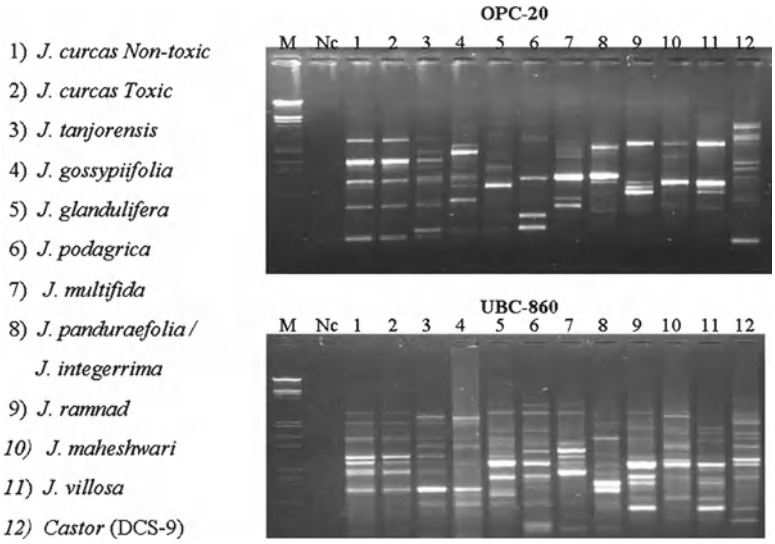

Fig. 22.3 Amplification of *Jatropha* species and castor using RAPD and ISSR primers (Source: Sujatha unpublished)

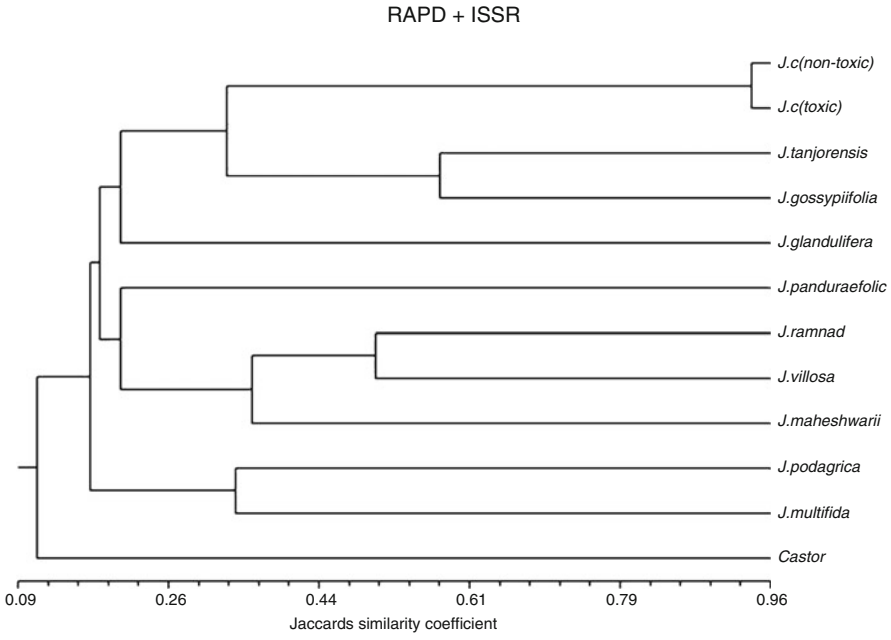

Fig. 22.4 Dendrogram based on RAPD+ISSR markers representing relationships of *Jatropha* species with castor (Source: Sujatha unpublished)

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Recent studies showed that the transfer efficiency of EST-SSRs was higher than that of genomic SSRs in cross-species/genera (Aggarwal et al. 2007). Wen et al. (2010) studied the transferability of 419 expressed sequence tag (EST)-SSRs and 182 genomic (G)-SSR primers that had been developed for cassava. The cassava-*Jatropha* cross-genera transferability values of 44.6% with EST-SSRs and 29.7% with genomic-SSRs were estimated to be high. The higher levels of transferability of EST-SSRs than that of G-SSRs reflect the conserved nature of coding sequences as compared with non-coding genomic DNA, and the fact that the mutation frequency of EST sequences is lower than that of genomic DNA sequences. Feng et al. (2009) reported a transfer rate of rubber EST-SSR markers to an extent of 93.3% in cassava, 90% in castor and 80% in amla. Based on these observations, rubber is considered to be relatively closer to cassava than castor or amla. Raji et al. (2009) reported a transferability rate of cassava EST-SSR markers to an extent of 15.3% in castor. EST-SSRs developed from *Jatropha* had a transferability rate of 47.0% in castor (Yadav et al. 2010), thus indicating their potential use in castor. Yu et al. (2011) demonstrated the transferability of SSR markers of rubber to *Ricinus, Manihot* and *Phyllanthus*.

Molecular phylogenetic analysis of Euphorbiaceae comprising 85 species of 83 genera, using plastid and nuclear DNA sequences (*rbcL, atpB, matK*, and 18 S rDNA) along with ovule and seed characters, showed the closeness of *Jatropha* to *Manihot, Croton* and *Hevea,* subfamily *Crotonoideae* rather than to *Ricinus* of the family *Acalyphoideae* (Tokuoka 2007). Moreover, the genus *Ricinus* was found close to genera *Chrozophora*, *Speranskia* and *Adriana* (Tokuoka 2007).

Zeng et al. (2009) produced the first collection of microRNAs (~21 nt non-coding RNAs) and their targets in Euphorbiaceae. About 85 conserved miRNAs in 23 families of castor were predicted, experimentally verified and characterized in cassava, castor, rubber tree and *Jatropha* during the normal seedling development both under cold and drought stresses. Of these, 58 miRNAs (68.2%) were found in other Euphorbiaceae taxa and the results revealed wide conservation of several miRNAs and their diverse functions in euphorbiaceous plants during seedling growth in response to abiotic stresses. About 18 miRNAs that responded to drought and cold stresses were identified. Euphorbiaceous plants are evolutionarily diversified and are characterized with high photosynthesis and high biomass. Studies on stress-related miRNAs offer excellent opportunities for understanding the adaptation mechanism of the euphorbiaceous plants to harsh and dynamic environments. These results demonstrate the potential value of various types of molecular markers for the development of genetic maps, assessment of genetic diversity, determination of phylogenetic relationships and for marker-assisted selection (MAS) breeding in the economically important Euphorbiaceae taxa.

Genomics

The Synthetic Genomics Inc. (SGI) and Asiatic Centre for Genome Technology (ACGT) completed *Jatropha* genome project with 100X coverage. Sequencing of the genome – using both the traditional Sanger sequencing and the next generation

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sequencing – has revealed that the *Jatropha* genome is approximately 400 million base pairs in size and is similar to that of the rice genome. Annotation of genomes to identify genes of interest helps in discovering the genetic variation using marker-assisted breeding, and thereby provide information on factors controlling oil synthesis, maximizing yield, biotic and abiotic stress tolerance and low-curcin variants. (http://greenbio.checkbiotech.org/news/.rst_jatropha_genome_completed_synthet-icgenomics_inc_and_asiati 511 c_ centre_genome_tech). The whole genome of *J. curcas* was also sequenced, using a combination of the conventional Sanger method and new-generation multiplex sequencing methods (Sato et al. 2011). Total length of the non-redundant sequences obtained was 285 858 490 bp consisting of 120 586 contigs and 29 831 singlets which accounted for ~95% of the gene-containing regions with an average G+C content of 34.3%. Comparison with genes of other plant species indicated a high degree of microsynteny with the genome of castor bean. In *Jatropha* 26,456 ESTs, 945 nucleotide sequences, 548 protein sequences and 148 predicted gene sequences are available in the NCBI database. Transcriptomic studies of Costa et al. (2010) in *Jatropha* generated 13,249 ESTs from the cDNA libraries of developing (7,320) and germinating (5,929) seeds. The length of ESTs, after trimming, ranged from 100 to 848 bp with an average size of 561.5 bp. They identified ESTs coding for enzymes involved in the synthesis and degradation of fatty acids and tri-acyl glycerols which influence oil quality. Transcripts coding for curcin, 2 S albumins and enzymes involved in the synthesis of terpenoids, which are associated with the phorbol ester biosynthesis pathways, were also identified.

The castor genome has recently been sequenced to 4.6X coverage using the whole genome shotgun strategy (Chan et al. 2010). A total of 83,464 nucleotide sequences, 63,398 ESTs, 63,073 protein sequences and 31,903 predicted gene sequences have been deposited in the public database. Also, genes involved in polyploidization, ricin family, oil metabolism and disease resistance were assembled and annotated. Lu et al. (2007) analyzed the ESTs derived from a full-length cDNA library of the castor developing endosperm, and identified 4,720 ESTs representing 1,908 unique sequences. The ESTs are enriched in genes encoding storage proteins, ricin, oleosins, as well as other housekeeping cellular components such as those for protein synthesis.

The other related taxa of the Euphorbiaceae are being sequenced to various levels and a lot of information is available in the public data base. In cassava, information on 2,907 nucleotide sequences, 83,028 EST sequences, 2,452 protein sequences and 226 predicted gene sequences is available. In *Hevea*, 2,162 nucleotide sequences, 13,050 ESTs, 654 protein sequences and 6 predicted gene sequences have been identified. These sequences, plausibly serve as a valuable resource for the development of genic microsatellites and EST-SSRs.

Conclusions

Genetic improvement of *J. curcas* can be accomplished through exploitation of the genetic variability available in the primary or secondary gene pools. Intensive studies carried out during the past 5 years on the assessment of genetic diversity using

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morphological and molecular markers revealed moderate levels of variability in the cultivar germplasm of *J. curcas* despite its enormous phenotypic plasticity. Nevertheless, vast scope exists for widening the genetic base through interspecific hybridization as the genus *Jatropha* encompasses about 170 species. Being the most primitive member of the genus, *J. curcas* crosses readily with several of the *Jatropha* species. Intergeneric hybridization is particularly useful for monospecific crops like castor rather than to *Jatropha* which has a large reservoir of untapped genetic potential in the wild allies. Hopefully, the wealth of information on molecular markers and genomic resources now available in the related taxa significantly and hopefully will accelerate the identification of molecular markers and trait genes to design customized cultivars endowed with superior agronomic attributes.

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Chapter 23
Breeding and Biotechnology of *Jatropha curcas*

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Abbreviations

AFLP	Amplified fragment length polymorphism
cTBP	Combinatorial tubulin-based polymorphism
IAC	Instituto Agronômico, Campinas, São Paulo, Brasil (Agronomic Institute), São Paulo, Brazil
ISSR	Inter-simple sequence repeat
ITAL	Instituto de Tecnologia dos Alimentos (Food Technology Institute) São Paulo Brazil
PCR	Polymerase chain reaction
RAPD	Random amplified polymorphic DNA
RT-PCR	Real-time reverse transcription PCR
SPAR	Simple primer amplification reactions
SSR	Simple sequence repeat

Introduction

The search for new sources of renewable energy in recent years has been driven by growth of human population and increasing shortage of fossil fuels. In addition, the global energy crisis, higher prices and pollution by fossil fuels has resulted in efforts to improve the quality of life and environmental sustainability.

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The Brazilian energetic matrix is one of the cleanest in the world since almost half of the energy (44.1%) consumed in the country is renewable in contrast to the global energy matrix for which 86.7% is due to non-renewable fossil fuels (EPE-Empresa de Pesquisa Energética, Brasil, 2012). The search for renewable energies has led to considerable development within the country and has reduced poverty by creating jobs. Although, the Brazilian energy matrix is still based on petroleum and its derivatives, the great difference in relation to other nations is undoubtedly the country´s potential to produce and use renewable energy, with 14.7% currently being provided by hydroelectric sources and 29.5% by biomass energy (EPE-Empresa de Pesquisa Energética, Brasil, 2012). Projections for 2030 indicate even greater growth of renewable sources, with the implementation of new hydroelectric plants and a regulated input of biomass energy. Of particular importance in the latter category is the strategic role of sugarcane and its complementation with other alternative energy sources, such as oil from oleaginous species for biodiesel production (Bronzatti and Iarozinski Neto, 2008).

The Brazilian interest in pioneering the development of renewable energy reflects the country's continental dimensions, tropical climate, abundant water resources, strong agricultural and energy potential, possible agricultural expansion and tradition concerning large scale agricultural production. This alternative energy production is based on ethanol from sugarcane and biodiesel from oilseeds. Brazil is widely recognized as a global leader in production, distribution and use of bio-ethanol from sugarcane (Nass et al. 2007). The knowledge and experience acquired from sugarcane ethanol production in the 1970s, when the National Alcohol Program (Pro-Álcool) was implemented, has encouraged the Brazilian government to increasingly invest in the exploitation of vegetable oils for biodiesel production through the National Program of Biodiesel Production and Use (PNPB) (Rodrigues 2005).

There is an increasing worldwide trend towards the creation of environmental laws that stipulate the use of renewable fuel in conjunction with fossil diesel. In Brazil, commercial diesel is currently a mixture of 5% biodiesel with fossil diesel (B5). Brazilian biodiesel production increased by 50% in 2010 compared to 2009, reaching approximately 2.4 billion liters. In the future, this demand will probably increase with a proposed ban on the importation of fossil diesel and a reduction in polluting gases that will require a commercial diesel mixture containing 20% biodiesel (B20) (Thame 2006).

When the issue of biodiesel in Brazil is considered, the potential for the cultivation of commodities, such as soybean and sunflowers is usually emphasized since these crops are generally produced on a large scale by large agribusiness companies, although their low yield per hectare is similar to that of other edible crop species. To satisfy the increasing demand for biodiesel there is a need to develop new agricultural options, such as *Elaeis guineensis* (oil palm), *Acrocomia aculeate* (palm tree macauba), *Orbignya speciosa* (Mart.) (palm tree babassu), *Raphanus sativus* (radish), *Brassica napus* L. var. *Oleifera* (rapeseed), *Crambe abyssinica* (crambe) and *Jatropha curcas* (physic nut). Among these oleaginous plants, *J. curcas* is currently considered an important crop because of its potential for biodiesel production, its oil quality and, more importantly, it does not compete with crops used in food production (Rajore and Batra 2005; Li et al. 2008; Carels 2009; Basha

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and Sujatha 2009; Gomes et al. 2010). According to Marques and Ferrari (2008), the yield per hectare is ~2,000 l and can be increased significantly with selective breeding. *J. curcas* seeds contain 25–40% oil with a predominance of oleic fatty acid in triglycerides, a factor that increases the value of these seeds because of the great oxidative stability conferred to biodiesel.

In addition to the productivity factor, other characteristics make *J. curcas* a promising alternative for exploitation in intensive and extensive agricultural activities, including tolerance to drought, wide adaptation to different soil and climatic conditions, ease of propagation, rapid growth and low cost of production; the latter being an important factor for its economic viability, especially on family farms.

On the other hand, some characteristics of *J. curcas* are completely inadequate for supplying biodiesel on the scale required by the Brazilian market; these characteristics include its biotype (excessive plant height, extensive branching on the stem that requires regular pruning), time required to reach stable productivity after planting (3–4 years), non-uniform fruit maturation and continuous flowering during the summer and autumn of tropical countries in the southern hemisphere. In addition, although *J. curcas* is considered a rustic and resistant plant, several studies have shown a high susceptibility of this species to various diseases and pests.

Thus, the successful use of *J. curcas* in biodiesel production requires appraisal of these problems and optimization of the species potential. This can be achieved by investing in appropriate technologies and especially in selective breeding in order to develop cultivars with exceptional agronomic, phytosanitary and phytochemical characteristics that this species does not currently have.

Reproduction Biology and Phenology

A sound knowledge of the (1) reproduction model, (2) flower biology and morphology, (3) characteristics of natural and artificial pollination and (4) mechanisms of fertilization is critical for developing a successful program of selective breeding. It is also essential to understand the physiological conditions (temperature, photoperiod and water) required to obtain the complete phenology (seed-to-seed cycle) of the target-species.

The reproductive system of *J. curcas is* monoecious, with unisexual flowers in the same inflorescence (Divakara et al. 2010). Despite the emergence of new inflorescences in the terminal buds of branches grown in the same year, the flowering is discontinuous, with fruits of different ages and levels of fruit dehiscence being observed in the same inflorescence. In addition, flowering occurs throughout the summer and autumn. Consequently, fruit production is not concentrated in a specific period of the year; this characteristic can increase the cost of manual harvesting and prevent mechanical harvesting.

J. curcas is deciduous, losing its leaves in the autumn and winter or in situations of abiotic stress. In nature, this behaviour may be advantageous to the plant by helping it

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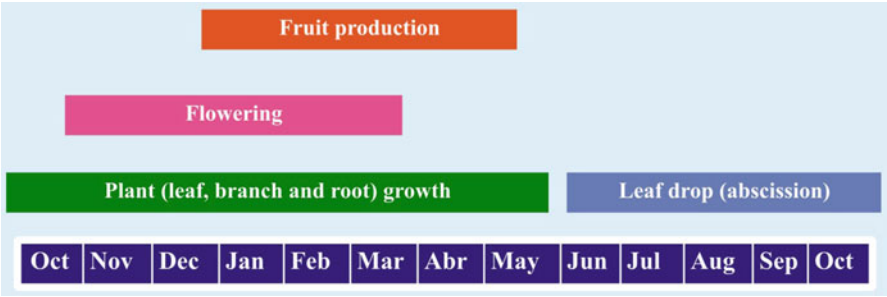

Fig. 23.1 Annual phenological cycle of *J. curcas* in São Paulo state, Brazil (Adapted from http://www.iica.org.uy/data/redpa_documentos/248642.pdf, accessed in May 2009)

to restrain pathogens and pests aggressions. However, periodic physiological rest leading to cyclic leaf drop would prohibit continuous fruit production throughout the year under a scheme of intensive agriculture.

Although *J. curcas* is self-compatible allowing self-fertilization and fruit development by geitonogamy, fruit production occurs predominantly via xenogamy since the opening of female and male flowers in the same inflorescence is not synchronized. Some studies suggest that the species shows protandry, with male flowers opening before female flowers (Raju and Ezradanam 2002; Luo et al. 2007b). In Brazil, protogyny has been observed, i.e., pistillate flowers open before staminate flowers (Saturnino et al. 2005). In both instances, this pattern of asynchronous opening of male and female flowers favours cross-fertilization or xenogamy (Heller 1996; Luo et al. 2007a). Pollination is entomophilous and the pollinators are mainly ants, bees and flies (Raju and Ezradanam 2002). Fruits are formed in clusters of about 10 green fruits, 2–3 cm in length and ovoid in form, each fruit usually has three carpels with one seed per carpel.

Bhattacharya et al. (2005) and Pranesh et al. (2010) reported apomixis in 32.0% and 28.5% of *J. curcas*, respectively. Under experimental conditions in Brazil (Janaúba, Minas Gerais), a much lower rate of apomixis (5%) was observed by Juhász et al. (2009). In contrast, preliminary results under our experimental conditions in Campinas (São Paulo, Brazil) revealed no apomixis in our germplasm available at *Instituto Agronômico de Campinas* (IAC), São Paulo, Brazil (Rufino et al. 2010).

The production of *J. curcas* seeds begins around the tenth month after planting, but only reaches full production in the third or fourth year (Arruda et al. 2004). Phenology is almost annual (Fig. 23.1). Flowering is one of the main phenological stages in the production of *J. curcas* oil since the number of female flowers and their fertilization determine the number of fruits and seeds that will develop. Flowering normally starts after a dormant period. In Brazil, this occurs after the winter when temperature and precipitation are low. After induction, flowering continues for prolonged periods, depending on the availability of water in the soil. According to Jongschaap et al. (2007), limited nutrient availability can interrupt flowering.

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The Genus *Jatropha*

The genus *Jatropha* includes approximately 175 species that vary considerably in pest and disease tolerance, oil fatty acid content, flowering and fruiting pattern. A few varieties of *J. curcas* have been described in different countries: (1) the Cape Verde variety that, according to some authors, has a worldwide distribution; (2) the Nicaragua variety that produces only a few, large fruits and (3) the non-toxic Mexican variety that is devoid of phorbol esters and occurs in natural populations of Mexico (Ferrari et al. 2009). According to Popluechai et al. (2009), some artificial interspecific hybrids obtained from crosses between *J. curcas* and *J. integerrima* also do not contain significant levels of phorbol esters and are therefore considered non-toxic. *J. platyphylla* Müll. Arg., another species of *Jatropha* found in Mexico, also has negligible toxicity level in seeds and oil (Makkar et al. 2011), which allow its use for human consumption.

The phorbol esters found in *J. curcas* seeds are potent cancer promoters (Goel et al. 2007). Non-toxic *J. curcas* varieties can be economically more interesting to the small producer, who can use the press cake resulting from oil extraction for animal feed, whereas toxic varieties would be most practical for the large producer, who can return this residue to the field in the form of bio-fertilizers, depending on the geo-economic reality. Recycling press cake from both toxic and non-toxic varieties increases the value of growing *J. curcas*, although the use of press cake as animal feed would probably provide the greatest aggregate value. Other *Jatropha* species of interest include *J. glandulifera*, *J. gossypiifolia*, *J. integerrima*, *J. multifida*, *J. podagrica* and *J. tanjorensis.* All of these species are widely distributed in India, whereas *J. curcas*, *J. integerrima* and *J. glandulifera* are native to the Americas (Sunita et al. 2005). *J. podagrica* is a shrub commonly found in Africa, Asia and Latin America (Olapeju et al. 2007) and *J. tanjorensis* is native to India, where it is found in only a few districts of Tamil Nadu and is reported to be a natural interspecific hybrid of *J. curcas* and *J. gossypiifolia* (Prabakaran and Sujatha 1999). *Jatropha multifida* and *J. platyphylla* occur naturally in Mexico; *J. multifida* is a popular landscape plant in Southern Florida (Yotam et al. 2000) while *J. integerrima*, *J. multifida* and *J. podagrica* are important ornamental plants.

Germplasm Collection, Characterization and Availability

The establishment of *J. curcas* as a commercially viable crop in Brazil requires the development of a suitable genetic breeding program. The success of this breeding program depends on a germplasm bank that is representative of the genetic variability in *J. curcas* (nuclear collection). This in turn requires the use of accessions from many different backgrounds in terms of soil and climatic conditions, topography, endemic occurrence of pests and diseases, etc.

The expansion of this germplasm bank by adding new accessions from various parts of the world (especially from the center of origin and species diversity

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for this genus), together with adequate conservation, evaluation and exploration of the germplasm bank based on careful morphological, chemical and molecular characterization, will provide a strong basis for developing elite varieties capable of sustained high crop production in different agro-climatic regions. However, the center of origin and diversity of *J. curcas* has not been well defined (Henning 2009). Carels (2009) stated that the oldest fossil remains of the genus *Jatropha* were found by Berry (1929) in geological formations in Peru. On the other hand, recent studies using molecular markers such as RAPD (Ram et al. 2007; Gupta et al. 2008; Pamidiamarri et al. 2009; Ikbal Boora and Dhillon 2010), ISSR (Basha and Sujatha 2007; Kumar and Sharma 2008), AFLP (Tatikonda et al. 2009), SSR (Sun et al. 2008) and SPAR (Ranade et al. 2008) have revealed low genetic diversity among accessions from most of the countries evaluated. Since the highest genetic diversity has been found in Mexico and other Central and South American countries, one expects these regions to be the center of origin of *J. curcas*.

In Brazil, several studies investigated the genetic variability among accessions. Grativol et al. (2010) used ISSR markers to assess the genetic variability of 332 accessions of *J. curcas* from eight states in Brazil and found that the accessions were closely related, but had greater genetic diversity than accessions from other countries. Accessions from Natal in the Brazilian state of Rio Grande do Norte were the most diverse, which suggested that they could be a useful source of genetic diversity for breeding programs of *J. curcas* worldwide.

The genetic diversity of accessions in the IAC germplasm bank has been analyzed using molecular markers such as RAPD, ISSR and SSR, as well as the novel, relatively unexploited technique of *combinatorial tubulin-based polymorphism* (cTBP; Breviario et al. 2007). The cTBP method uses size variations of the first and second introns of plant β-tubulin genes to assess genetic variability (Popluechai et al. 2009). The results obtained using these different markers have revealed moderate genetic diversity within and among Brazilian accessions of different origins (Carqueijo et al. 2010).

However, morphological and chemical evaluations of these accessions have revealed genetic variability in plant characteristics, such as plant size, number of fruits per bunch, uniformity of fruit maturation, total oil content, fatty acid composition and phorbol ester content. Other authors have reported large variations in oil content (27.8–39.0%) and seed weight (44–79 g) in Indian accessions from different areas (Wani et al. 2006; Kaushik et al. 2007; Rao et al. 2008).

Despite the extensive variations in agro-morphological and chemical properties reported in different studies, we have observed limited variability among the *J. curcas* genotypes evaluated so far, particularly with respect to the genes involved in the mechanisms of tolerance to biotic and abiotic stress. At present, we are searching for sources of genetic resistance to major diseases, such as (1) rust caused by the fungi *Phakopsora jatrophicola* and *P. arthuriana*; (2) powdery mildew caused by the fungus *Oidium hevea*; (3) anthracnose or leaf spot caused by the fungi *Colletotrichum gloeosporioides* and *C. capsici*; (4) gummosis

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or soft rot of the stem base caused by the fungus *Phytophthora* spp. and (5) pests such as the white mite (*Polyphagotarsonemus latus*), red mite (*Tetranichus* spp.), bug (*Pachycoris torridus*) and leafhoppers (*Empoasca* spp.). The identification of these and other genes of interest in related species for introgression in *J. curcas* through interspecific hybridization is a promising strategy that we are currently exploring at IAC.

Although most interspecific hybrids show pre- and/or post-zygotic genetic incompatibility, some of the parent combinations that we have tested have shown compatibility, especially in crosses of *J. curcas* with *J. integerrima* (21.1% natural fruit set). Other interspecific crosses have shown strong genetic incompatibility. In such cases, the use of appropriate procedures to overcome the post-fertilization barriers has allowed us to obtain unprecedented segregating generations that represent a genetic reserve equal to or greater than that of a *J. curcas* germplasm bank. As discussed below, other biotechnological tools can also be used to expand the genetic variability and exploit the allelic variation.

Model of Ideal *J. curcas* Plant for Use in Brazil

Exploitation of *J. curcas* as a new agricultural option for large-scale biodiesel production in Brazil will depend on a radical change in the plant biotype and this may also lead to a change in crop management.

Drastic yearly pruning (50–100 cm) to limit plant size is widely used in Brazil and other countries that produce *J. curcas* seeds. Despite larger and more uniform production due to yearly renewal of the canopy, this practice is costly and labor intensive requiring the elimination of pruned branches to decrease infection rate by opportunistic pathogens.

A suitable alternative to this procedure would be to develop semi-dwarf or dwarf plants by selection from the available germplam, self-fertilization or interspecific hybrids. Such plants could greatly delay the need for pruning and provide a more uniform production. This in turn would allow the use of mechanical harvesting that would lead to a reduction in the cost of oil per hectare. The reduced production due to dwarf biotype would be compensated by a larger plant density per hectare. Depending on actual plant size, planting in double rows could also be tested. Spacing of 1.0 m × 1.0 m × 1.5 m or 1.0 m in double rows with 3–4 m between the double rows (to allow mechanized farming practices) could improve crop management and produce at least the same yield as with the conventional system, but without the need for systematic pruning.

Other economic characteristics, such as higher productivity per plant, more synchronous fruit maturation, crop concentration in one or few period(s) during the year, early maturation, resistance to biotic and abiotic stress, oil yield per plant, fatty acid composition in relation to oil quality and lack of toxicity (by reducing or eliminating phorbol esters) could be combined with dwarfism.

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Genetic Breeding

The first step in our breeding program involved the (1) establishment of a germplasm bank, (2) undertaking of a morphological and molecular characterization of the available accessions, (3) introduction of proper methods for germplasm manipulation and (4) expansion of the germplasm bank by adding several *J. curcas* accessions and other wild and congeneric species, such as *J. integerrima*, *J. podagrica, J. multifida, J. pohliana* and *J. gossypiifolia*. The IAC germplasm bank currently has about 1,200 *J. curcas* accessions from different geographic regions that include nine Brazilian states (Alagoas, Bahia, Ceará, Mato Grosso do Sul, Minas Gerais, Pará, Rio Grande do Norte, São Paulo and Tocantins) and four other countries (China, Costa Rica, India and Mexico). The other species in the germplasm bank are originated from the Brazilian states of Bahia, Ceará, São Paulo and from Costa Rica. Two general approaches developed by the IAC team have been used in manipulating this germplasm and are being applied to the *J. curcas* breeding program, i.e., (1) the exploitation of the pre-existing genetic variability in *J. curcas* and (2) the introgression of genetic variability from congeneric species of the *Jatropha* genus into *J. curcas*.

Strategy I: Exploitation of the Existing Variability in J. curcas

This strategy is based on recurrent or intra-population selection with mass selection in the S0 population (germplasm bank), and also between and within progenies of self-fertilization (S) with genealogical method.

Since selection involves a choice among several possibilities, it is necessary to maximize the potential combinations available to identity the most appropriate genotypes for a given situation. This process of potential maximization involves the largest possible variability for any characters. Thus, the exploitation of the genetic variability in *J. curcas* requires its prior investigation and characterization with morphological, chemical and molecular markers. The understanding of the genetic variation associated with plant size, branching pattern, flower sex ratio, pest and disease resistance, drought tolerance, toxicity and yield is paramount for the selective breeding of *J. curcas*. Estimation of the inter-character correlation, narrow sense heritability (additive variance) and potential genetic gain are necessary to manage selective breeding.

The next step in assessing the existing genetic variability in *J. curcas* involves separation of the environmental effect(s) to confirm the superiority of the preselected accessions. The genetic gains can be fixed through successive self-pollinations or, alternatively, by culturing microspores or ovules in order to obtain homozygous plants. Methods for cloning *ex vitro* (by cuttings) and *in vitro* (micropropagation) have received greater attention in order to allow the evaluation of experimental clones in different soil and climatic conditions. The development of appropriate protocols for the micropropagation of selected plants with the possibility

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of using bioreactors for massive somatic embryo multiplication could quickly provide genetically uniform seedlings on a commercial scale. In addition, compatibility testing between clones could be used to create a clonal garden for seed production of superior genotypes selected at any stage of the breeding program, thereby accelerating the generation of a new cultivar.

Selection of Elite Genotypes

Agro-morphological evaluations of all accessions in the IAC germplasm bank have revealed marked variability within and among accessions of different regions for the following characteristics: number of basal branches (up to 50 cm from the soil surface), percentage of basal branches with fruiting (uniformity), total number of bunches or inflorescences per plant and the number of fruits in the best developing bunch.

The oil yields of the IAC germplasm bank accessions of different origins ranged from 11.3% to 39.7% (mean: 31.5%), with the most abundant fatty acids being oleic, linoleic, palmitic and stearic acids; of these, oleic (C 18:1) and linoleic (C 18:2) acids were the most abundant (Ferrari et al. 2009). The oleic acid/linoleic acid ratio influenced the iodine index as a consequence of the degree of fatty acid unsaturation and, therefore, of the oxidative stability of oil and biodiesel obtained from this oilseed. Thus, a high oleic/linoleic ratio indicates the formation of an excellent biodiesel (Mittelbach and Remschmidt 2004).

The phorbol ester content of our samples ranged from 0.4 to 8.97 mg/g, indicating the occurrence of plants with low toxicity and high toxicity seeds (Ferrari et al. 2009). Since the mean phorbol ester content of non-toxic Mexican genotypes is 0.11 mg/g, we considered the IAC genotype with a phorbol content of 0.4% to have low toxicity.

Characterization of some genotypes in the IAC germplasm bank confirmed the extent of genetic variation in the characteristics reported above. Based on these evaluations, we selected 42 genotypes that showed the best performance in relation to agronomic and chemical traits that included small size, greater uniformity of production, higher number of fruits per bunch, higher yield and oil quality (higher content of oleic acid), lower levels of phorbol esters and genotypes that responded best to micropropagation methods (organogenesis and embryogenesis *in vitro*). All of the selected plants were cloned and transplanted to irrigated fields for further testing in order to assess the extent to which environmental factors influenced genetic effects.

Self-pollination of Elite Genotypes

Genotypes bearing the traits of interest common to different accessions of the germplasm bank were self-fertilized manually to yield a total of more than 100 progenies (S_1 generation) that were subsequently evaluated by focusing on the

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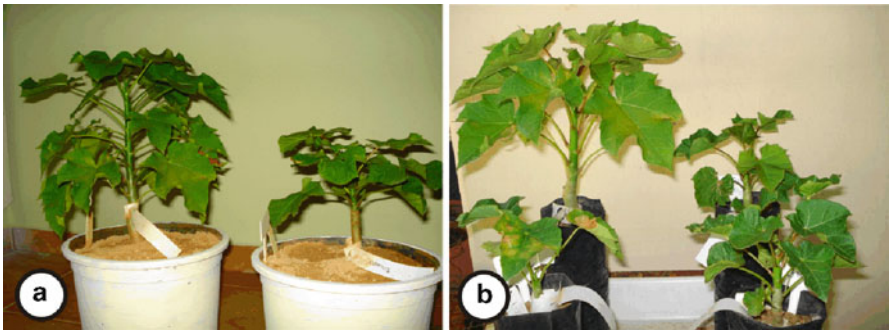

Fig. 23.2 S_1 generation plants selected from the progenies of self pollination. (**a**) Plant of normal size (*left*), dwarf plant (*right*). (**b**) Contrast between one normal (*left*) plant and three dwarf plants

demand for inbred plants. We identified a single dwarf genotype in the S_1 generation from the self-fertilization of a normal plant that segregated following the ratio of one dwarf biotype for six normal biotypes (Fig. 23.2). Apart from their very small size, the biotype of dwarf plants also showed shortening of the internodes and morphologically different leaves.

The individuals selected for the characteristics of interest in the first generation of self-pollination (S_1) were self-fertilized again to obtain the S_2 generation, and so on. Simultaneously with each generation of self-pollination (S_1, S_2…S_n), we undertook biparental crosses (intraspecific hybridization) to examine the uniformity of the phenotypic variance in populations of hybrid origin *(J. curcas* x *J. curcas)*, similar to the cryptic hybrid method used in corn. By using this approach, we explored how homozygosity during inbreeding can reveal deleterious alleles that may lead to interesting phenotypes, such as phorbol ester-free, male-sterile, pistillate or gynoic dwarf plants, etc. Heterosis can be exploited in hybrid combinations (diallels) between plants obtained after successive self-pollination. In our future breeding programs, we plan to explore the heterosis in genetic backgrounds of smaller plants (semi-dwarfs), looking for genetic vigor in characteristics related to oil production.

The probability of obtaining homozygous genotypes as well as heterotic hybrid combinations (specific combining ability) increases with successive generations of self-pollination. Inbreeding can produce small, weak plants with lower productivity. However, the dense cultivation of these plants can compensate this effect and actually result in greater productivity per unit of area. Combining this feature with other advantages, such as a greater uniformity in fruit maturation may allow the use of mechanical harvesting in large plantations.

In vitro propagation of genotypes selected in the S_0, S_1...S_n, F_1, BC_1, BC_2...BC_n generations will expedite quality seedling production from genotypes identified as superior in some stage of the genetic improvement program.

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Strategy II: Introgression of Genetic Variability into J. curcas

To accelerate the process of production of new varieties with a narrow or unknown genetic background, such as that of *J. curcas*, it is necessary to increase the genetic variability by looking for valuable traits in congeneric species. Many loci of interest, such as those involved in pest and disease resistance or related to fatty acid metabolism, are unavailable for selective breeding because of interspecific barriers. When present, post-zygotic genetic barriers can be overcome by techniques such as *in vitro* embryo rescue associated with backcrosses. Although applied to interspecific hybrids involving only a small number of *Jatropha* species, we have successfully used this strategy in our breeding program. Other biotechnological tools used to increase genetic variability, such as tissue culture and genetic engineering techniques, are being developed in view of their future incorporation to the IAC *Jatropha* genetic breeding programs.

Interspecific Hybridization

Interspecific hybridization among *Jatropha* species may be an excellent option for increasing the genetic variability of *J. curcas* and for producing varieties with desirable characteristics (greater and more uniform productivity, tolerance to biotic as well as abiotic stress) since these agronomic traits also occur in some related species. Reddy et al. (1987) first performed crosses between *Ricinus communis* and *Jatropha* species (*J. curcas, J. glandulifera, J. multifida, J. hastata, J. podagrica* and *J. gossypiifolia*). They noted inter incompatability in all interspecific combinations with differences in pollen germination showing *J. curcas* to be closer to *J. gossypiifolia* and *J. glandulifera*.

Parthiban et al. (2009) conducted crosses between *J. curcas* and the following wild species: *J. podagrica, J. gossypiifolia, J. multifida, J. integerrima, J. villosa, J. tanjorensis, J. glandulifera* and *J. maheshwarii*. They noted total incompatibility of most of these species with *J. curcas*, except for *J. integerrima,* with which it was possible to obtain some interspecific F_1 hybrids. Similar result was obtained earlier by Sujatha and Prabakaran (2003). Basha and Sujatha (2009) examined the $BC_1 \times F_1$ and $BC_2 \times F_1$ progenies of these F_1 hybrids and found significant gains in oil content, oleic and linoleic acid content as well as interesting characteristics related to seed production. In particular, *J. integerrima, J. podagrica* and *J. multifida* could contribute for reduced toxicity of the endosperm protein, improved plant architecture, better oil quality and content as well as greater drought resistance (Sujatha 2006; Basha and Sujatha 2009). However, the broad crosses among these species resulted in limited success, perhaps because of the existence of pre- or post-zygotic barriers. According to Parthiban et al. (2009), an understanding of the biological nature of the cross incompatibility barriers should allow successful production of new hybrids. The use of appropriate biotechnologies should make possible to obtain such rare hybrids (discussed below).

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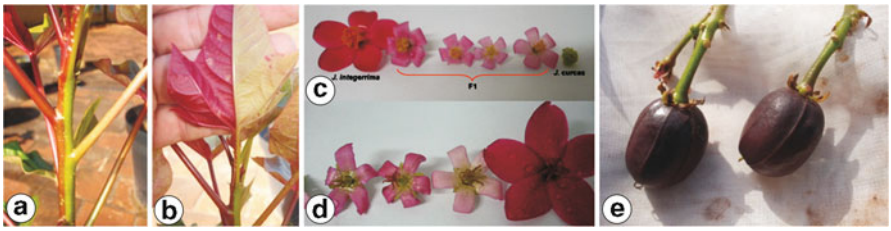

Fig. 23.3 F_1 hybrids from *J. curcas* (♀) and *J. integerrima* (♂) crosses. Segregation for levels of pigmentation (anthocyanins) in the abaxial portion of petioles (**a**) and leaves (**b**). Segregation for male (**c**) and female flowers (**d**) according to coloration and size. Segregation for fruit coloration including dark purple fruits (**e**)

We have used interspecific hybridization to generate genetic variability in *J. curcas* by the introgression of interesting loci found in five wild species: *J. integerrima*, *J. multifida*, *J. podagrica*, *J. gossypiifolia* and *J. pohliana*. The verification of the existence and the type of genetic incompatibility prevailing in each interspecific cross was done by fluorescent microscopy of pollen germination and pollen tube growth in pollinated pistils (Moreira et al. 2011a) since the absence of pollen tube development in pistil readily indicates a pre-zygotic barrier. In parental combinations where there is no pre-zygotic barrier, the *in vitro* embryo rescue technique has been successfully used to generate new genotypes.

To date, we have obtained more than 80 F_1 hybrids from *J. curcas* (♀) and *J. integerrima* (♂) crosses. Compared with other interspecific crosses that we have done, this cross showed the highest rate of fruit production (21.1% of fruit set). The rate of seed germination ranged from 14.5% to 40.0% for fruits collected in the senescent phase and from 80.7% to 83.5% for fruits collected in the intermediate stage of ripeness characterized by fruit yellowing (Rufino et al. 2011a).

Our findings indicated extensive morphological variability in this F_1 hybrid population, with plants exhibiting different sizes (heights): dwarf (± 20 cm), semi-dwarf (>20–60 cm), medium (>60 cm to 1.5 m) and tall (>1.5 m) (Rufino et al. 2011a). Petioles of the F_1 population also showed considerable genetic variability. High levels of pigmentation (anthocyanins) were found in the abaxial portion of the leaves and petioles and there was enhanced degree of pigmentation in flowers that ranged from pink to dark purple (Fig. 23.3). The size and number of female and male flowers per bunch also varied considerably. The reproductive structures of all F_1 progeny evaluated were similar to the parental male. There was segregation for fruit coloration, with the production of green, light purple and dark purple fruits (Fig. 23.3). About 3% of the chimeric plants had shoots or branches with different colors on a same plant, which is hypothetically due to transposons activation in somatic cell lines, known to be frequent in *J. curcas* (Costa et al. 2010).

Other crosses revealed rates of incompatibility far greater than that observed in crosses of *J. curcas* with *J. integerrima.* For example, a *J. curcas* cross with *J. multifida* yielded a fruit set rate of 7.7% with only four viable hybrids in the progeny, whereas a *J. curcas* cross with *J. podagrica* showed an even greater rate of

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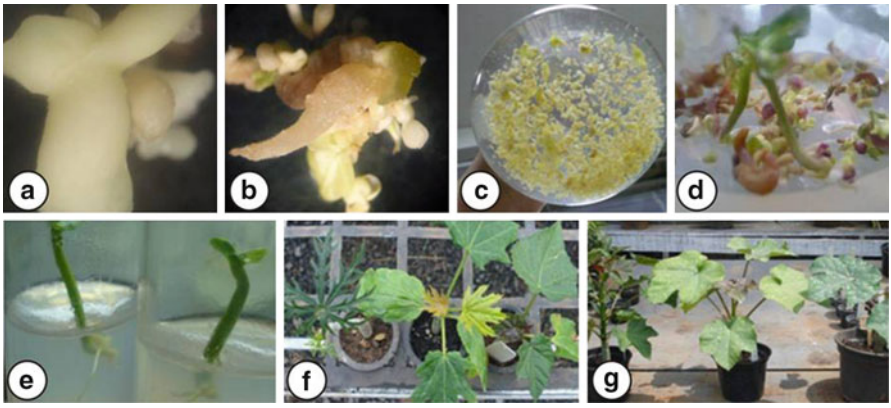

Fig. 23.4 In vitro embryo rescue. Induction of somatic embryos via direct (**a**) and indirect (**b**) embryogenesis; multiplication phase (**c**); germination (**d**) and conversion phases (**e**) acclimatization of rare hybrids, *left*: *J. multifida* (♂), *centre*: F_1 hybrid, *right*: *J. curcas* (♀) (**f**) greenhouse grown rare hybrids, *left*: *J. curcas* (♀), *center*: F_1 hybrid, and *right*: *J. podagrica* (♂) (**g**)

incompatibility (0.4% of fruit set) and only one hybrid plant in the progeny. Crosses of *J. curcas* with *J. pohliana* and *J. curcas* with *J. gossypiifolia* were totally incompatible. In these cases, these rare hybrids could, however, be obtained by the *in vitro* embryo rescue technique. With this approach, hundreds of interspecific hybrids plants have been generated and later multiplied by somatic embryogenesis in the IAC laboratories of biotechnology (Fig. 23.4). Unprecedented genotypes with rare genetic combinations were generated as described above and provide a rich genetic material for use in our breeding program.

Recent studies of gene expression using RT-PCR and chemical evaluation of embryo cultured *in vitro* have revealed different contents of oil, fatty acids and phorbol esters depending of the culture conditions. These studies are being developed together with the Institute of Biosciences from the University of São Paulo (USP) in São Paulo (Brazil).

Backcross Between *J. curcas* and Interspecific Hybrids

The F_1 interspecific hybrids obtained by crossing *J. curcas* (♀) and *J. integerrima* (♂) were backcrossed with *J. curcas* plants previously selected for agronomic and chemical characteristics to obtain the $BC_1 \times F_1$ progenies. Our results showed a high rate of fixation (31%) and germination (85.9%) in this backcross. However, the survival rate of plants after germination decreased to 38.9%, with only 140 $BC_1 \times F_1$ plants available at the moment. These backcrossed plants showed marked variation in their morphology and pigmentation of leaves and stems. There was extensive phenotypic segregation in the morphological traits, with the majority of $BC_1 \times F_1$ individuals being *J. curcas* like, in their phenotypic appearance, with a complete

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absence of anthocyanin pigmentation in their leaves and petioles (Rulfino et al. 2011b).

The next cycle of directed crosses will emphasize a second backcross using the dwarf *J. curcas* plants as female parents, with low or total absence of phorbol esters, increased fruit production per bunch, higher oil yield and quality, etc. Simultaneously, self-pollinations progenies ($S_1 \times BC_1 \times F_1$) will be obtained to begin the fixation of alleles of the desired characteristics. The following year, these generations will be evaluated for agronomic traits and toxic constituents. Then, additional cycles of backcrossing ($BC_2 \times F_1$) and self-pollinations ($S_2 \times BC_1 \times F_1$) will be carried out to obtain promising gene combinations for future experiments. Bi-parental crosses within and between each generation of backcrossing and self-pollination will be done to combine desirable characters and/or to explore heterosis.

Biotechnological Approaches for the Genetic Improvement of *J. curcas*

J. curcas is xenogamic and highly heterozygous for most traits of interest, which implies a high degree of segregation. Consequently, breeding programs requires several years to obtain a cultivar. According to Carvalho et al. (2008), the small genome size (416 Mb), chromosome number (2n = 22), ease of vegetative manipulation and transformation favour the application of biotechnological approaches in the genetic improvement of *J. curcas*. A potential benefit of biotechnological intervention in *Jatropha* breeding is the possibility of introducing genetic changes more rapidly in order to meet the growing demand for stable and improved genetic material. The main biotechnological techniques of potential use in improving *Jatropha* include tissue culture, genetic engineering and molecular genetic analysis.

Tissue Culture

Tissue culture techniques allow the culture of various plant tissues and organs *in vitro*, including leaves, embryos, anthers, pollen, ovary, ovules and other tissues. These techniques are very promising as tools to speed up the process of breeding in *Jatropha.* For example, *in vitro* cloning of elite genotypes helps to ensure uniform performance among parental lines and keeps genetic gain from selective breeding. Micropropagation of *J. curcas* by shoot multiplication and regeneration from different tissues has been reported by various authors (Sujatha and Dhingra 1993; Sujatha 1996; Sujatha and Mukta 1996; Sardana et al. 2002; Deore and Johnson 2008). In addition, somatic embryogenesis has also been demonstrated in *J. curcas*, which offers an opportunity for large scale propagation of elite genotypes (Jha et al. 2007). Other *in vitro* techniques including genetic manipulation through transgenic

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approach can also be used to speed up a breeding program or to expand the genetic variability of the germplasm involved and are briefly reported below.

In Vitro Culture of Anthers or Ovules

The *in vitro* culture of anthers or ovules is used to produce haploid plants that can be transformed in diploid individuals, homozygous for all genes, by colchicine treatment during chromosome replication. This procedure has great potential to eliminate lethal alleles from parent lines and accelerate the fixation of elite genotypes. However, very little work has been done in this direction.

In Vitro Embryo Rescue

In the presence of post-fertilization barriers, hybrid embryos obtained after fertilization degenerate during their growth because of inadequate endosperm development followed by embryo abortion (Segeren et al. 1993). This problem can be overcome by *in vitro* embryo rescue. This technique can be used to obtain plants from embryos that are too weak to develop into seedling on their own or to save embryos from interspecific crosses in which post-zygotic incompatibility made their natural development difficult. These hybrid embryos can grow further into shoots and plants when rescued *in vitro*. However, successful embryo rescue depends largely on the maturation stage of embryos and the composition of culture medium (Sharma et al. 1996).

Optimization of culture medium and growth conditions as well as the best embryonic stage suitable for development into whole plantlets are major challenges particularly when an incompatibility barrier compromises embryonic development shortly after pollination. We have successfully used embryo rescue to obtain rare hybrids in crosses of *J. curcas* with *J. multifida* and *J. curcas* with *J. podagrica* (Moreira et al. 2011a), both of which show high rates of post-zygotic genetic incompatibility in most parental combinations (Moreira et al. 2011b). This approach has also been successfully applied to crosses of compatible parental combinations between *J. curcas* and *J. integerrima*, to obtain hybrids with rare genetic combinations that do not exist in nature. So far, we were not successful in obtaining hybrids between *J. curcas* and *J. gossypiifolia* and between *J. curcas* and *J. pohliana* using this technique.

In Vitro Fertilization and Somatic Hybridization

In the presence of a pre-zygotic barrier, pollen germination on the stigma and pollen tube elongation are interrupted so that there is no fertilization and no embryo formation. One possibility to overcome this barrier is to use *in vitro* fertilization or protoplast fusion (Ishizada 2008). Our recent studies have revealed that most

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crosses between *J. curcas* and *J. pohliana* showed pre-zygotic incompatibility. In this case, the techniques just outlined will be necessary to obtain hybrid plants between these species. However, the nature of incompatibility between *J. curcas* and *J. gossypiifolia; J. podagrica;* and *J. multifida* could be pre-or post-zygotic, depending on the parental genome combination. A sound knowledge of such incompatibility is important when selecting the biotechnological procedure to be employed in obtaining interspecific hybrids between *Jatropha* species.

In Vitro Mutagenesis

The exposure of *J. curcas* explants to physical (gamma or UV radiation) or chemical mutagens can produce morphological, physiological and biochemical mutants (Pandey and Datta 1995; Dwimahyani and Ishak 2004; Punia 2007).

Genetic Engineering

Genetic engineering has been widely used to obtain transgenic plants and has great potential for *Jatropha* breeding because of the possibility to introduce interesting genes, such as those for aquaporin (*JcPIP2*) and betaine aldehyde dehydrogenase (*JcBD1*) (Zhang et al. 2007, 2008), which play an important role in the rapid growth of *J. curcas* under dry and saline conditions, respectively. Among other candidate genes for engineering, one may cite the (1) stearoyl-acyl carrier protein desaturase, which is an enzyme of fatty acid biosynthesis in higher plants that also plays a key role in their ratio of saturated to unsaturated fatty acids and (2) genes involved in the production of toxic diterpenes (phorbol ester) that could be knocked out to yield non-toxic cultivars. However, the use of this technology in *J. curcas* requires the prior development of an efficient *in vitro* regeneration protocol for a specific elite genotype; and this approach has been studied by several authors (Sujatha and Dhingra 1993; Sujatha and Mukta 1996; Jha et al. 2007; Deore and Johnson 2008; Marques et al. 2008; Franco et al. 2010). The protocol of shoot regeneration from undifferentiated tissues needs to be adapted to the plasmid vector of interest in order to optimize the efficiency of transformation for the genotype studied. We are currently studying such adaptations. Li et al. (2006) were the first to establish a highly efficient genetic transformation procedure for *J. curcas* via *Agrobacterium tumefaciens* infection of cotyledon disc. Despite their enhanced rate of *in vitro* regeneration, these explants are not clonal, which means that cotyledons do not allow the conservation of the characteristics of a candidate elite genotype through the process of gene introgression by genetic transformation. Thus, we are currently developing a transformation protocol based on young leaves as explants for this purpose (unpublished data).

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Molecular Markers

Changes in genome nucleotide sequence from an individual to another provide a *molecular phenotype* of the differences between these two individuals. To play the role of *molecular traits*, *molecular markers* must have Mendelian inheritance. These molecular changes may or may not be anonymous and are detected in different ways, depending on the molecular technique used. In our studies of *J. curcas*, we have used several types of molecular markers to (1) characterize the genetic diversity of the IAC germplasm bank accessions, (2) identify apomictic plants and (3) identify intraspecific and interspecific hybrids.

We have analyzed the genetic diversity of 103 genotypes that varied widely in several morphological and agronomical traits and represented accessions from different geographic origins in Brazil (the states of Alagoas, Bahia, Ceará, Mato Grosso do Sul, Minas Gerais, Pará, Rio Grande do Norte, São Paulo and Tocantins) as well as from China, Costa Rica, India and Mexico. Three types of markers (SSR, ISSR and cTBP) were used in this analysis. As explained above, cTBP is a marker based on the presence of polymorphism in introns of genes in the tubulin B family (Bardini et al. 2004; Breviario et al. 2007). In the case of SSR markers, we used the 12 loci reported by Pamidimarri et al. (2009) whereas the ISSR markers were analyzed using the seven loci described by Grativol et al. (2010). We also used two loci as cTBP markers. In general, the diversity in the IAC germplasm accessions was low to moderate, and the intrapopulation variation was similar to or greater than that observed among Brazilian localities. ISSR and cTBP were more informative than microsatellites (SSR) in this study. Five samples of each species (*J. curcas, J. podagrica, J. multifida, J. gossypiifolia, J. pohliana* and *J. integerrima*) were evaluated by using cPTB markers. Preliminary results showed that cTBP markers can be used to differentiate species and to assess intraspecific genetic variability, especially in *J. curcas*. Like ISSR and SSR, cTBP may therefore be useful in creating core collections and in choosing appropriate intra- and interspecific crosses in *J. curcas* breeding programs.

So far, no apomictic *J. curcas* plants have been found in the IAC germplasm under our experimental conditions. Field experiments were conducted thrice during the same year. A few days after flower emasculation, flower bunches were protected to assess rate of fruit set. The field results have recently been confirmed by RAPD analysis. For this, we used 23 families with six plants each (one female parent and five F_1 plants). Of the families studied, at least one of the individuals showed genetic polymorphism. This result, although not yet conclusive, indicates the occurrence of gametic recombination and excludes the hypothesis of apomixis in the plants analyzed to date (unpublished observations).

The early confirmation of hybrids by using molecular markers is an important strategy in this breeding program, especially when there is no possibility of using morphological markers in newly germinated seedlings or, particularly, backcross plants, which may exhibit morphological features very similar to the target spe-

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cies (*J. curcas* in this case). We (Carqueijo et al. 2010) and others (Basha and Sujatha 2009) have used RAPD to identify interspecific hybrids of *Jatropha* (F_1 and BC_1 generations). RAPD confirmed the hybrid nature of the 80 F_1 individuals that we obtained and of the 36 backcrossed plants ($BC_1 \times F_1$) analyzed so far (out of a total of 90), thus confirming the usefulness of RAPD in such analyses (unpublished data).

Conclusions

J. curcas is incipient with respect to genetic improvement for enhanced productivity, oil yield per unit area and other characteristics of interest discussed above. By using the strategies outlined in this chapter, we have combined and improved the main features of *J. curcas* necessary for cultivation in small, medium and large rural properties in Brazil, as part of a program to meet the growing demand for biodiesel in this country. *J. curcas* can be improved by assessing the variation in wild congeneric species and selecting elite genotypes in self-pollinization or hybrid generations. Biotechnological and molecular biological techniques have been integrated into the genetic improvement of *J. curcas* through conventional breeding to reduce the growth time and increase the efficiency of breeding. The constant technique of development and optimization, scaling up of elite genotype micropropagation, alien gene transfer through interspecific hybridization and biotechnological contribution through somaclonal variation and gene transfer by genetic transformation are very important for trait domestication. In addition, we have recently imported valuable genetic material in the form of phenotypically different, non-toxic (phorbol ester-free) plants and pistillate plants from Mexico, the probable centre of origin of *J. curcas*. In order to enhance plant productivity, we plan to introgress the pistillate trait in our genetic background and then explore the specific combining capacity (heterosis).

The use of modern tools such as DNA sequencing methods to determine the complete or partial sequence of the *J. curcas* genome (Synthetic Genomics Inc. and Asiatic Centre for Genome Technology; Sato et al. 2010; Gomes et al. 2010; Costa et al. 2010) is another important advance in this field. Genome annotation to identify genes of interest should be helpful in assessing genetic variation by allowing the use of marker-assisted breeding to accelerate and complement conventional breeding programmes (Gomes et al. 2010). This approach can provide information on factors involved in controlling oil synthesis, maximizing yield, inducing tolerance to biotic and abiotic stresses and producing varieties with low-curcin or phorbol ester levels. Thus, important genes may be identified in the genomes of *Jatropha* spp. and transferred to *J. curcas* by genetic transformation. We are already optimizing protocols that will allow us to master this technology for future assessment of this promising perspective.

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Chapter 24
Conservation Strategies and Management of *Jatropha* Germplasm

J. Radhamani and Natarajan Sivaraj

Introduction

Crop diversity is part of the biological diversity and includes the resources that contribute to people's livelihood by providing food, feed, medicine, fiber, clothing, shelter and energy. Hence, it contributes towards achieving the global objectives of food security, poverty alleviation, environment protection, and sustainable development. Genetic variation must be conserved and effectively utilized to combat new pests and diseases, and to produce better adapted varieties for the changing environments. Conservation and utilization of plant genetic resources are important components of *ex situ* collections. The conserved germplasm accessions need to be characterized for important morpho-agronomic characters and these trait specific germplasm are to be distributed to bonafide researchers for utilization in crop improvement programs all over the world. Exiguous use of germplasm has been observed in breeding programs, mainly due to lack of information on economic traits.

The genetic diversity is a pre-requisite for any crop improvement programme and forms the basis for meaningful and effective conservation for their specific traits. Therefore, the selection of lines with rich potential valuable traits and significant morphological differences would help in quickly identifying the cultivars from diversity rich areas for effective conservation and also for future utilization.

This potential of biofuel production can be further increased with intensification of cultivation and genetic improvement and domestication of natural genetic resources variability that will help in selection and distribution of germplasm/

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selected superior clones to all stakeholders (co-operators) to promote cultivation and sustainable use. Availability of information on conservation strategies and the management of conserved germplasm would promote storage of seed material, cross fence and long distance exchange of seed material among various stakeholders (co-operators) including farmers and evaluation for identification of superior types and sources of desired traits.

Germplasm Conservation

Conservation of plant diversity is of utmost importance in ensuring protection of healthy environment over the globe and also for meeting basic human needs of food, nutrition, health care, clothing and fuel. Although around 2,50,000 plant species have been described so far and a much larger number still remains to be duly recognized, yet only 3,000 species are grown for their human use since the beginning of agriculture, considered to be nearly 10,000 years ago. Germplasm conservation is defined as the management of human use of the biosphere so that it may yield the sustainable benefit to present generations, while maintaining its potential to meet the needs and aspirations of future generations.

Conservation Approaches

The Convention on Biological Diversity (CBD) within its broader framework defines two conservation strategies *in situ* conservation and *ex-situ* conservation. Both methods are highly complementary measures, which can be judiciously integrated so that the conservation efforts are effectively realized for sustainable release of useful variability. These approaches can be effectively utilized for the conservation of *Jatropha* species.

In-Situ Conservation

Conservation of genetic resources within their ecosystem and natural habitat ensures the maintenance and recovery of viable populations of species in their natural surroundings. The *in situ* conservation, which involves protection or keeping aside the ecosystems or habitat harbouring biodiversity from human intervention is good for mainly forestry species, wild relatives of crop species, forage crop species, medicinal and aromatic plants and the species that are under various levels of threat or about to extinct, because they cannot be grown outside their habitat or they are the members of complex ecosystems, like tropical forest trees, that have high dormancy or associated with highly specialized breeding system. To meet the set objectives the following approaches are being followed:

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1. **Ecosystem approach:** It involves conservation of ecosystems large enough to ensure self-perpetuation and evolution of organisms. Under this, disturbance of habitat can cause degradation of vegetation that may disturb the stability of plant components. Thus, survival and conservation of genetic resources is greatly influenced by ecological imbalances and may result in change in species/population composition. Since there is only a limited variability available in India, this conservation approach may be suitable for *Jatropha* species. This type of approach is prevalent in forest areas of Tamil Nadu and Uttarakhand, India.
2. **Habitat approach:** It helps to manage the target species or group of species in its original habitat and can adopt any of the following methods-Managed forests (Sacred groves), Nature reserves with multiple uses, Preservation plots, Wildlife sanctuaries, Habitat or National parks, On-farm conservation and Agro-ecosystem through preservation of peasant cultivation as dynamic system. *Jatropha* is conserved on-farm in the centre of origin and other regions, because it is used as a hedge. Since the cultivation system does not seem to change, genetic erosion may not be an important concern at present. On-farm conservation, apart from allowing evolution to continue, contributes as well to the conservation of diversity at all levels (landscape, ecosystem, among and within species) and it is therefore highly strategic. It does contribute to empower the farmers to better exercise control over their crop genetic resources, major biological and livelihood assets. Another major advantages of on- farm conservation is related to its conduciveness in safeguarding the traditional knowledge associated to biodiversity which is an integral part of people's social and cultural identity (CUBIC 2000), and which is fundamental for celebrating and appreciating crop diversity now and in the future. Lastly, on-farm conservation is a powerful instrument to allow the implementation of benefit sharing as recommended by the CBD which has in fact recognized the continued maintenance of traditional varieties on-farm as an essential component of sustainable agricultural development (UNEP 1992). This approach is being tried in Erode, Karnataka and Andhra Pradesh.

India has a land area of 329 million ha, of which more than 19% is under forest cover. The country is distinguished into 16 phytogeographical zones classifying the major vegetation types of the country (Gadgil and Meher-Homiji 1990). Sunil et al. (2008, 2009) assessed jatropha germplasm *in-situ* and described the diversity and distribution in the South East Coastal Zone of India. Traditional, *in-situ* conservation of PGR through human intervention in India can be traced back to ancient times with the establishment of sacred groves and protection of plants around the temples of those species that are either part of religious practices or of medicinal value. During the post-Independent period since 1947, approximately, 4.2% of the total geographical area has been put under the programmes of conservation of biodiversity. This was achieved with the establishment of biosphere reserves, habitat-parks, wild life parks and sanctuaries, protecting the diversity under natural habitat following ecosystem or habitat approach. The plant biodiversity of economically important or domesticated species is being conserved with the establishment of gene sanctuaries for certain species and their wild relatives and on-farm conservation of farmers/traditional varieties and landraces.

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Clonal Repositories/Field Gene Banks

Plant genetic resources of different fruit plants/tree species and many ornamentals are maintained by vegetative propagation in order to maintain their genetic make-up true to the type. In case of fruit germplasm, usually conservation is done in the field gene banks which were earlier known as varietal collection, clonal repositories or living collection. Field gene bank is a facility where clonal materials are conserved as living collections in field, orchard or in plantation. The reasons for retaining clonal lines relate to maintenance of heterozygosity and adapted complexes, inability to set seed early, long duration of juvenile stages and production of recalcitrant and/or large seed (Towill 1988). Many collections of tree species including their wild relatives are found in botanical gardens and arboreta, but no collection is complete. Almost all wild species of fruits/agroforestry species in India are being maintained in the forest, which face danger of extinction due to indiscriminate felling of plant materials for various uses like wood, timber, bark and fruits, etc. *In-situ* conservation may involve many disciplines: ecology, agronomy and resource management, soil science, genetics, entomology and plant pathology.

In field gene banks, the plant genetic resources are kept as live plants that undergo continuous growth and require continuous maintenance. They are often used when the germplasm is either difficult or impossible to conserve as seeds (i.e. when no seeds are formed, seeds are recalcitrant or seed production takes many years, as for many tree species) or the crop is reproduced vegetatively.

Field gene banks provide an easy and ready access to the plant genetic resources, for characterization, evaluation or utilization, while the same material conserved in the form of seeds, *in vitro* or cryo must be germinated or regenerated and grown before it can be used. They are also useful for conserving vegetatively propagated genotypes that commonly produce variants (genetic variation) since these can be more easily identified and rouged out in the field than *in vitro*. Field gene banks however, are generally more expensive to maintain, requiring more labour, more inputs and more space (land) than other methods of conservation. They also have higher levels of risk from natural disasters and adverse environmental conditions like drought, floods or attacks from pests and diseases. Important plus trees with good oil content are being maintained at several field gene banks in India and other countries (Tables 24.1 and 24.2).

There is limited genetic variability in the Indian germplasm of *J. curcas* while wide variability has been observed between Indian and Mexican genotypes (Basha and Sujatha 2007). Genetic improvement is possible only by improving of crop features such as seed size, morphology, oil content, synchronization of fruit maturity, toxicity, digestibility and resistance to pest and diseases. Breeding programmes and conservation strategies are principally ongoing in India, China, Thailand, Philippines, Mexico, Guatemala, Africa, Egypt and lately started in Brazil (Carels 2009).

The *Jatropha* variety in Nicaragua has fewer, but larger fruits. A non-toxic variety of Mexico which is free from phorbol esters could be a potential source of oil for human consumption and the seed cake can be a good protein source for human and livestock (Becker 1996). These identified varieties can be introduced in breeding

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Table 24.1 Some field gene banks of *Jatropha* in India

S. no.	Institute	State
1	Directorate of Oilseeds Research, Hyderabad	Andhra Pradesh
2	NBPGR Reg. Station, Hyderabad	Andhra Pradesh
3	Central Research Institute for Dryland Agriculture, Hyderabad	Andhra Pradesh
4	The Energy and Resources Institute, Shillong	Meghalaya
5	S.D. Agricultural University, Sardarkrushinagar	Gujarat
6	CCS Haryana Agricultural University, Hisar	Haryana
7	National Botanical Research Institute, Uttar Pradesh	Haryana
8	Himalayan Forest Research Institute, Shimla	Himachal Pradesh
9	NBPGR Reg. Station, Ranchi	Jharkhand
11	NBPGR Reg. Station, Thrissur	Kerala
12	NRC for Agroforestry, Jabalpur	Madhya Pradesh
13	Dr Panjabrao Deshmukh Krishi Vishwavidyalaya, Akola	Maharashtra
14	NBPGR, Issapur farm, New Delhi	New Delhi
15	Thapar University, Patiala	Punjab
16	M.L.Sukhadia University, Rajasthan	Rajasthan
17	NBPGR Reg. Station, Jodhpur	Rajasthan
18	University of Rajasthan, Jaipur	Rajasthan
19	Forest College and Research Institute – TNAU	Tamil Nadu
20	M.S.Swaminathan Research Foundation, Chennai	Tamil Nadu
21	Madurai Kamaraj University, Madurai	Tamil Nadu
22	Forest Research Institute, Dehradun	Uttarakhand
23	High Altitude Plant Physiology Res. Center, Srinagar	Uttarakhand

Table 24.2 Status of some field genebanks of *Jatropha* in other countries

S. no.	Centre	No. of collections in FGB
1	Burkina Faso, Centre National de Semences Forestieres (CNSF), 01 BP 2682, Ougadougou 01	12 provenances from Burkina Faso
2	Cape Verde, Institute Nacional de Investigacaoe Desenvolvimento Agfario (INDIA) CP 84, Praia	13 provenances
3	Cost Rica, Centro Agronomico Tropical de Investigacion y Ensenanza (CATIE), PO Box 7170, Turrialba	3 provenances from Costa Rica

programmes and exploit their full potential in developing trait specific germplasm of jatropha.

Seed Orchard

Seed orchard is an intensively managed plantation of specifically arranged trees for the mass production of genetically improved seeds to create plants, or seeds for the establishment of new man made forests. Seed orchards are a common method of mass-multiplication for transferring genetically improved material from breeding populations to production populations (forests) and in this sense are often referred

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to as "multiplication" populations. A seed orchard is often composed of grafts (vegetative copies) of selected genotypes, but seedling seed orchards also occur mainly to combine orchard with progeny testing. Seed orchards are the strong link between breeding programs and plantation establishment. They are designed and managed to produce seeds of superior genetic quality compared to those obtained from seed production areas, seed stands, or unimproved stands.

In first generation seed orchards, the parents usually are phenotypically-selected trees. In advanced generation seed orchards, the seed orchards are harvesting the benefits generated by tree breeding and the parents may be selected among the tested clones or families. It is efficient to synchronize the productive life cycle of the seed orchards with the cycle time of the breeding population. In the seed orchard, the trees can be arranged in a design to keep the related individuals or cloned copies apart from each other. Seed orchards are the delivery vehicle for genetic improvement programs where the trade-off between genetic gain and diversity is the most important concern. The genetic gain of seed orchard crops depends primarily on the genetic superiority of the orchard parents, the gametic contribution to the resultant seed crops, and pollen contamination from outside seed orchards. Seedling seed orchard has been established at AFRI, Jodhpur with 116 accessions of plus tree collections of *Jatropha.*

Village Conservation Areas

The decline of biodiversity in agricultural landscapes due to increasing demand for agricultural land and intensified farming is of increasing concern (Matson et al. 1997; Clough et al. 2007). Consequently, conservation of plant species within patches of marginal and degraded areas has been considered to be a step towards reversing this trend (Huston 1993). Furthermore, these areas are excellent reserves for conservation of agro biodiversity of forestry species which is vital for maintaining a stable agro-ecosystem (UNEP 1999). Conservationists prioritize marginal and degraded areas as a mechanism to reduce competition with food production (Bai and Dent 2006). Worldwide critical fuel shortages accompanied with high prices as well as the global issues of climate change due to global warming has prompted governments and non-governmental organization to search for alternative sources of energy, which are renewable, safe and non-polluting. In this regard, renewable vegetable fuels such as *J. curcas* are being promoted for cultivation in marginal and degraded lands (Jimu et al. 2009). Most of the marginal areas in Andhra Pradesh, Tamilnadu, Uttar Pradesh, Orissa, Chattisgarh, Madhya Pradesh, India are used for the conservation of *J. curcas.*

Ex-Situ Conservation

Most of the genetic resources for food and agriculture have been conserved following *ex situ* approaches. Based on genetic material that refers to "any material of plant origin of actual or potential value for food and agriculture, which includes

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reproductive or vegetative propagating material containing functional units of heredity", suitable ex-situ approaches may be adopted for cost effective conservation to meet the desired objectives. This has been achieved by perpetuating sample population in genetic resources centres, botanical gardens, tissue culture repositories, genebanks for seed propagated species and conservation of pollen, embryo and other plant parts/organelles, etc. Conservation of seed propagated plants is relatively easy for seeds with orthodox type of storage behavior, i.e., the viability can be maintained by drying the seeds and storing at low temperatures and *Jatropha* shows orthodox seed storage behavior. The ex-situ preservation of orthodox seeds in genebank has been the most effective strategy for plant genetic resources conservation. Today, about six million accessions are conserved worldwide in more than 1,000 genebanks as *ex-situ* germplasm collections including 5.27 lakh accessions maintained in field genebanks. Fifteen of the genebanks have long-term facilities (FAO 1998). There is a basic advantage in following this approach that the availability of seed although in small quantities will be ensured even after 2–3 human generations. It is nevertheless scientifically accepted that the *ex-situ* mode of conservation does not provide a panacea for conserving crop genetic resources. It has been found to be associated with inadequate sampling procedures during field collecting, lack of representation in the genebanks of the whole range of diversity of a given crop and its close relatives, etc. Besides this, the storage of seeds involves the freezing of evolutionary processes, thus preventing new type or levels or resistance to evolve, because the plants are not allowed to respond to selective pressures of environment.

The *ex-situ* conservation approaches can be classified into:

1. **Plant conservation**: Botanical garden, herbal garden, arboreta, etc.
2. **Seed conservation**: Storage of desiccation tolerant seeds at low temperatures
3. ***In vitro* conservation:** Maintenance of aseptic cultures of cell, tissue and organs
4. **Cryopreservation**: Preservation of desiccation sensitive seed, organ and cultures using cryogenics
5. **DNA conservation**: Genomic DNA, DNA libraries, Gene constructs

Seed conservation has vital role in preservation of genetic variability as it is simple to handle, cost-effective and capability of maintaining genetic stability over long time periods. Seed conservation is a popular tool for germplasm conservation at the global level. The most important components of managing *ex-situ* germplasm include well established procedures for collection/assembly, characterization, conservation and sound scientific approaches for effective utilization of conserved germplasm. The goals of *ex-situ* conservation in *Jatropha* can be achieved in a variety of ways.

Types of Conservation

Short-Term

Seeds can be shade dried and stored up to 2 years in sealed plastic containers, paper bags or muslin cloth bags at 18–20°C and 45% RH.

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Medium-Term

Seeds can be shade dried and stored up to 5 years in cloth bags, metal cans and plastic jars at 4–10°C and 35% RH. Active collections are kept at medium term storage which are immediately available for distribution, utilization and multiplication. The seeds which do not satisfy the quality and quantity and to be regenerated are also stored in medium term module. The seeds are equilibrated to 8–10% moisture content at these conditions. Medium-term storage of *Jatropha* accessions is available at NBPGR Regional Stations at Thrissur, Hyderabad, and Jodhpur.

Long-Term (−18°C)

Seeds can be dried and stored for long term in sealed aluminium foil pouches at −18°C with 6–8% moisture content on fresh weight basis for long term. The base collections are stored in long term for posterity. This method is being practiced at NBPGR, New Delhi for storage of *Jatropha* germplasm which is discussed in detail.

Conservation at National Gene Bank, New Delhi

The National Gene Bank (NGB) is responsible for the conservation of seeds on a long term basis, as base collection for posterity. In addition, it provides technical support to the network in planning, development and operation of medium-term gene bank facilities, in human resource development and in providing the accessions for the regeneration of active collections. It is supported by a network of 10 Regional Stations in different agro-ecological zones of the country and 59 Crop based, National Active Germplasm Sites (NAGS).

Seed Conservation

The seeds are best stored at subzero temperature with 3–7% moisture content (IBPGR 1985) depending on species as the acceptable standards. Conservation of genetic resources is a high priority in the national context, as a global plan of action activity or a convention on biological diversity requirement. The National Plant Genetic Resources programme in India has already developed elaborate guidelines for sending germplasm for long term conservation in its gene bank, which maintains international standards and also ensures long term viability of the material conserved after due processing. The following points are check listed before sending the material for *ex-situ* conservation:

1. Well-developed and physiologically mature seeds.
2. Free from insects, weed seeds and diseases.

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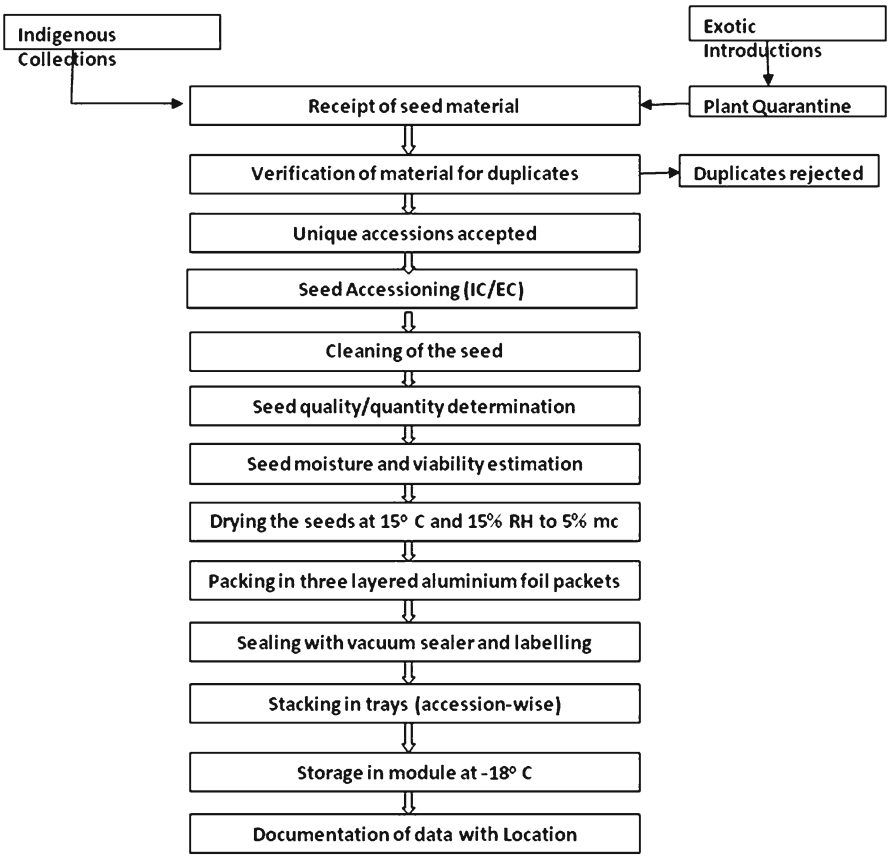

Fig. 24.1 Flow chart for the processing of *Jatropha* seed material in NGB

3. Clean and free from under sized, shrivelled, immature and discoloured seeds.
4. Properly labelled and packed to avoid damage during transit.
5. Untreated with chemicals.
6. Send to gene bank for storage at the earliest possible (immediately after harvest).
7. Accompanied with the minimum passport data.

Processing of Seed for Long-Term Conservation

The cleaned seeds are dried to low moisture contents (6–7%) and stored in hermetically sealed moisture impervious containers at sub-zero temperatures (−18°C). The steps involved in processing the seed material for Genebank depicted in the flow chart (Fig. 24.1).

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Identification of Physiologically Mature Seed and Collection of Material

In *J. curcas*, the flowering takes place in the months of July-August and the fruit matures in the months of October-November. The mature fruits are generally collected in the months of October-November. The excised seeds without any mechanical damage are collected. Seed collectors should aim to ensure that seeds are collected/ harvested at peak quality so that their longevity in the seed bank is optimal. Ideally the seeds should be collected when they are on the point of natural dispersal. Since the flowering in *Jatropha* is asynchronous all the fruits do not mature at the same time. Hence, harvesting should be done periodically.

Extraction of Seeds

Germplasm collection in *Jatropha* species is undertaken by selecting the fully ripened, physiologically mature fruits which can be assessed by some of the field indicators such as picking up the fruits when they just begin to dehisce, brownish black in colour, fully ripened (about 70 days after anthesis), seed moisture content 12–18% on fresh weight basis. The fruits should be picked up by shaking the branches or with basket attached to a stick directly from the tree. Collection of fallen fruits from the tree should be avoided to avoid fungal infection.

Transport and Unpacking of the Seed-Lots

*Jatropha s*eeds are large in size and prone for fungal infections and hence, the collected fruits/ seeds are unpacked immediately upon arrival. This is done within a clean (and relatively contained) area, in case live insects are present. Collections are checked for signs of seed damage (caused by insects) and to assess whether immediate cleaning is required, as with wet fruits. Any seed-lot containing live insects is separated further for quarantine treatment/ salvaging. The majority of collections are placed in the walk-in drying room in muslin cloth bags at 15°C and 15% RH.

Seed Cleaning

Accessions may arrive as cleaned seeds, or fruits (all broadly termed here as seed-lots or seed collections). Seed sample sizes are usually small compared to other crop species. Therefore, seed wastage must be minimized. Seed collections need to be clean for several reasons:

- There needs to be reasonable reduction of plant bulk by extracting the smallest storable entity, usually the seed, without incurring any damage to the collection.

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- Removal of unnecessary plant matter reduces the risk of disease carry-over.
- Empty and/or insect-infested seeds are removed where possible from the seed collection, as these 'incompetent' seeds complicate the interpretation of germination results and quantity determination as well as adding unnecessary bulk.
- Cleaning removes contaminants of the collection, especially inert matter.

All cleaning is done by hand, with the aid of some simple equipment. Automated cleaning can impart a degree of seed damage that may lead to loss of viability.

X-ray Analysis or Cut-Test

X-ray analysis can be an invaluable tool in determining the status of seed samples before, during or after seed cleaning. It is a means of estimating the proportion of empty, poorly developed or insect-infested seeds present in a subsample of the main collection. Where the X-ray analysis reveals an easily removable fraction of empty seeds, the collection is recleaned. X-ray analysis will often show the internal structure of a seed, including the size and position of the embryo. A fairly small subsample of seeds (approximately 50) can be analyzed to give an acceptable estimate of the quality of the whole collection. The X-rayed sample is not returned to the batch; it is discarded, used for display or, occasionally and in the case of very small accessions, used for a germination test.

Seed Quantity Determination

Several methods are used to estimate the number of seeds within accessions. The determination and method is recorded on the Seed Bank Database, which, where appropriate, calculates the final seed quantity. The method used to estimate quantity depends on a number of factors:

1. If there are only a few seeds (less than about 300), they are counted individually.
2. A collection may be counted entirely by a seed counting machine if it is clean and has suitably sized seeds. However, this method is too slow for very large collections.

Seed Moisture content determination: For gene bank purpose, seed moisture content is generally expressed on wet weight basis. Even a small change in the moisture content has a large effect on the storage life of seeds. Seed moisture content is determined by low constant temperature oven drying method (103 ± 2°C for 16 h).

Seed Viability Assessment

1. **Seed viability (Germination method)**

Two different types of substrata can be used to assess the seed viability by germination method.

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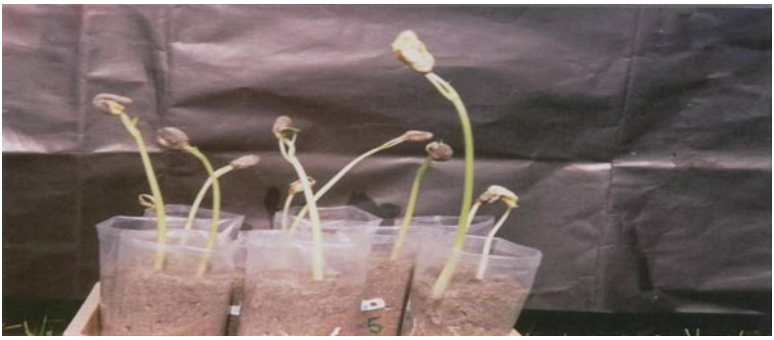

Fig. 24.2 Seed germination of *J. curcas* over sand in polybags

Fig. 24.3 Seed germination of *J. curcas* in between the folds of germination paper towels

- Over sand (Fig. 24.2)
- In between the folds of germination towel (Fig. 24.3)

The standard germination test can be conducted by placing the seeds in replicates of 50 seeds each which are maintained at 27 ± 2°C with 80–90% relative humidity in the seed germinator. In the fully mature seeds, the radicle starts emerging on the third day of plating. The seeds were considered germinated only when both radicle and plumule emerges completely. The percentage germination is calculated based on the count of normal seedlings. The seedlings are evaluated on the 15th day of plating for estimating the seed germination percentage. Planting the seeds in between the folds of germination paper is much simpler and economical.

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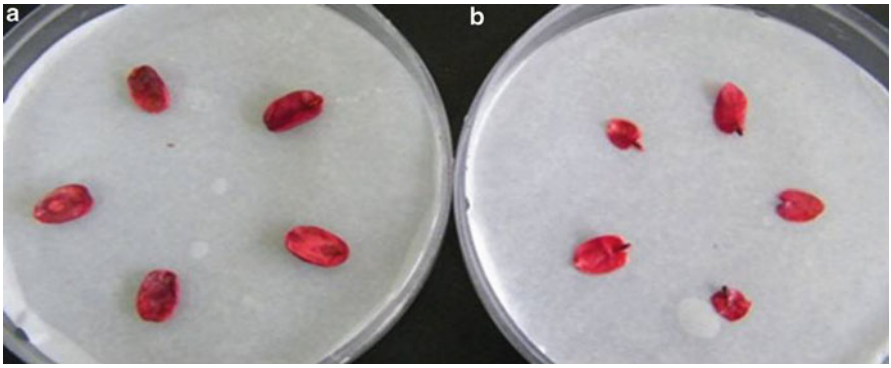

Fig. 24.4 The seed staining pattern of viable seeds and embryos in *J. curcas*

Quick Viability Test (Triphenyl Tetrazolium Chloride Test) for Testing Seed Viability

Assessment of seed viability by Triphenyl tetrazolium chloride (TTC) solution is the quick and best method of testing viability but the concentrations of TTC, duration of staining, optimum temperature for staining are important factors for proper assessment. Seeds are soaked in water overnight followed by immersion in 1.0% tetrazolium chloride solution at 35°C for 6–8 h which produce good staining pattern for viability assessment in *Jatropha* seeds (Fig. 24.4), indicating 92–96% viable seeds which was at par with viability (%) recorded through the lab germination test. Therefore, the TTC staining technique can be recommended for assessment of seed viability and quality of *Jatropha* seeds of a lot before taking large scale plantations.

Accessioning

Once the seed qualifies for quality and quantity it is given a national unique identity based on the passport data. The introduced germplasm are assigned exotic code (EC) numbers while indigenous germplasm collected from within the country are assigned indigenous code (IC) numbers.

Drying, Packaging and Cold Storage

After cleaning and quantity determination is completed, the accessions are moved to a drying room which is maintained at 15% relative humidity (RH) and 15°C, an

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internationally accepted regime for drying to avoid any adverse effects of drying on the initial quality and subsequent longevity (Cromatry 1984). Seeds in muslin cloth bags are placed loosely in slatted plastic crates to facilitate drying. Seed lots remain within the room for 1–3 weeks by which time they should have reached equilibrium moisture with the air. Before being packaged ready for storage, their moisture status is checked. *Jatropha* seeds are packed once they attain a moisture content of 5–7%. The passport data along with the national identity is documented. The minimum data is printed on the labels which are pasted on the respective packed seed packets. The processed accessions are arranged in plastic baskets and placed in cold storage vault at −18°C at a defined location in the gene bank.

Germplasm Documentation

Data documentation is the most important and dynamic activity. Correct and reliable recording of data, its documentation and information transfer is as important as proper handling of germplasm itself. Proper documentation of plant genetic resources allows the best use of data, its easy retrieval and usefulness. This way the documentation of plant genetic resources is very useful in disseminating information on germplasm holdings more effectively and for a specific purpose enabling one to a search information having desired traits so that the selected accessions could be used for breeding programmes for developing new traits. It also acts as a source of information to assist in planning and operation of any gene bank activity. For documentation of gene bank holdings in *National Gene Bank* (NGB), two types of information files are used: passport data descriptors and gene bank management descriptors.

The passport data descriptors include name of crop, taxonomic code, cultivar, national Id, collector_no, other_Id, location in the gene bank while gene bank management descriptors include seed quantity, seed viability, seed moisture content, etc. Being the most important component of National Gene Bank, it is accomplished efficiently through a well managed computer network.

Some Other Advanced Methods for Conservation

In-Vitro Conservation

Although several techniques have been developed for micro-propagation of *Jatropha* germplasm, initiatives in the direction of conservation are limited. Micro-propagation is advantageous in *Jatropha* which facilitates the availability of planting materials irrespective of the seasonal fluctuation, supply of high quality planting materials, based on the selection of explants from highly reliable good mother plants, Supply of disease-free plants, If the selected mother plant is disease resistant, progenies

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supplied are also disease resistant plants, production of large number of plants (seed stock) in relatively smaller area, preservation and maintenance of germplasm in germplasm bank.

Meristem cultures have assumed a significant place in the domain of plant tissue culture due to its role in clonal propagation as well as in the production of virus-free plants. Moreover, apical meristems are less differentiated and genetically more stable which makes this an ideal system for germplasm preservation (D'Amato 1975; Kartha 1985b; Sakai 1985).Two basic approaches are followed to maintain germplasm collections *in-vitro* (1) minimal growth, and (2) cryopreservation (Scowcroft 1984). Minimal growth conditions for short to medium term storage can be followed in several ways – reduced temperature and/or light; incorporation of sub-lethal levels of growth retardants, induction of osmotic stress with sucrose or mannitol, and maintenance of cultures at a reduced nutritional status particularly, reduced carbon, reduction of gas pressure over the cultures, desiccation and mineral oil overlay. The advantage of this approach is that cultures can be readily brought back to normal culture conditions to produce plants on demand. However, the need for frequent sub culturing may pose a great disadvantage including contamination of cultures as well as imposition of selection pressure with subsequent change in genetic make-up due to somaclonal variation.

Preservation of Embryos

The data available in literature regarding survival of embryos and their subsequent regeneration into plants, points that their most important use could be for conservation of (a) difficult to store seed species (b) species in which less number of seeds are produced, (c) hybrid embryos produced out of incompatible crosses, and (d) haploid germplasm. For cryopreservation, different types of embryos have been used, i.e., zygotic, somatic, nucellar and the pollen embryos obtained from androgenic anthers which could be successfully cryopreserved (Bajaj 1985).

Freeze Preservation of Pollen

Cryopreservation of pollen is easy and has been considered of great value in supplementing the usual germplasm preservation techniques by seed and clonal storage and for enriching haploid gene pools. Pollen banks are efficient, economical and space saving, compared to maintaining live field collections. Pollen storage was primarily developed as a tool for controlled pollination of asynchronous flowering genotypes, especially in fruit tree and agroforestry species (Alexander and Ganeshan 1988). In addition pollen storage has also been considered as an emerging technology for genetic conservation (Roberts 1975; Omura and Akhima 1980; Withers 1991). Conservation of pollen (eg., under organic desiccation freeze drying, low

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temperature) is primarily used for facilitating hybridization when flowering is asynchronous or for use in next season. Thus, it can help in better utilization of available genetic resources. Pollen can easily be collected and cryopreserved in large quantities in relatively small space. In addition, exchange of germplasm through pollen poses less quarantine problems compared with seed or other plant propagules.

Cryopreservation of Seeds

Cryopreservation at the temperature of liquid nitrogen (LN) (−196°C) offers the possibility for long-term storage with maximal phenotypic and genotypic stability (Steponkus 1985). This method being relatively convenient and economical, large number of genotypes and variants could be conserved and thus maximize the potential for storage of genetically desirable material. Similarly, the seeds/ embryos of *Jatropha* can be cryopreserved

DNA banks may One day be a practical method of conserving germplasm, although at present technical difficulties in storing entire genomes and the inability to regenerate individuals from DNA alone mean that this technique is, as yet, not operational.

These techniques can be additionally exploited to conserve and maintain the trait specific germplasm of *Jatropha* and for easy exchange across the border.

Management of *Jatropha* Seeds in NGB

Presently *long term storage* (LTS) at National Gene bank holds 1989 accessions of *J. curcas* germplasm from different agro-climatic zones of India. A total collection of 1,074 accessions have been made under the network institutions of micro-mission under DBT Project whereas 638 accessions have been assembled/collected by NBPGR head quarters and its regional stations and 277 accessions from other different sources (Table 24.3). Maximum collections were assembled/ collected from Southern India (40.4%) followed by Northern India (34.9%) (Fig. 24.5). The DIVA-GIS map showing the collection sites of the above conserved *Jatropha* germplasm is presented in Fig. 24.6.

Table 24.3 Seeds collected/assembled in India

Different sources	No. of accessions
Network institutions of micro-mission under DBT	1,074
NBPGR, Regional stations	638
Others	277
Total	**1,989**

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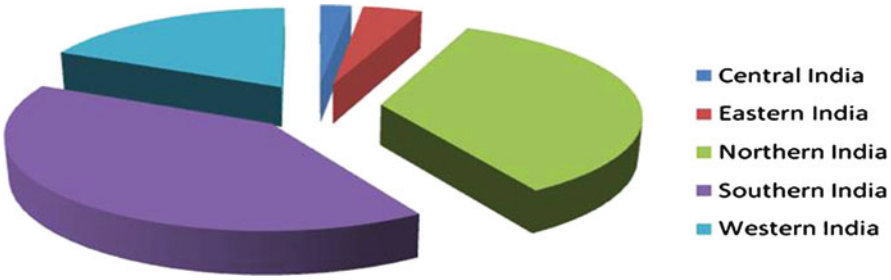

Fig. 24.5 Representation of *Jatropha* accessions from different regions of India

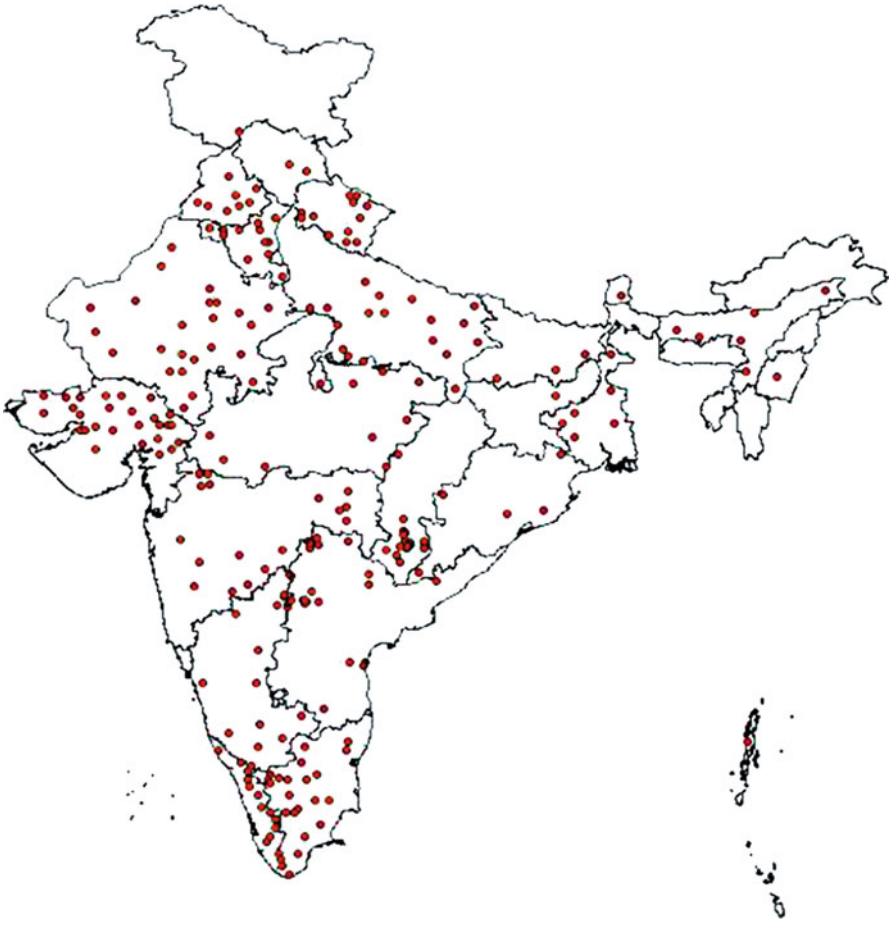

Fig. 24.6 DIVA-GIS map showing the collection sites of *Jatropha* germplasm conserved in the *National Gene Bank* (NGB)

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Inventorization and Documentation of Passport Data and Gene Bank Data of Specific Traits

All the accessions collected and assembled for the gene bank were allocated the national identities and both the passport data and the gene bank data have been compiled on the excel data sheet with 30 different characteristics for easy retrieval.

Identification of Trait Specific Germplasm

The passport data available has been studied for the trait specific characteristics of the *Jatropha* germplasm. The characteristics were mainly specified either for the tree or the seed oil content. Under the tree characteristics, 32 accessions have been identified for various characteristics such as highly branched, good bearing, fruits in cluster, high seed yield, high test weight, low plant height, higher stem girth, etc. (Table 24.4). The seed oil content has been categorized under three heads *viz.* the oil content with ≥30% and the second group with 35–40% and the third group with ≥40% oil. So there were 24, 93, 20 accessions in first, second and third categories of seeds making the total trait specific accessions for oil content to be 137. These

Table 24.4 Trait specific germplasm available in NGB

S. no.	Specific traits	No. of accessions
1	10–15 ft, highly branched, good bearing	5
2	Fruits in cluster with heavy bearing	1
3	Fruits containing four seeds, with large bold seeds	1
4	Higher stem girth and high canopy	1
5	Seed yield, oil content and test weight	132
6	High canopy, more no. of capsules, higher test weight	2
7	High seed yield and test weight	1
8	Higher no. of primary branches, higher test weight	1
9	More height, higher test weight, higher stem girth and high canopy	3
10	High seed yield, more no. of tertiary branches, high test weight	1
11	High oleic acid, O/L Ratio, cetane no. and oil content	1
12	Higher seed yield, oil content and test weight	1
13	Higher plant canopy and higher test weight	1
14	More number of secondary branches and higher plant height	2
15	Low number of secondary branches, low height and low stem girth	7
16	Higher seed yield and oil content and broad stem girth	1
17	Plant canopy and higher test weight	1
18	High yielding	14
19	Disease resistant	34
20	Drought tolerance	63
21	30–40% oil content	137
	Total	**410**

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potential valuable traits can be further exploited by plant breeders for the development of improved cultivars.

In addition there were four varieties of *Jatropha* in the LTS facility which comprise of Hansraj, SKN- Big, Chhatrapati and Urlikanchan where the seed oil content varied from 44% to 48% on kernel basis and protein content from 44% to 47%. The refractive index was in the range of 1.46–1.47.

Registration of Plant Germplasm

With the objective of giving credit to the scientists who have developed or identified promising experimental material (including parents of inbred lines) or promising germplasm and to facilitate flow of germplasm among the scientists working in crop improvement programs, the ICAR initiated germplasm registration mechanism in 1996 to register such promising germplasm. The information regarding these germplasm is put for public domain, which has become a mandatory requirement to safe guard the national resources with respect to IPR. Under this mechanism 6 *Jatropha* accessions have been registered (Table 24.5) in which two are registered for varied flower colours with continuous flowering from Directorate of Oilseeds Research, Hyderabad and other two are registered for high oil content promising (40.6 and 42%) oil content from NBPGR Regional Station, Hyderabad.

Conclusions

The past two decades have witnessed the development of new techniques for plant germplasm conservation which offers new options and permit conservation of diversity in the form of seeds, pollen, embryos and *in-vitro* cultures (Chandel et al. 1988). The rapid strides made in the past few years with regard to these novel approaches have enhanced the value of gene banks and clonal repositories. Despite substantial data, it is difficult to recommend universally applicable storage protocols for any specific species especially in case of tree species. This necessitates concerted research efforts on the study of behaviour of cells and cell constituents at all the stages of development and conservation. In *Jatropha*, several molecular tools can give an insight of the genetic distinctness of the crop in the centre of origin and other regions of its introduction which necessitates characterization of accessions with broader geographical background and comparison of genetic relationships with morphological characteristics in order to identify genetically divergent material to develop genetically superior stocks. Since the tree species has an easy adaptation to marginal and semi marginal lands, community level conservation can be encouraged by providing incentives to respective communities. Different species of *Jatropha* are valued for various economic traits *viz.* variation in fatty acid profile, photoperiod insensitivity,

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Table 24.5 *Jatropha* germplasm Registered in NGB

S. no.	National identity	Donor/other identity	INGR no.	Year	Pedigree	Dev. Institute	Trait
1	IC427819	Soumya	4117	2004	*J. curcas* x *J. integerrima*	DOR, Hyderabad	Pink flowers with continuous flowering
2	IC427820	Swetha	4118	2004	*J. curcas* x *J. integerrima*	DOR, Hyderabad	White flowers with continuous flowering
3	IC541650	BAAS-51	8086	2008	BAAS-51	NBPGR RS, Hyderabad	High oil content (40.6%)
4	IC537939	SNES-45	8087	2008	SNES-45	NBPGR RS, Hyderabad	High oil content (42%)
5	IC566227	FC&RI-HC32	9036	2009	(*J. curcas* (MTP1) X *J. integerrima*)	FCRI, TNAU, Mettupalayam, Tamil Nadu	For brownish purple colour fruit
6	IC566228	FC&RI-HC21	9037	2009	(*J. curcas* (MTP1) X *J. integerrima)*	FCRI,TNAU, Mettupalayam, Tamil Nadu	For oblong fruit

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higher oil content, resistance to insects, heavy fruit bearing (Banerji et al. 1985; Sujatha 1996, 2006). There is thus an immense scope for transfer of beneficial traits from other *Jatropha* species to *J. curcas* for heavy bearing, high oil content, desired oil quality, earliness, reduced toxicity of endosperm proteins and wider adaptability through efficient conventional and biotechnological approaches.

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Part IV
Biotechnology

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Chapter 25
Large Scale Micropropagation of *Jatropha curcas* for Quality Germplasm

Aneesha Singh and J. Chikara

Introduction

Clonal propagation through tissue culture has the potential to provide high multiplication rates of uniform genotypes, resulting in short-term production gains. As research progressed, tissue culture media became more defined and new tissue culture techniques were applied to *J. curcas* for a variety of purposes. These techniques have not yet been widely used for large-scale commercial operations. However, propagation of clones of *J. curcas*, selected for high oil content, provides the potential for rapidly establishing high oil yielding plantations.

Proliferation through axillary buds for plant regeneration is based on pre-existing meristems. In this method, axillary buds present in the axils are stimulated to develop into a branch. This technique exploits the natural ontogenic route of branch development by lateral (axillary) meristems. In vitro propagation through meristem culture is also the best possible means of virus elimination. A summary of reports on axillary shoot bud proliferation of *J. curcas* and species is given in Table 25.1.

Different methods of preparing cuttings in *J. curcas* have been reported (Camellia et al. 2009; Dhillon et al. 2009) but cuttings have their own limitations as they are prone to diseases and seasonal variation. Moreover, the technique cannot be used in case of generation of large number of plants from a single plant. The available planting material is indeterminate with variability in yield components and oil content, which are strongly influenced by environment (Heller 1996). Since *Jatropha* is in the stage of domestication, it is of prime importance to develop superior genotypes for commercial exploitation.

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Table 25.1 Studies on in vitro axillary shoot bud proliferation in *J. curcas*

S. no.	Species	Explants	Reference
1	*J. curcas* (non-toxic)	Nodal segments	Sujatha et al. (2005)
2	*J. curcas*	Shoot tips	Rajore and Batra (2005)
3	*J. curcas*	Nodal segments	Kalimuthu et al. (2007)
4.	*J. curcas*	Nodal segments	Datta et al. (2007)
5	*J. curcas*	Nodal segments	Murali and Sreenivasachar (2007)
5	*J. curcas*	Nodal segments	Shrivastava and Banerjee (2008)

Stages Involved in Micropropagation

A successful micropropagation protocol proceeds through a series of stages, each with a specific set of requirements. These are (1) initiation of aseptic cultures, (2) shoot multiplication, (3) rooting of microshoots, (4) in vitro hardening, and (5) field transfer of tissue culture raised plants.

Initiation of Aseptic Cultures

The success of plant tissue culture as a means of plant propagation is greatly influenced by the nature of the culture medium used i.e. the macro- and micro-element composition, vitamin mixture, sugar concentration, growth regulator composition and climatic conditions (Murashige and Skoog (MS) 1962; Staba 1969). The suboptimal culture medium may cause physiological disorders like reduced multiplication rate, hyperhydricity or tissue death (Barghchi and Alderson 1996; Van der Salm et al. 1994).

The choice of explant for initiation of culture is largely dependent on the method to be adopted for in vitro propagation. Explants with vegetative meristems are often suitable for enhanced axillary branching. The most commonly used explants are the nodal segments and shoot tips, wherein the axillary buds and shoot tips are made to proliferate to form multiple shoots.

For initiation of aseptic cultures, a thorough knowledge of the physiological status and the susceptibility of the plant species to different pathological contaminants are required. For most of the explants, the commonly adopted procedure involves surface disinfection of initial explants with a systemic fungicide, Bavistin/2–4 drops of liquid soap solution or 2% Teepol for 10–20 min prior to surface sterilization. They were surface-sterilized in 0.1% $HgCl_2$ (w/v) (Rajore et al. 2002; Lin et al. 2002; Rajore and Batra 2005; Deore and Johnson 2008; Shrivastava and Banerjee 2008) for 10–25 min followed by repeated washing (five times) with sterile distilled water. Following sterilization, the explants were trimmed (~1.0 cm) at the base and cultured with the cut surface in contact with the culture medium. Nodal segments were placed on MS medium supplemented with auxin and cytokinins for axillary

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shoot bud induction. Different cytokinins are known to give different morphological responses, which can partially be attributed to the differential mineral acquisition. Cytokinins are reported to play an important role in plant response to elements like Mn^{2+}, K^{+}, Na^{+}, Ca^{2+} and Cl^{-}, phosphate and nitrogen levels (Mudliar and Bharti 1984). Kinetin (Kn) treated nodal explants of *J. curcas* showed minimum variations in the endogenous macro-nutrient levels, when compared to control (MS medium). High nutrient accumulation was associated with the TDZ treatment. This could be a result of the relatively less 'dilution' of the mineral content in cultures exposed to TDZ. Reduced growth with increased mineral acquisition may also be considered as a response to the stress (Singh 2009).

Shoot Multiplication

Shoot multiplication is the most crucial stage of micropropagation. The success of a micropropagation protocol, to a large extent depends on the rate and mode of shoot multiplication. In vitro shoot proliferation and multiplication are based on media formulations containing cytokinins as the major PGRs. MS medium supplemented with BAP and IAA induced 6–12 multiple shoots (Reddy et al. 2009; Fig. 25.1a), IBA and Kn combination induced 30 multiple shoot buds (Datta et al. 2007), IAA with other additives induced three multiple shoots (Rajore and Batra 2007) and BAP + Kn + IAA induced 45 multiple shoots (Kalimuthu et al. 2007). Shrivastava and Banerjee (2008) reported induction of 100 shoots per explant on medium with BAP in combination with IBA. Activated charcoal when added to the culture medium promoted high frequency of multiple shoots in shoot tip cultures of *J. curcas* (Rajore and Batra 2005).

In Vitro and Ex Vitro Rooting of Microshoots

The in vitro rooting capacity depends on the interaction of internal and external factors. Shoots failed to induced root in either full or half-strength MS basal medium without PGRs in most of the studies (Rajore and Batra 2005; Kalimuthu et al. 2007; Datta et al. 2007) except few (Sujatha and Mukta 1996). In most of the earlier reports, varying concentrations of different auxins were used for root induction. However, Sujatha and Mukta (1996) reported rooting of microshoots on growth regulator free solidified medium. However, when 1.0–3.0 cm elongated shoots were placed on MS medium with 0.3–3.0 mg l^{-1} IBA, varying percentage of rooting was reported (Rajore and Batra 2005; Datta et al. 2007; Dubey et al. 2010). Addition of phloroglucinol (200 μM) to the medium enhanced the frequency of rooting to 76.7% (Kumar et al. 2010). Shrivastava and Banerjee (2008) reported that rooting was comparatively better in half strength MS medium with IBA. IAA (1.0 mg l^{-1}) was found to be a more suitable hormone for root induction than IBA in *Jatropha*

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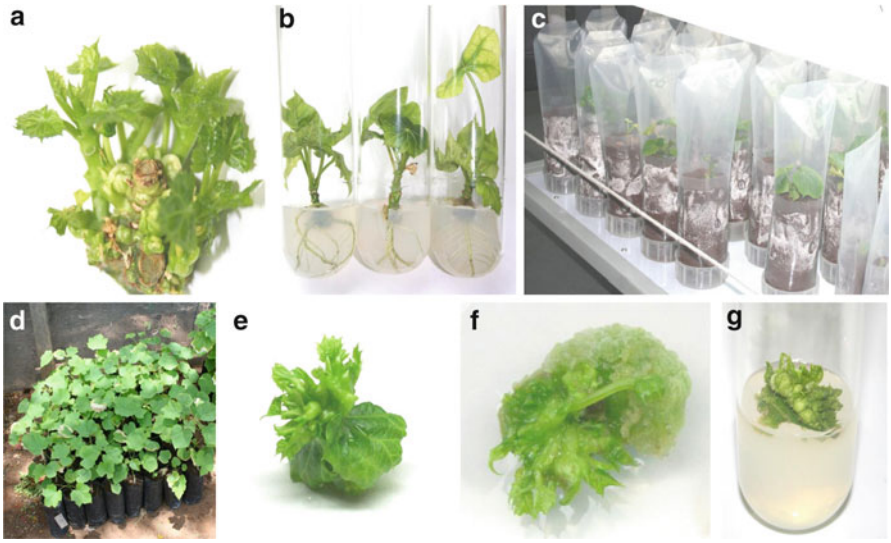

Fig. 25.1 Micropropagation of *J. curcas* using different (**a**) multiple shoot induction upon sub culture of shoot buds, (**b**) in vitro rooting (**c**) in vitro hardening, (**d**) micropropagated plantlets in nursery (**e**) leaf, (**f**) regeneration of callus, (**g**) and cotyledonary leaf explant

(Kalimuthu et al. 2007). More than two auxin combinations of IBA, IAA, and NAA were found to be best for rooting (Reddy et al. 2008, Fig. 25.1b). Few attempts have also been made for root induction under ex vitro conditions. Singh et al. (2010) developed a new method for ex vitro rooting of microshoots wherein shoots were encapsulated with sodium alginate. This protected the shoots from direct stress of soil and improved survival percentage when transferred to ex vitro conditions.

Hardening and Field Transfer

Successful acclimatization of micropropagated plants and their successive transfer to the field is a crucial step for commercial exploitation of in vitro technology. Various methods for hardening the rooted plantlets are known which are mainly based on the theory of gradual reduction of humidity around the plants and altering plant metabolism from partial dependence to complete independence on an external carbohydrate source. Plantlets were established within 1–2 months, which includes in vitro hardening in green house conditions (Fig. 25.1c). Survival rate was more than 85% in nursery/field conditions (Fig. 25.1d). Micropropagated plants were transferred to field to assess their performance as compared to cuttings and seedlings with reference to seed yield and it was observed that tissue culture raised plants were superior to cuttings and seed raised plants in the field. In the years

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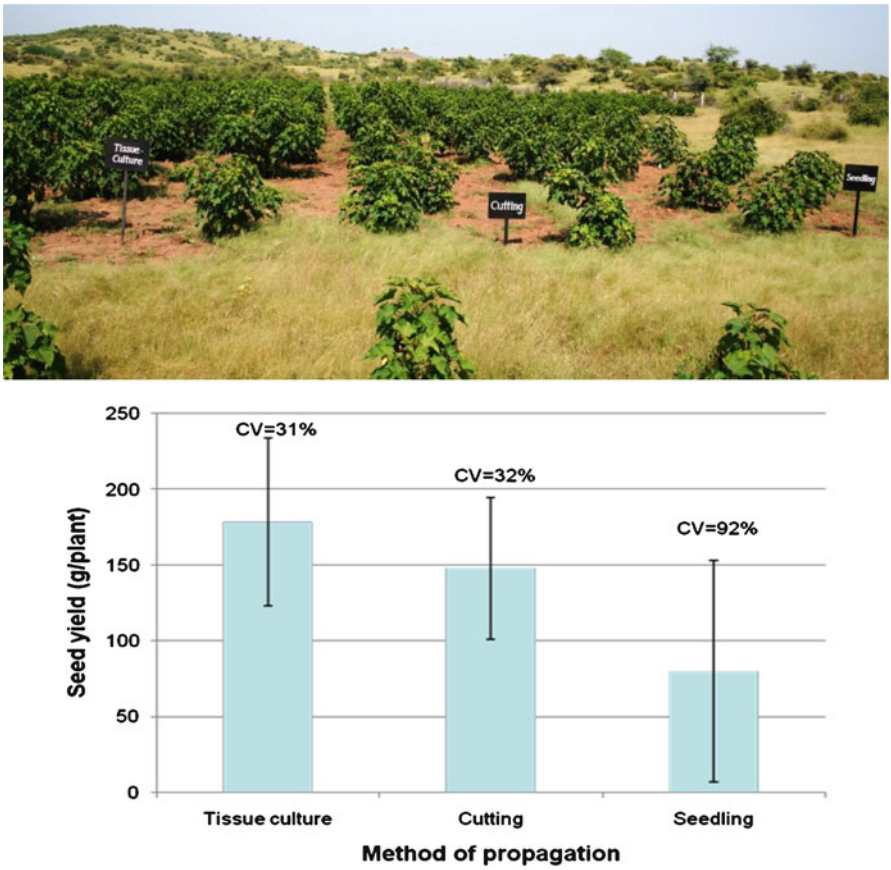

Fig. 25.2 Comparative performance of micropropagated, cuttings and seedlings (2008–2009) raised from single genotype (N = 10) (DOP: Aug, 06)

(2007–08 and 2008–09), plants raised through cuttings and tissue culture yielded higher with less coefficient of variation than seed raised plants (Fig. 25.2).

Plant Regeneration and Organogenesis

In vitro plant regeneration is often the most important step for successful implementation of various biotechnological techniques used for plant improvement programs. Induction of adventitious shoots and regeneration from callus cultures are of importance for induction of somaclonal variation in *Jatropha* due to lack of significant variability in the germplasm. A number of reports are available which indicate rapid regeneration and multiplication through organogenesis/somatic embryogenesis in

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Table 25.2 Somatic embryogenesis using different explants of *J. curcas*

S. no.	Explant	Reference
1	Cotyledonary leaf	Kalimuthu et al. (2007)
2	Leaf	Jha et al. (2007)
3	Callus	Sardana et al. (2000)

Table 25.3 Organogenesis using different explants of *J. curcas*

S. no.	Species	Explants	Reference
1	*J. curcas*	Hypocotyls, leaf, petiole	Sujatha and Mukta (1996)
2	*J. curcas*	Epicotyls	Qin et al. (2004)
3	*J. curcas* (non-toxic)	Leaf	Sujatha et al. (2005).
4	*J. curcas*	Cotyledonary leaf	Kalimuthu et al. (2007); Li et al. (2008); (Kumar et al. 2010b)
5	*J. curcas*	Leaf	Rajore and Batra (2007);Deore and Johnson (2008);Misra et al. (2010); Kaul et al. (2010); Kumar et al. (2010)
6	*J. curcas*	Petiole	Dubey et al. (2010); Kumar et al. (2010a)
7	*J. curcas*	Embryo	Varshney and Johnson (2010)
8	*J. curcas*	Hypocotyl, epicotyl	Kaewpoo and Te-chato (2010)

Jatropha. Organogenesis refers to the process of differentiation by which plant organs are formed adventitiously from the unusual points of origin of an organized explant where preformed meristems are lacking. Basically, there are two modes of organogenesis (1) Direct formation of buds from excised explants, and (2) indirect regeneration of plants via callusing. Direct organogenesis ensures clonal fidelity whereas; indirect organogenesis often shows genetic erosion. The regulation of organogenesis in vitro can be achieved by different types of manipulation.

Among the cytokinins, TDZ was found to be better than BAP for direct organogenesis (Reddy et al. 2008). Successful direct organogenesis through adventitious shoot regeneration and somatic embryogenesis using leaf, petiole and cotyledonary leaf explants has been reported in *J. curcas* (Dubey et al. 2010; Kumar et al. 2010, a, b; Misra et al. 2010; Kaul et al. 2010) (Fig. 25.1e.g; Tables 25.2and 25.3). Various other attempts were made for successful callus induction and callus mediated regeneration (Sardana et al. 2000; Lin et al. 2002; Lu et al. 2003; Weida et al. 2003; Wie et al. 2004; Sharma et al. 2006; Qin et al. 2006; Rajore and Batra 2007) (Fig. 25.1f). Several workers reported the formation of shoot primordials on calli derived from leaf explants using appropriate combinations of auxins and cytokinins. The use of MS medium with additives like adenine, additional copper, citric acid, glutamine, L-arginine, PVP and citric acid is reported (Shrivastava and Banerjee 2008; Datta et al. 2007; Rajore and Batra 2005; Varshney and Johnson; 2010; Kaul et al. 2010).

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Sujatha and Mukta (1996) developed a protocol for regeneration in *J. curcas* through callus derived from hypocotyl, leaf and petiole tissues. The major problem associated with callus-mediated regeneration is induction of somaclonal variations, a potential drawback when objective is to maintain clonal fidelity of the elite plants. Fidelity of regenerants obtained from epicotyl and hypocotyl explants was confirmed by flow cytometry and plantlets produced were uniform (Kaewpoo and Te-chato 2010). There is need to study the genetic fidelity of callus generated plantlets using molecular finger printing techniques.

These reports indicate that shoot regeneration in *J. curcas* could be obtained from leaves, hypocotyls, epicotyl, embryo, cotyledonary leaf and petioles.

Conclusions

Rapid production of disease-free elite clones and faster introduction of novel genotypes with desirable traits are of urgent need in *J. curcas* improvement programmes. Hence, various in vitro propagation techniques are of immense importance. At present, there are several reproducible protocols available for in vitro propagation of *J. curcas*. However, the new challenges that are faced today by the tissue culture industry are cost competence. Therefore, it is necessary to bring about further improvements in the existing protocols including ex vitro rooting that minimize the cost of plant regeneration and lead to commercialization of technology. In vitro propagation of *J. curcas* via somatic embryogenesis offers a great potential for rapid propagation and improvement. Direct regeneration protocols using leaf explants could be effectively used in maintaining the clonal fidelity of elites and in genetic transformation programs.

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Chapter 26
Jatropha Tissue Culture: A Critical Review on Present Scenario and Future Prospects

Nitish Kumar and Muppala P. Reddy

Introduction

Energy is an important source of development as it aims at human welfare covering household, agriculture, transport and industrial complexes. Like other natural resources, energy sources are renewable as well as non-renewable. Non-renewable hydrocarbons are being used as the major energy sources world over. Their rapid depletion has brought the situation where, the whole world is facing energy crisis, thus compelling the scientific community to search for renewable sources. One potentially promising option includes bio-fuel, which is renewable and do not contribute to the greenhouse effect and helps in reduction of particulate matter (PM), carbon mono-oxide (CO), unburned hydrocarbons and SO_2 emissions which are essential for the improvement of air quality. In Europe and USA, biodiesel is produced predominantly from edible oils, which are cultivated on arable lands. About 130 million hectares of eroded wasteland exist in India, which is currently unsuitable for food production. The recognition that *Jatropha* oil can yield an exceptional quality biodiesel has led to a surge of interest in *Jatropha* across the globe, more so in view of the potential for avoiding the dilemma of "food vs. fuel" (Ghosh et al. 2007).

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Besides biodiesel, *Jatropha* cultivation can make considerable impact in promoting organic farming through use of deoiled seed cake. The fruit shells having a calorific value equivalent to coal can be made into briquettes and used in solid fired boilers and other applications as a substitute for fossil fuel.

Jatropha is a genus of approximately 175 succulent plants, shrubs and trees from the family Euphorbiaceae, native to Central America and has become naturalized in many tropical and subtropical areas, including India, Africa and North America (Fairless 2007). *J. curcas* also called physic nut is considered as one of the best candidates for future biodiesel production (Heller 1996; Fairless 2007). Biodiesel prepared from *J. curcas* has been successfully tested in both mobile and stationary engines without modification in any of the engine parts. Now there is a surge of interest in *J. curcas* as a biodiesel "miracle tree" to help alleviate the energy crisis and generate income in rural areas of developing countries (Martin and Mayeux 1985; Jones and Miller 1991; Openshaw 2000; Francis et al. 2005; Mandpe et al. 2005; Ghosh et al. 2007). Low yields and significant differences reported in seed yield and oil content (Kaushik et al. 2007; Ghosh et al. 2007; Jongschaap et al. 2007) have been the limiting factors for large scale cultivation of this crop.

Genetic improvement of this plant is now a major target for scientists. Two major approaches i.e., agricultural practices and biotechnological approaches have been suggested for realization of the potential of the crop. The biotechnological approaches to address the problem include propagation of selected high yielding genotypes using micropropagation/tissue culture techniques and introgression of desirable agronomic traits through genetic engineering. Biotechnological crop improvement thus, appears to be one of the time effective, alternative approach wherein, transgenic production assumes importance. For improvement through transgenic approach, efficient plant regeneration protocol from isolated plant cells or tissue is a prerequisite. The present review, will briefly discuss the recent findings and thoughts in these areas with particular emphasis on micropropagation.

Micropropagation

J. curcas is primarily propagated through seeds and significant variation in seed yield and oil content was observed in plantations raised through seeds. The seed viability and rate of germination are low and quality seed screening is another laborious task thus, propagation through seed may not provide quality planting material for sustainable agriculture. It was also observed that huge quantities of seeds are being utilized for raising the planting material. Alternatively, vegetative propagation techniques could help in raising quality planting material while seed can be diverted for biodiesel preparation. Propagation can also be carried out without losing the traits by stem cuttings but the limitations in generation of large scale planting materials are (a) availability of sufficient quantity of material, and (b) vegetative propagation is seasonal. Thus, conventional propagation through seeds

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is not reliable and vegetative propagation by stem cuttings is inadequate to meet the demand. Therefore, improvement of *J. curcas* by modern methods of agro-biotechnology is of interest world-wide. This has increased the importance and need for developing micropropagation methods to facilitate large scale production of true-to-type plants and for the improvement of the species using genetic engineering techniques.

Micropropagation is an alternative method of vegetative propagation, which is well suited to the multiplication of elite clones, offers many advantages, is not limited by the number of selected elite genotypes, produces pathogen-free plants and can provide a commercial production system within a limited time frame and space. The techniques can also be used for genetic improvement of the species. Micropropagation is accomplished by several means, i.e. multiplication of shoots from different explants such as shoot tips or axillary buds; or direct formation of adventitious shoots or somatic embryos from tissues, organs or zygotic embryos.

Several efforts have been made to establish protocols for micropropagation and regeneration from different explants of *Jatropha*. The earlier investigations were confined to endosperm culture of *J. panduraefolia* (Syn. *J. integerrima*). A continuously growing tissue culture of mature endosperm of *J. panduraefolia* was obtained on white's medium supplemented with 2,4-dichlorophenoxyacetic acid (2,4-D), kinetin, and yeast extract (YE) (Srivastava and Johri 1974; Srivastava 1997). In *J. panduraefolia*, the mature endosperm needs the association of an embryo for its proliferation (Srivastava 1997). Organogenesis from proliferated endosperm cultures and formation of triploid plantlets has also been reported in *J. panduraefolia* (Johri and Srivastava 1973).

Among the species studied, *J. integerrima* expressed great propensity for caulogenesis that occurred at high frequencies (75–100%) from all explants including floral tissues (Sujatha and Dhingra 1993; Sujatha and Reddy 2000; Sujatha et al. 2000). High regenerability of *J. integerrima* supported studies on understanding the biochemical and histological changes that occur during in vitro organogenesis (Sujatha et al. 2000). These studies reveal the scope for improvement of *J. curcas* through somatic hybridization in interspecific crosses limited by crossability barriers. Further, the tissue culture protocols serve as a prelude for improvement through biotechnological tools as well.

Micropropagation of J. curcas

Low and unstable seed yield, non availability of superior clones, shortage of cuttings has necessitated the development of micropropagation techniques in *J. curcas.* Protocols for axillary bud proliferation or shoot tip multiplication have been developed from different meristematic tissues (Sujatha and Mukta 1996; Sardana et al. 1998; Lin et al. 2002; Rajore et al. 2002; Sujatha et al. 2005; Rajore and Batra 2005; Sharma et al. 2006; Qin et al. 2006; Datta et al. 2007; Kalimuthu et al. 2007; Shrivastava and Banerjee 2008; Thepsamran et al. 2008; Singh 2009). In all the

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studies, benzyl aminopurine (BAP), kinetin, have been used in combination with or without auxins and up to 100% efficiency of shoot bud induction was achieved. The pioneering work of Sujatha and Mukta (1996) revealed the importance of BAP and indole-3-butyric acid (IBA) combination for achieving shoot multiplication from shoot tips. Kalimuthu et al. (2007) reported that BAP, kinetin and indole-3-acetic acid (IAA) combination is more efficient in multiple shoot bud induction from shoot tip and nodal segments. According to Datta et al. (2007), axillary shoot bud proliferation was best initiated on Murashige and Skoog's (MS) basal medium supplemented with 22.2 μM BAP and 55.6 μM adenine sulphate, in which cultures produced 6.2 shoots per nodal explant. It is also reported that BAP is more efficient as compared to other cytokinins in multiple shoot bud induction from shoot tip and nodal segments (Sujatha et al. 2005; Kalimuthu et al. 2007; Datta et al. 2007). Regeneration in *J. curcas* is also reported to be highly genotype dependent and thidiazuron (TDZ) was more efficient as compared to BAP in shoot regeneration (Kumar 2009; Singh 2009; Kumar et al. 2010a, b, c, 2011a, b; Kumar and Reddy 2010, 2012; Singh et al. 2010; Sharma et al. 2011). Regeneration protocols through shoot regeneration and somatic embryogenesis are available for *J. curcas* (Sujatha and Mukta 1996; Sardana et al. 2000; Lu et al. 2003; Wei et al. 2004; Sujatha et al. 2005; Rajore and Batra 2007; Jha et al. 2007). All the above studies reported were either through callus-mediated regeneration or direct shoot morphogenesis with interspersed callus. Despite the availability of regeneration systems from different explants of *J. curcas*, the presence of intermediary callus or callus-mediated regeneration is least desired for the production of true-to-type plants. Recently, direct regeneration using different types of explants has been reported (Deore and Johnson 2008; Kumar 2009; Dubey et al. 2010; Kumar and Reddy 2010, 2012; Kumar et al. 2010a, b, c, 2011a, b; Singh et al. 2010; Khemkladngoen et al. 2011; Sharma et al. 2011). In all these studies, cytokinins (BAP, TDZ or both) have been used in combination with or without auxins (IAA and IBA) and up to 100% regeneration efficiency was achieved. According to Kumar (2009), besides concentrations and combinations of plant growth regulators (PGRs), regeneration efficiency also depends on orientation, source, type of explants, and genotype used (Mazumdar et al. 2010). The percentage response of explants forming shoot buds increased with increase in the concentration of TDZ (Deore and Johnson 2008; Kumar 2009; Kumar et al. 2010a, b, c, 2011a, b; Kumar and Reddy 2010, 2012; Sharma et al. 2011). In vitro explants had higher rates of regeneration efficiency and more number of shoot buds as compared to in vivo explants (Kumar and Reddy 2010, 2012 Kumar et al. 2010a, b, c, 2011a, b; Sharma et al. 2011). The regeneration efficiency and number of shoot buds were higher in cotyledonary leaf and petiole as compared to mature leaf and petiole (Kumar 2009) and higher in leaf than petiole segements (Sujatha and Mukta 1996; Kumar 2009). The regeneration efficiency and number of shoot buds were higher in horizontally placed petiole explants as compared to vertically placed explants (Kumar and Reddy 2010).

The percentage of rooting differed significantly and depending upon the concentrations and combinations of auxins used *viz.* IBA, IAA and NAA, the best rooting was observed on half strength MS medium supplemented with 15 μM IBA, 5.7 μM

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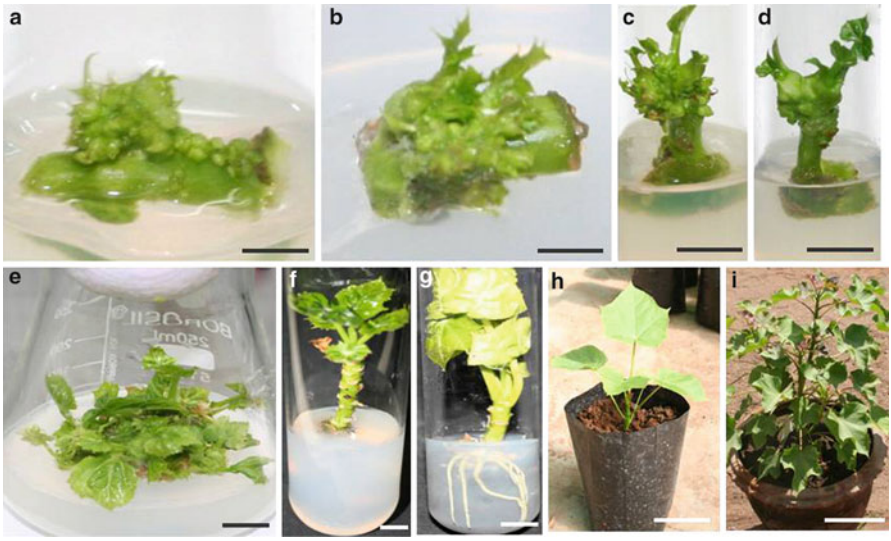

Fig. 26.1 Direct shoot bud induction from petiole explants of *J. curcas.* Direct shoot bud induction from (**a**) in vitro petiole in horizontal position (bar 5 mm), (**b**) in vivo petiole in horizontal position (5 mm), (**c**) in vitro petiole in vertical position (bar 5 mm) and (**d**) in vivo petiole in vertical position on MS medium with 0.5 mg l^{-1} TDZ after 6 weeks (bar 5 mm). (**e**) Shoot proliferation of induced shoot buds on MS medium with 2 mg l^{-1} kinetin + 1 mg l^{-1} BAP + 1 mg l^{-1} NAA after 4 weeks (bar 100 mm). (**f**) Elongation of proliferated shoot on MS medium with 0.5 mg l^{-1} BAP and 1.5 mg l^{-1} IAA after 6 weeks (bar 1 mm). (**g**) Development of roots at the base of elongated shoot on half strength of MS medium with 2% sucrose + 3 mg l^{-1} IBA + 1 mg l^{-1} IAA + 1 mg l^{-1} NAA + 0.25 mg l^{-1} activated charcoal after 4 weeks (bar 1 mm). (**h**) Regenerated plants in polybags after 4 weeks (bar 100 mm). (**i**) Regenerated plant in pot after 6 month under natural condition (bar 100 mm) (Source: Kumar and Reddy 2010)

IAA, 5.5 μM NAA (Kumar and Reddy 2010). Rooting frequencies were high and rooting was successful on growth regulator-free medium (Sujatha and Mukta 1996; Jha et al. 2007), 5.5 μM NAA (Sujatha et al. 2005) and 0.5–1.5 μM IBA (Datta et al. 2007; Li et al. 2008). Rooted plants survived with more than 80–90% success (Kumar 2009; Kumar and Reddy 2010, 2012; Kumar et al. 2010a, b, c, 2011a, b; Singh et al. 2010; Sharma et al. 2011). An example of complete regeneration protocol from petiole (Fig. 26.1) and cotyledonary leaf (Fig. 26.2) explants are shown.

The high cost of production is one of the limitations on the practical application of micropropagation for commercial application. Ex vitro rooting is a promising method, skipping the phase of in vitro rooting and reduces the time significantly besides reducing the cost. Singh et al. (2010) reported a method for direct rooting of elongated *J. curcas* shoots in the soil by encapsulating the shoots in growth hormones, sodium alginate and calcium chloride matrix.

Effect of nickel and copper on regeneration from leaf explants has been investigated (Sarkar et al. 2010; Khurana-Kaul et al. 2010) and Sarkar et al. (2010) observed that percent regeneration decreased with increase in addition of nickel

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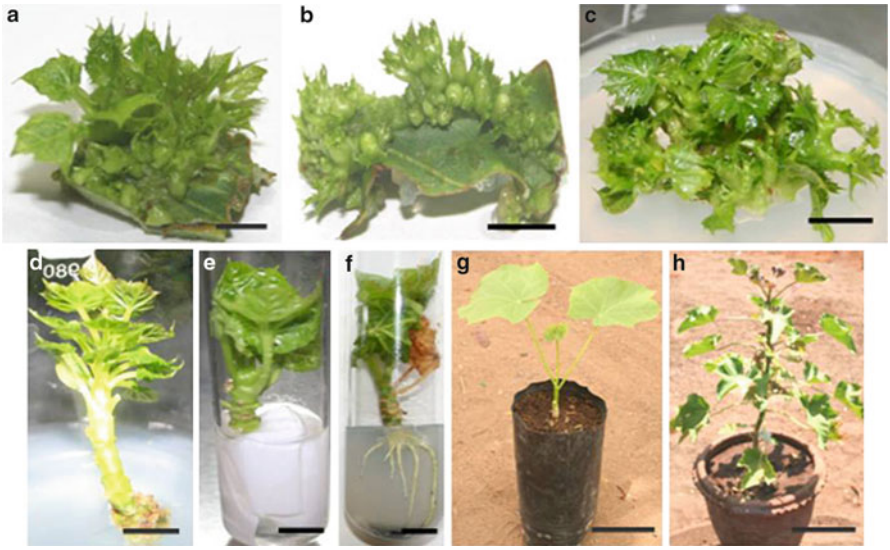

Fig. 26.2 Shoot regeneration from cotyledonary leaf explants of *J. curcas.* Direct organogenesis from (**a**) in vitro cotyledonary leaf explant (bar 5 mm), (**b**) in vivo cotyledonary leaf explant (bar 5 mm) on MS medium with 0.5 mg l^{-1} thidiazuron (TDZ) after 6 weeks. (**c**) Shoot proliferation of induced shoot buds on MS medium with 2 mg l^{-1} kinetin (Kn) + 1 mg l^{-1} 6-benzyl aminopurine (BAP) + 1 mg l^{-1} α-naphthaleneacetic acid (NAA) after 4 weeks (bar 100 mm). (**d**) Elongation of proliferated shoot on MS medium with 0.5 mg l^{-1} BAP + 1.5 mg l^{-1} indole-3-acetic acid (IAA) after 6 weeks (bar 5 mm). (**e**) Elongated shoot cultured on half strength basal MS liquid medium supplemented with 3 mg l^{-1} indole-3-butyric acid (IBA) + 1 mg l^{-1} IAA + mg l^{-1} NAA for root induction (bar 5 mm). (**f**) Development of roots at the base of auxins treated elongated shoot on half strength basal MS medium with 0.25 mg l^{-1} activated charcoal after 4 weeks (bar 1 mm). (**g**) Regenerated plant in polybag after 4 weeks (bar 150 mm). (**h**) Regenerated plant in pot soil after 6 month under natural condition (100 mm) (Source: Kumar et al. 2010c)

(1.0 mM) to the medium (Fig. 26.3). According to Khurana-Kaul et al. (2010) significant improvement in shoot bud induction was observed when the concentration of copper sulphate was increased to 10 times the normal MS level.

Micropropagation of J. curcas (Non-toxic)

J. curcas as an energy plant has attained great attention in recent years; however, oil and deoiled cake are toxic despite having the best protein composition (Makkar et al. 1998). A non-toxic variety reported from Mexico is found suitable for human consumption and its innocuous nature was established using experimental models (Makkar and Becker 1997). Cultivation of the non-toxic variety of *J. curcas* is more important as it can provide oil and seed cake for live stock providing additional value to the crop. However, the major limitation with this variety is low and incon-

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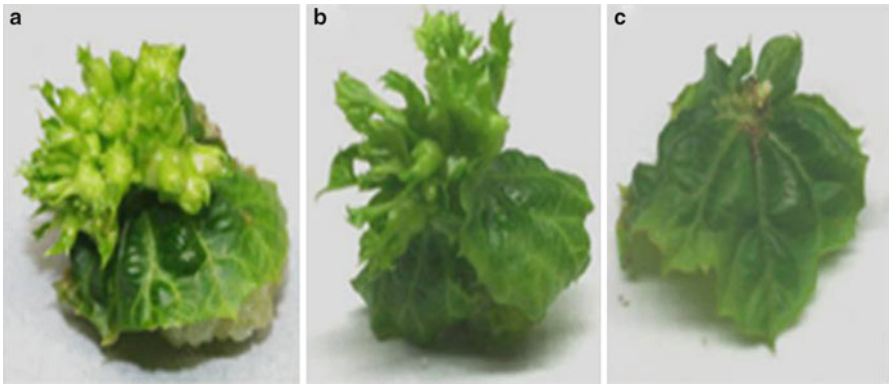

Fig. 26.3 Regeneration of shoots buds from leaf explants in nickel amended MS medium containing 0.5 mg l^{-1} TDZ (**a**) control with no nickel; (**b**) 0.01 mg l^{-1} nickel; (**c**) 0.1 mg l^{-1} nickel (Source: Sarkar et al. 2010)

sistent yield. To improve the species, micropropagation techniques have been initiated (Sujatha et al. 2005). Kumar et al. (2010a) using TDZ achieved direct shoot regeneration from different explants of non-toxic *J. curcas* (complete regeneration from petiole explants of non-toxic *J. curcas* is shown in Fig. 26.4). The regeneration efficiency and number of shoot buds were higher in horizontally placed explants as compared to vertically placed explants (Kumar et al. 2011b).

Somatic Embryogenesis in *J. curcas*

Somatic embryos, which are bipolar structures, arise from individual cells and have no vascular connection with the maternal tissue of the explants. Embryos may develop directly from somatic cells (direct embryogenesis) or development of recognizable embryogenic structures is preceded by numerous, organized, non-embryogenic mitotic cycles (indirect embryogenesis). Somatic embryogenesis has a great potential for clonal multiplication. Under controlled environmental conditions, somatic embryos germinate readily, similar to their seedling counterparts. Jha et al. (2007) reported an efficient protocol for regeneration through somatic embryogenesis. Frequency of embryogenic callus formation was 56.0% with embryo conversion rate of 80% producing an average of 58.5 somatic embryos per callus. Globular somatic embryo induction was observed after 4–6 weeks of culture initiation and conversion of somatic embryos to germinated plantlets took 4–6 weeks. Somatic embryogenesis was associated with induction of a low frequency of secondary embryogenesis which also matured into whole shoots. Varshney and Johnson (2010) reported efficient plant regeneration from immature embryos of *J. curcas* and it was found that the size of embryo is critical for the

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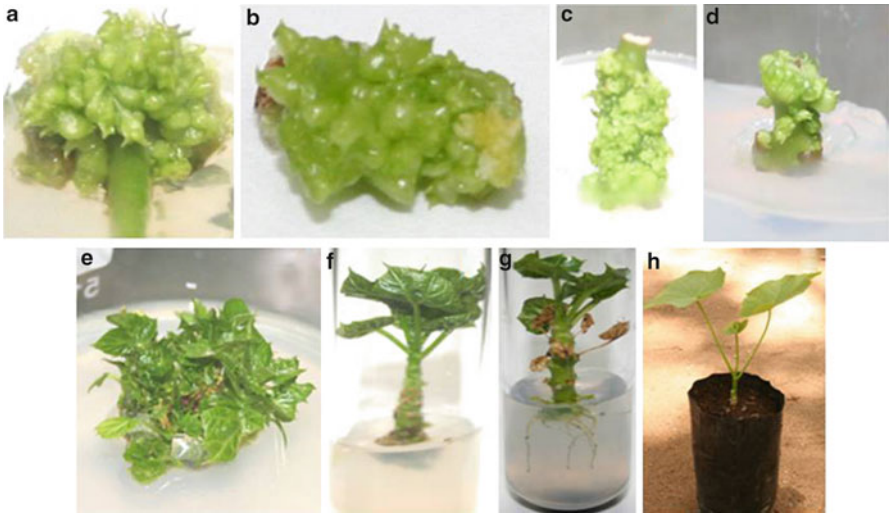

Fig. 26.4 Direct shoot bud induction from petiole explants of non-toxic *J. curcas.* Direct shoot bud induction from (**a**) in vitro petiole in horizontal position, (**b**) in vivo petiole in horizontal position, (**c**) in vitro petiole in vertical position, and (**d**) in vivo petiole in vertical position on MS medium with 0.5 mg l^{-1} TDZ after 6 weeks. (**e**) Shoot proliferation on MS medium with 2 mg l^{-1} Kinetin + 1 mg l^{-1} BAP + 1 mg l^{-1} NAA after 4 weeks. (**f**) Elongation of shoot on MS medium with 0.5 mg l^{-1} BAP and 1.5 mg l^{-1} IAA after 6 weeks. (**g**) Development of roots on half-strength of MS medium with 3 mg l^{-1} IBA +2 mg l^{-1} IAA +1 mg l^{-1} NAA + 0.25 mg l^{-1} activated charcoal after 4 weeks. (**h**) Regenerated plant in polybag (Source: Kumar et al. 2011b)

establishment of callus. Immature embryos (1.1–1.5 cm) obtained from the fruits collected 6 weeks after pollination showed a good response of morphogenic callus induction (85.7%) and subsequent plant regeneration (70%) with the maximum number of plantlets (4.7/explant) on MS medium supplemented with IBA (0.5 mg l^{-1}) and BA (1.0 mg l^{-1}). The above medium when supplemented with growth adjuvants such as 100 mg l^{-1} casein hydrolysate, 200 mg l^{-1} L-glutamine and 8.0 mg l^{-1} $CuSO_4$ resulted in an even higher frequency of callus induction (100%). Plant regeneration (90%) with the maximum number of plantlets (10/explant) was achieved on MS medium supplemented with 500 mg l^{-1} polyvinyl pyrrolidone, 30 mg l^{-1} citric acid, 1 mg l^{-1} BA, 0.5 mg l^{-1} Kn, and 0.25 mg l^{-1} IBA. It was observed that plantlet regeneration could occur either through organogenesis of morphogenic callus or via multiplication of pre-existing meristems in immature embryos. The age of immature embryos and addition of a combination of growth adjuvants to the culture medium appear to be critical for obtaining high regeneration rates. Well-developed shoots rooted on half-strength MS medium supplemented with 0.5 mg l^{-1} IBA and 342 mg l^{-1} trehalose. Recently, Cai et al. (2011) developed an efficient method for somatic embryogenesis in three accessions collected from China, India and Indonesia. Indirect somatic embryogenesis was achieved when endosperm tissue and immature embryos between 0.5 and

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1.0 cm in length were cultured in a medium with 2,4-D, preferably at 5–10 mg l^{-1}, followed by a shift to a hormone-free medium supplemented with glutamine and asparagine. Production of secondary embryos was improved by supplementing KNO_3, glutamine and asparagine. 2,4-D (0.1–0.2 mg l^{-1}) and PEG 8000 (5–10%) were essential for maintenance of embryogenic calli in liquid medium. Regeneration took as short as 3 months using the suspension cultures and over 95% of the regenerated trees were able to flower and set seeds with no discernable morphological abnormality.

Conclusions

Jatropha is seen as a very promising option for the production of bio-fuel from degraded land and is universally accepted as an energy crop. However, the adoption and implementation of the concept has advanced comparatively slowly so far due to non availability of high yielding genotypes for large scale plantations. Conventional breeding has to be integrated with latest biotechnological approaches to introduce some of the traits such as higher seed yield, oil content, synchronous maturity and early flowering to make the technology tailor made. More in depth studies on exploitation of somaclonal variations, mutations, doubled haploids, and genetic transfer for improvement of *J. curcas* for agronomically desirable traits assumes importance. Application of information from *Jatropha* genome project can be used for further improvement through genomics. The great potential of micropropagation for large-scale multiplication needs further studies to make the technology sufficiently robust to allow commercial application by improving the rooting and also including more diverse accessions and low-cost tissue culture techniques to reduce the unit cost plant production without compromising the quality. Oil production and esterification in vitro is an unexploited area and worth pursuing.

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Chapter 27
Tissue Culture Studies of *Jatropha* Species: A Review

E. Chamundeswari, S. Goverdhan, and N. Rama Swamy

Introduction

Jatropha is a large genus comprising more than 170 species. The commonly occurring species in India are *J. curcas, J. glandulifera, J. gossypiifolia, J. multifida, J. nana, J. panduraefolia, J. integerrima, J. villosa, J. heynei, J. maheshwarii, J. tanjorensis* and *J. podagrica.* Most of these species are wild but some are ornamentals; except for *J. curcas* and *J. glandulifera* which are oil yielding species (Swarup 2004). The seeds of Jatropha contain 30–40% oil with a fatty acid pattern similar to that of edible oils (Gubitz et al. 1999). Jatropha oil contains linoleic and oleic acids which together account for up to 80% of the oil composition. Palmitic and stearic acids are the other fatty acids present in this oil. *J. curcas* has attracted particular attention as a tropical energy plant. The seed oil of *J. curcas* can be used as a diesel engine fuel, for it has characteristics close to that of the fossil fuel, diesel. *J. curcas* seed yields approach 6–8 MT ha^{-1} with approximately 37% oil (Deore and Johnson 2008). In view of its importance in production of biodiesel, according to our literature survey, several reviews on in vitro micropropagation have been published in the last decade on *J. curcas* (Sujatha and Mukta 1996; Weida et al. 2003; Sujatha et al. 2005; Sujatha and Prabakaran 2003; Datta et al. 2007; Deore and Johnson 2008; Misra and Misra 2010; Mukherjee et al. 2011). Reddy et al. (1986) studied the efficiency of callus induction from leaf explants in four *Jatropha* species *viz., J. podagrica*, *J. gossypiifolia*, *J. glandulifera* and *J. curcas* using various auxins in different concentrations and combinations (MS + NAA + 2,4-D; MS + 2,4-D 0.5 + Kn1.0). Goverdhan (1996) made preliminary in vitro studies of *J. gossypiifolia* (two varieties), *J. glandulifera, J. podagrica, J. multifida, J. integerrima* and *J. tanjorensis* using various explants. To our knowledge, there is no review on other *Jatropha* species, and we report the work on in vitro micropropagation in other species of *Jatropha* except *J. curcas.*

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In Vitro Micropropagation

Plant tissue culture technologies are now being routinely used for micropropagation, secondary metabolites, germplasm conservation, etc. Micropropagation without an intervening callus phase is advantageous over conventional vegetative propagation in terms of quantity, quality and economics (Altman and Loberant 1998). In general three modes of in vitro plant regeneration have been in practice, organogenesis, embryogenesis and proliferation of axillary and apical meristems. The difference mainly matters, when it relates to the genetic stability of the resulting micropropogated plants; the obvious option then would be axillary and shoot meristem proliferation and somatic embryogenesis through which true-to-type of plants are developed. In vitro micropropagation has been proven in the recent past as a means for rapid multiplication of the planting material. Different basal media, plant growth regulators, media additives and carbohydrate sources are being used to manipulate culture conditions for in vitro propagation of commercially important plant species like *Jatropha.*

J. panduraefolia Andr.

Srivastava (1971) and Srivastava and Johri (1974) worked on the tissue culture of *J. panduraefolia* and could raise triploid plants from endosperm cultures. The mature endosperm was cultured on White's medium supplemented with 2, 4-D + kinetin + yeast extract (YE) for induction of callus. These calli were transferred on to WB medium with NAA + kinetin + casein hydrolysate (CH). They observed differentiation of shoot buds and roots all around the callus.

In vitro morphogenesis of *J. panduraefolia* was studied by Chamundeswari (1997). Different types of explants *viz.,* leaf, shoot apex, and zygotic embryos were cultured on MS (Murashige and Skoog 1962) medium fortified with different concentrations and combinations of plant growth regulators (Table 27.1). Shoot bud proliferation through callus was observed from shoot meristem cultures and zygotic embryos.

J. gossypiifolia L. var. *elegans*

A perennial ornamental shrub up to 3 m tall, native to Brazil. Young leaves are purple in the variety *elegans* whereas all the parts are green in variety *gossypiifolia.* The variety *gossypiifolia* possesses fewer hairs in the fruit and the fruit size will be larger.

Goverdhan (1996) studied *J. gossypiifolia* var. *elegans* and noted good callogenesis from leaf explants on MS medium supplemented with 0.5 mg l^{-1} NAA with 2.0 mg l^{-1} BAP and 3.0 mg l^{-1} kinetin. A combined treatment of two cytokinins and an auxin *viz.,* BAP + KN + NAA in two concentrations were tested. In MS + KN 2.0 mg l^{-1}, BAP 1.0 mg l^{-1} and NAA 0.5 mg l^{-1} the shoot apex explants of

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Table 27.1 In vitro morphogenesis from different explants of *J. panduraefolia* Andr.

Explant	Concentration of PGRs (mg l^{-1})	Morphogenesis
Leaf	1 BAP + 2 KN + 1 NAA	Callus
Shoot apex	3 AS + 3 BAP	Callus, with formation of shoot bud primordial
	2 BA + 2 KN	Formation of multiple shoots after 4 weeks
	1 AS + 1 TDZ	Multiple shoot buds at base of shoot apex
Embryo	2 BAP + 1 KN + 1 NAA	Shoot with callus
	1 BAP + 2 KN + 1 IBA	Growth of shoot

BAP 6-benzylamino purine, *KN* kinetin, *NAA* naphthaleneacetic acid, *AS* adenine sulphate, *TDZ* thidiazuron, *IBA* indolebutyric acid

J. gossypiifolia var. *elegans* produced good amount of callus. However best response with respect to callogenesis was seen in MS + KN 3.0 mg l^{-1}, BAP 1.0 mg l^{-1}, NAA 0.5 mg l^{-1}. Shoot apex, cotyledon explants of *J. gossypiifolia* var. *elegans* produced good amount of callus in medium with adenosine, and in combined treatment of putrescine 0.5 mg l^{-1} + KN 1.0 mg l^{-1} + NAA 2.0 mg l^{-1}.

In vitro morphogenetic events from different explants *viz.,* leaf, cotyledons, hypocotyls, shoot meristems was studied and only callus was induced from all the explants studied except shoot meristems (Chamundeswari 1997). Zygotic embryos of *J. gossypiifolia* var. *elegans* were cultured on MS medium augmented with various concentrations and combinations of plant growth regulators. At 2 mg l^{-1} adenine sulphate, shoot primordia were initiated from shoot meristem cultures.

J. gossypiifolia var. *gossypiifolia*

Shoot apex and hypocotyl explants of *J. gossypiifolia* var. *gossypiifolia* produced roots on medium with 1.0 mg l^{-1} *putrescine* (Put) + 1.0 mg l^{-1} *spermine* (SP) and 1.0 mg l^{-1} *spermidine* (SPM) (Goverdhan 1996).

In vitro morphogenesis from different explants of *J. gossypiifolia* var. *gossypiifolia* was studied by Chamundeswari (1997). Seed and seedling explants *viz.,* leaf, cotyledon, shoot apex, zygotic embryo, and endosperm were cultured on MS medium supplemented with various combinations and concentrations of plant growth regulators. These included BAP+KN+NAA, BAP+KN+IBA, BAP+KN+IAA, and BA+KN. Greenish white callus was induced from all the explants used. Whereas shoots were developed from shoot meristem cultures at 2 mg l^{-1} adenine sulphate, 1 mg l^{-1} BAP, and 2 mg l^{-1} IBA from endosperm cultures and plantlets from zygotic embryo cultures.

J. glandulifera Roxb

J. glandulifera is a small bushy shrub found all over India and has greenish yellow flowers and green leaves. It has glandular hairs only in axis of leaves. Biological activity of shoots against cancer is reported (Hussain et al. 1992).

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Goverdhan (1996) reported callus from leaf explants of *J. glandulifera* on subculture in MS medium supplemented with BAP 1.0 mg l^{-1} + NAA 0.5 mg l^{-1} and after 6 weeks rhizogenesis occurred. Shoot apex of *J. glandulifera* produced bunch of roots from basal region in BA 2.0 mg l^{-1} however, MS + BAP 1.5 mg l^{-1} + NAA 0.5 mg l^{-1}; MS + BAP 2.0 mg l^{-1} + NAA 0.5 mg l^{-1} produced more compact callus without any organogenesis. Shoot apices of *J. glandulifera* grown on MS + Put 0.5 mg l^{-1} + KN 1.0 mg l^{-1} + NAA 2.0 mg l^{-1} gave identical response in terms of callusing ability in 6 weeks and roots developed from the base of shoot apex. Other combinations (MS + SPM 0.5 mg l^{-1} + KN 1.0 mg l^{-1} + 2,4-D 2.0 mg l^{-1}; MS + SP 1.0 mg l^{-1} + BAP 1.5 mg l^{-1}; MS + SP 1.0 mg l^{-1} + BAP 2.0 mg l^{-1}; MS + SP 2.0 mg l^{-1} + BAP 2.0 mg l^{-1} and MS + SP 2.5 mg l^{-1} + BAP 2.0 mg l^{-1}) were found to be excellent in enhancing callusing. Shoot apex explants of *J. glandulifera* produced good amount of callus on MS + KN 2.0 mg l^{-1} and MS + BA 2.0 mg l^{-1} + KN 2.0 mg l^{-1} and compact translucent callus from anthers of *J. glandulifera* on MS + NAA 5.0 mg l^{-1}. Callus derived from leaf explants of *J. glandulifera* sub cultured in MS + Put 0.25, 0.5, 1.0. 2.0, and 3.0 mg l^{-1} gave identical response in terms of callusing ability. The callus was cream in colour, compact and translucent without organogenesis.

In vitro morphogenesis from different explants of *J. glandulifera* was studied by Chamundeswari (1997). Various explants *viz.,* leaf, cotyledon, shoot meristems, anthers, pistil, zygotic embryos and endosperm were cultured on MS medium augmented with different concentrations and combinations of growth regulators. The combination of growth regulators tested include 2,4-D, NAA, BAP+NAA, BAP+KN+NAA, KN+2,4-D, KN+BAP. White compact callus induction was noted from all the explants cultured. Whereas shoot bud proliferation was observed from shoot meristems (Fig. 27.1) at 1 mg l^{-1} BAP+2 mg l^{-1} KN+1 mg l^{-1} IAA and also at 2 mg l^{-1} AS and from zygotic embryos on 15 mg l^{-1} CM and 3 mg l^{-1} BAP.

J. tanjorensis Ellis and Saroja

This species is a natural interspecific hybrid between the two highly incompatible species *J. curcas* and *J. gossypiifolia.* Since there was no seed set, propagation through tissue culture technology is useful. Prabakaran and Sujatha (1999) have attempted the in vitro micropropagation of the species. Leaf explants were cultured on MS medium fortified with 0.5–5.0 mg l^{-1} BA in combination with 0.5–1.0 mg l^{-1} IBA. Shoot regeneration was induced on medium with BA at concentrations of 2.0–10.0 mg l^{-1} and best regeneration was obtained on medium with 5.0 mg l^{-1} BA. The number of shoots per responding calli was maximum from leaf discs initially cultured on medium containing 10.0 mg l^{-1} BA and 0.1 mg l^{-1} IBA.

Goverdhan (1996) using leaf and shoot apex explants was able to produce compact callus on MS medium fortified with BA 1.0–2.0 mg l^{-1} + IAA 1.0 mg l^{-1} without further response.

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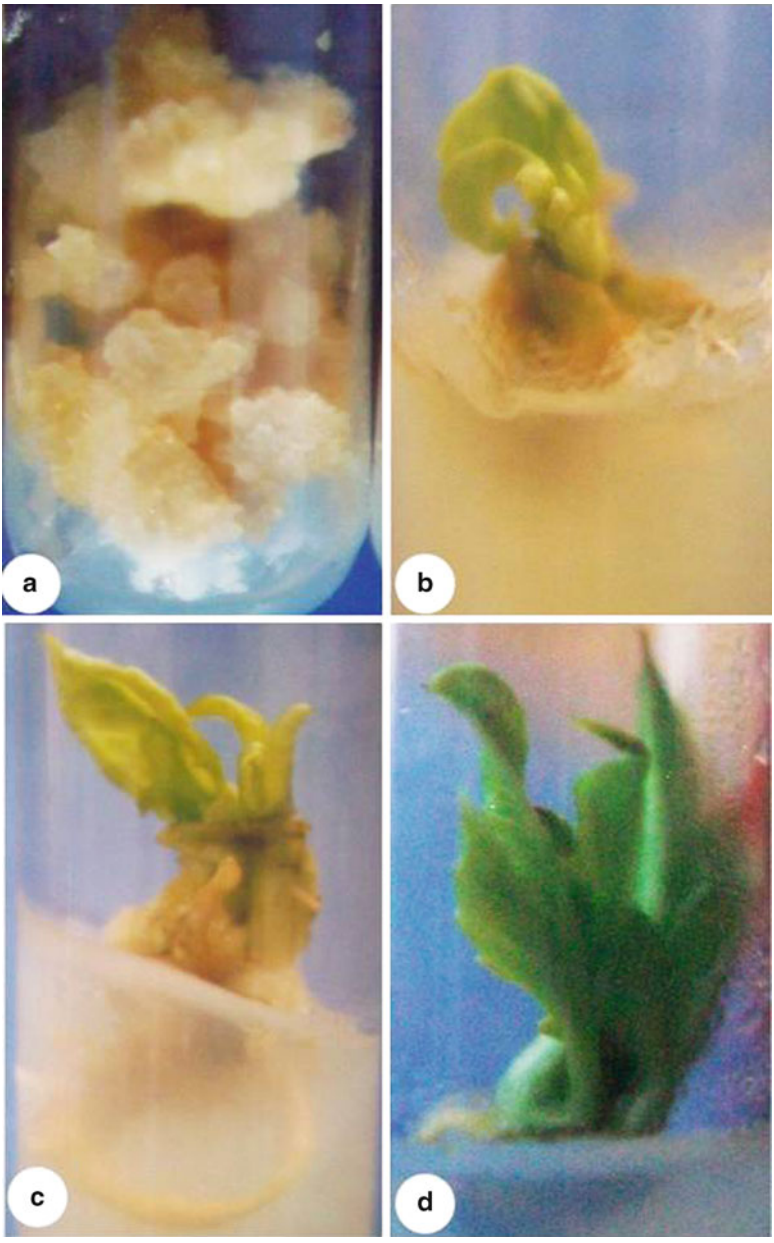

Fig. 27.1 In vitro morphogenesis in *J. glandulifera.* (**a**) White nodular callus from leaf explants on MS + BAP + NAA, (**b**) Multiple shoots and roots from zygotic embryo on MS + 2 mg l^{-1} BAP + 1 mg l^{-1} Kn + NAA, (**c**). Shoot meristem culture on MS + 1 mg l^{-1} BAP + 2 mg l^{-1} Kn + 1 mg l^{-1} IAA. (**d**) The same after 6 weeks of culture

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Different explants *viz.,* leaf, cotyledon, shoot meristems and zygotic embryos of *J. tanjorensis* were cultured on MS medium fortified with various plant growth regulators and their combinations (BAP+IAA, BAP+NAA, BAP+KN+NAA, BAP+KN+IAA, 2,4-D+NAA). In all these combinations only greenish white compact callus formation was observed by Chamundeswari (1997).

J. integerrima Jacq.

An erect ornamental shrub, native to West Indies, grows up to 6 m tall, sparingly pubescent. This is a drought tolerant perennial, ornamental shrub with bright crimson red flowers.

Sujatha and Dhingra (1993) have developed the protocol for regeneration from hypocotyl, stem, leaf and peduncle explants on MS medium supplemented with BAP, zeatin, and kinetin at several concentrations plus 0.1 mg l^{-1} IBA. Of the three cytokinins tested BA (0.5–2.0 mg l^{-1})+0.1 mg l^{-1} IBA was most effective in inducing multiple shoot buds. According to their observation, BA (1.0 mg l^{-1}) and IBA (0.1 mg l^{-1}) were the best for in vitro propagation of *J. integerrima* through direct induction of adventitious shoots from hypocotyl, stem, peduncle and leaf explants.

The ability of adventitious shoot induction from hypocotyls, stem, peduncle and leaf explants of *J. integerrima* on MS medium fortified with 0.1–2.0 mg l^{-1} BAP, kinetin or zeatin in combination with 1.0 mg l^{-1} IBA were compared (Sujatha and Reddy 2000). BA was the most effective cytokinin for induction of shoots from various explants studied. The highest shoot regeneration potential was observed from stem and leaf segments (Sujatha and Reddy 2000).

Sujatha et al. (2000) have also studied the biochemical and histological changes during in vitro organogenesis in *J. integerrima* using hypocotyl explants. Hypocotyl explants were cultured on 0.01–2.0 mg l^{-1} BA in combination with 0.5 mg l^{-1} IBA. Shoot proliferation was observed on MS medium supplemented with 0.1–2.0 mg l^{-1} in combination with 1.0 mg l^{-1} IBA. During in vitro regeneration, peak activities of alkaline phosphatase, peroxidase and polyphenol oxidase were observed at day 14 indicating their involvement in the formation of meristematic centers. While protein accumulation and acid phosphatase activity were found maximum at day 28 in *J. integerrima* (Sujatha et al. 2000).

Chamundeswari (1997) worked on in vitro micropropagation from different explants of *J. integerrima* by using various concentrations and combinations of plant growth regulators. Various types of explants *viz.,* leaf, cotyledon, zygotic embryo, shoot meristems, anthers and endosperm were cultured on MS medium supplemented with different plant growth regulators and their combinations (BAP+IAA, BAP+NAA, BAP+KN+NAA, BAP+KN+IAA, 2,4-D+NAA). Greenish white compact callus induction was observed from all the explants except zygotic embryos in which shoot formation was observed at 1 mg l^{-1} NAA+2 mg l^{-1} KN+1 mg l^{-1} BAP.

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J. multifida L.

A glabrous shrub, native to South America. Cultivated in gardens, parks for ornamental foliage and flowers and known as coral plant. It is known as 'malai aman akku' in Tamil.

In vitro morphogenesis from various explants was studied in *J. multifida* (Chamundeswari 1997). The explants *viz.,* leaf and shoot meristems were cultured on MS medium augmented with different concentrations and combinations of growth regulators. In all the concentrations and combinations, callus was induced with different texture and coloration where as single shoot with callus development was observed from meristem cultures.

J. multifida leaf explant showed little response in the combined treatment of BAP 2.0 mg l^{-1} + NAA 1.0 mg l^{-1}, and KN 1.0 mg l^{-1} + BAP 2.0 mg l^{-1} + NAA 1.0 mg l^{-1} (Goverdhan 1996).

J. podagrica Hook.

Small shrub, up to 1 m height, native of Panama. Often found in conservatories, gardens and parks but thrive well in rich soil fully exposed to the sun and regularly watered.

Various types of explants *viz.,* leaf, cotyledon, shoot apex, zygotic embryo, endosperm of *J. podagrica* were cultured on MS medium fortified with different concentrations and combinations of plant growth regulators. Callus was induced from all the explants used. Whereas shoot elongation was found at 3 mg l^{-1} AS +3 mg l^{-1} BAP from meristem cultures and also from embryo cultures (Chamun deswari 1997).

Leaf explants of *J. podagrica* on MS + BAP 2.0 mg l^{-1} + 2,4-D 1.0 mg l^{-1} + GA_3 1.0 mg l^{-1} produced light cream coloured nodular callus (Goverdhan 1996).

The growth and development of *J. podagrica* zygotic embryos in vitro were studied (Jesus et al. 2003). Disinfected embryos (0.2–0.5 cm long) were cultured on Murashige and Skoog's culture medium salts (50%, 75% and 100%) and GA_3 (gibberellic acid at 0, 0.14, 0.43, 1.30 or 3.90 μM). The growth and development of embryos were most pronounced in the MS medium containing 50% nutrient salts without GA_3.

Spera et al. (1997) studied the effect of different concentrations of kinetin (1.0 mg l^{-1}) and 2,4-D (4.0 mg l^{-1}). The treatment that induced the best callus production was 1.0 mg l^{-1} kinetin + 2.0 mg l^{-1} 2,4-D. Regardless of the kinetin concentration used, there was an increase in callus weight at 2,4-D concentrations of 1.0 and 2.0 mg l^{-1}.

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Fig. 27.2 Micropropagation in *J. elliptica.* (**a**) In vitro cultivated plant with flowers and fruits, (**b**) induction of multiple shoots from shoot tips, (**c**) in vitro rooting, (**d**) acclimatization of plants after 40 days. (*Source*: Campos et al 2007)

J. elliptica (Pohl) Muell. Arg

J. elliptica is a medicinal herb from Brazilian cerrados used on folk medicine against severe itches, snake bites and syphilis. Seed germination is low and this species is being over exploited leading to its endangering. Campos et al. (2007) have developed the protocol for in vitro micropropagation of the species. Nodal

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segments of *J. elliptica* were cultured on 0.1/0.5 mg l^{-1} IAA in combination with 5–15 mg l^{-1} BAP. More number of multiple shoots was recorded at 0.5 mg l^{-1} IAA + 15 mg l^{-1} BAP. For in vitro rooting different concentrations of IBA/NAA (1.0–5.0 mg l^{-1}) was used. Profuse rhizogenesis and lengthy roots were found at 5.0 mg l^{-1} NAA (Fig. 27.2; Campos et al. 2007).

J. nana Dalz.

A small, sparingly branched shrub, 30–45 cm high, found in stony and waste places near Poona and Mumbai, being apparently endemic to the Deccan, dwarf of African type. Tissue culture work on this species endemic is necessary so that using modern plant molecular biotechnologies, hybrids can be produced with *J. curcas* or with any other related species.

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Chapter 28
Genetic Transformation of *Jatropha curcas*: Current Status and Future Prospects

Nitish Kumar, Muppala P. Reddy, and Mulpuri Sujatha

Introduction

The massive use and dependence on fossil fuels will eventually lead to their depletion but, more importantly, the present applications and uses of fossil energy sources contribute significantly to altering the environment. Bioenergy, holds the greatest promise to contribute significantly to reduce the petroleum dependency and protect the environment. Biodiesel is a fast developing alternative eco-friendly fuel in many developed and developing countries of the world. Global biodiesel production is set to reach 24 billion liters by 2017 (Divakara et al. 2010). Shortage of edible oils for human consumption in developing countries does not favor its use, for biodiesel production. Many non-edible oil producing crops and plants have been considered for the purpose, and among these *Jatropha curcas* L., a member of Euphorbiaceae family has evoked considerable interest all over the tropics as a potential biofuel plant (Martin and Mayeux 1985; Jones and Miller 1991; Openshaw 2000; Francis et al. 2005; Mandpe et al. 2005; Ghosh et al. 2007; Divakara et al. 2010). Since,

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J. curcas does not compete with conventional crops for land, the dilemma of "food verses fuel" does not arise (Ghosh et al. 2007).

J. curcas has gained attention in tropical and sub-tropical countries and has spread beyond its current origin, because of its hardiness, easy propagation, drought endurance, high oil content, rapid growth, adaptation to wide agro-climatic conditions, and multiple uses of plant as a whole. It has an estimated annual production potential of 200,000 metric tonnes in India (Tiwari et al. 2007). *J. curcas* is propagated by seeds, seedlings, cuttings and micro cuttings. However, conventional propagation is limited by poor seed germination, scanty and delayed rooting of seedlings and vegetative cuttings and their availability in large numbers (Heller 1996; Openshaw 2000; Purkayastha et al. 2010). Large amount of variability has been observed even in plantations raised using the seeds from best performing accessions (Ghosh et al. 2007) and also seed yield is limited by abiotic and biotic stresses, especially salt, cold and insect pests (Kumar 2009). Therefore, additional genetic tools are required to explore the potential and to provide additional genetic gain. The traditional way to improve desirable traits of any species is by breeding, but, these techniques have limitations as these depend on sexual compatibility and often take 10–15 years to release a new variety due to extensive backcrossing. An assessment of genetic diversity using molecular markers disclosed, low inter-accessional variability amongst *J. curcas* germplasm (Sujatha et al. 2008; Sudheer et al. 2010; Shen et al. 2010). Therefore, alternative genetic manipulation tools, such as genetic transformation methods, are urgently required to provide additional tools for genetic improvement of this crop. Recent advances in genetic transformation and the availability of characterized genes with many of advantages have made it possible to transfer chimeric gene/genes of academic/agronomic importance to the genome of recipient species to produce transgenic progeny with desired characteristics. This technology may help bypass some of the limitations of classical breeding programmes and reduce the time required to produce improved varieties. Moreover, direct genetic transformation has become a method of choice for basic plant research as well as a principal technology for generating transgenic plants.

Genetic Transformation

In Vitro Culture and Regeneration

For genetic transformation of any crop/plant an efficient regeneration system is prerequisite. Efficient as well as reproducible plant regeneration protocols have been developed using different explants *of J. curcas* (Sujatha and Mukta 1996; Sardana et al. 2000; Wei et al. 2004; Rajore and Batra 2005, 2007; Sujatha et al. 2005; Jha et al. 2007; Deore and Johnson 2008; Kumar 2009; Kumar and Reddy 2010, 2012; Kumar et al. 2010a, b, c, 2011a, b; Singh et al. 2010; Sharma et al. 2011). Recombinant DNA technology and tissue culture, together with the recent gene transfer methods like *Agrobacterium*-mediated transformation, biolistics, electroporation, micro-injection, polyethylene glycol aided transformation, silicon carbide

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fibers and liposome methods now enable to target genes into plants even from distantly related organism like bacteria, virus, animals and even humans (Crossway et al. 1986; Fraley et al. 1986; Fromm et al. 1987; De la pena et al. 1987; Klein et al. 1987; Kaeppler et al. 1990). Of these, the most widely used methods of genetic transformation are the *Agrobacterium*-mediated and direct gene transfer using particle gun and both these methods have their own advantages and limitations (Potrykus 1991; Altpeter et al. 2005; Sharma et al. 2005).

Agrobacterium-Mediated Genetic Transformation

Agrobacterium-mediated transformation is preferred method of gene transfer for reasons like simplicity, cost effectiveness, little re-arrangement of transgene(s), ability to transfer relatively larger DNA segments (Hamilton et al. 1997), and preferential integration of foreign genes into transcriptionally active regions (Konez et al. 1989; Ingelbrecht et al. 1991), thereby ensuring proper expression of transgenes in plants (Hernandez et al. 1999), as compared to other methods. Efforts have been made to establish *Agrobacterium*-mediated genetic transformation protocols in *J. curcas* (Li et al. 2006, 2008b; He et al. 2009; Hui Zhu et al. 2010; Kumar et al. 2010b; Mazumdar et al. 2010; Pan et al. 2010; Zong et al. 2010). Li et al. (2006) were the first to initiate genetic transformation and attempted to determine the optimal conditions for gene transformation mediated by *A. tumefaciens*. The results showed that *J. curcas* was insensitive to the antibiotic cefotaxime, and sensitive to kanamycin, hygromycin, phosphinothricin. All the above three selective antibiotics synchronously suppressed the induction and the growth of callus, shoot organogenesis in a dose dependent manner. The type of explant and the physiological state of the explant had strong influence on the transient expression efficiency of β-glucuronidase (GUS) activity. Hypocotyl, cotyledon petioles and cotyledons from 14-day-old seedlings were more susceptible to *A. tumefaciens* infection. Li et al. (2008b) reported genetic transformation from callus cultures through *A. tumefaciens*-mediated transformation. The results indicated that the efficiency of transformation using the strain LBA4404 and phosphinothricin for selection was an improvement over the strain EHA105 and hygromycin. About 55% of the cotyledon explants produced phosphinothricin resistant calli on Murashige and Skoog (MS) medium supplemented with 1.5 mg l^{-1} benzyladenine (BA), 0.05 mg l^{-1} 3-indolebutyric acid (IBA), 1 mg l^{-1} phosphinothricin and 500 mg l^{-1} cefotaxime after 4 weeks of culture. Shoots were regenerated following transfer of the antibiotic resistant calli to shoot induction medium containing 1.5 mg l^{-1} BA, 0.05 mg l^{-1} IBA, 0.5 mg l^{-1} gibberellic acid (GA_3), 1 mg l^{-1} phosphinothricin and 250 mg l^{-1} cefotaxime, and about 33% of the resistant calli differentiated into shoots and rooted on 1/2 strength MS media supplemented with 0.3 mg l^{-1} IBA. About 13% of the total inoculated explants produced transgenic plants (Li et al. 2008b). He et al. (2009) also studied several factors affecting the transformation efficiency, such as the explant type, pre-culture and co-culture period, use of acetosyringone and density of *A. tumefaciens* and reported 67.7% efficiency in

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2-day pre-cultured and co-cultivated hypocotyl explants. Addition of acetosyringone showed a remarkable increase in transformation efficiency. Kumar et al. (2010b) reported a simple and reproducible protocol using *A. tumefaciens* and *J. curcas* leaf explants. *A. tumefaciens* strain, LBA 4404 harboring the binary vector pCAMBIA 1,304 having sense-dehydration responsive element binding (S-DREB2A), GUS and hygromycin-phosphotransferase (*hpt*) genes were used for gene transfer (Kumar et al. 2010b). They further reported that the *A. tumefaciens* strain, explant type, marker gene selection, bacterial incubation time, length of the pre-culture period of leaf explant in regeneration medium prior to infection, bacterial growth phase, bacterial cell density, method of wounding of leaf explants (glass beads, pricking with hypodermic needle), length of co-cultivation period, pH of the co-cultivation medium and acetosyringone concentration all play an important role in transformation efficiency. The highest transformation efficiency was achieved using 4-day pre-cultured, non-wounded leaf explants infected with *A. tumefaciens* culture corresponding to $OD_{600} = 0.6$ for 20 min, followed by co-cultivation for 4 days in a co-cultivation medium containing 100 mM acetosyringone, pH 5.7. With this protocol, primary transformants could be developed within 5 months from leaf explants with an overall transformation efficiency of 28%. The protocol optimized for explant preparation, co-cultivation, selection and recovery of whole plantlets is presented in Figs. 28.1, 28.2 and Table 28.1 (Kumar et al. 2010b). Mazumdar et al. (2010) observed that juvenile explants are more amenable to *A. tumefaciens*-mediated transformation as compared to other explants. Pan et al. (2010), also described *A. tumefaciens*-mediated transformation system of *J. curcas* cotyledon explants obtained from mature seeds and kanamycin selection with 30.8% transformation efficiency. Zong et al. (2010) introduced lateral shoot inducing factor (*LIF*) in *J. curcas* genome for promoting lateral branches using *A. tumefaciens* strain LBA4404 and young leaf explants with an overall transformation efficiency of 23.91%. Southern blot hybridization was performed which revealed single and multiple copy events and further confirmed that LIF was stably integrated into the *J. curcas* genome.

Microprojectile Bombardment-Mediated Genetic Transformation

Microprojectile bombardment, also called the biolistic method or the particle gun method has been used in many laboratories. The concept has been described in detail by Sanford (1988). The ability to deliver foreign DNA into regenerable cells, tissues or organs appears to provide the best method for achieving genotype independent transformation bypassing *Agrobacterium* host specificity. There are only two reports on genetic transformation in *J. curcas* using microprojectile bombardment (Purkayastha et al. 2010; Joshi et al. 2011). Purkayastha et al. (2010) established genetic transformation system in *J. curcas*, using bombardment of particles coated with plasmid pBI426 with a GUS-NPT II fusion protein under the control of a double 35 S cauliflower mosaic virus (CaMV) promoter. The β-glucuronidase (GUS) activity in shoot apices was significantly affected by the gold particle size, bombardment pressure, target distance, macrocarrier travel distance, number of bombardments, and

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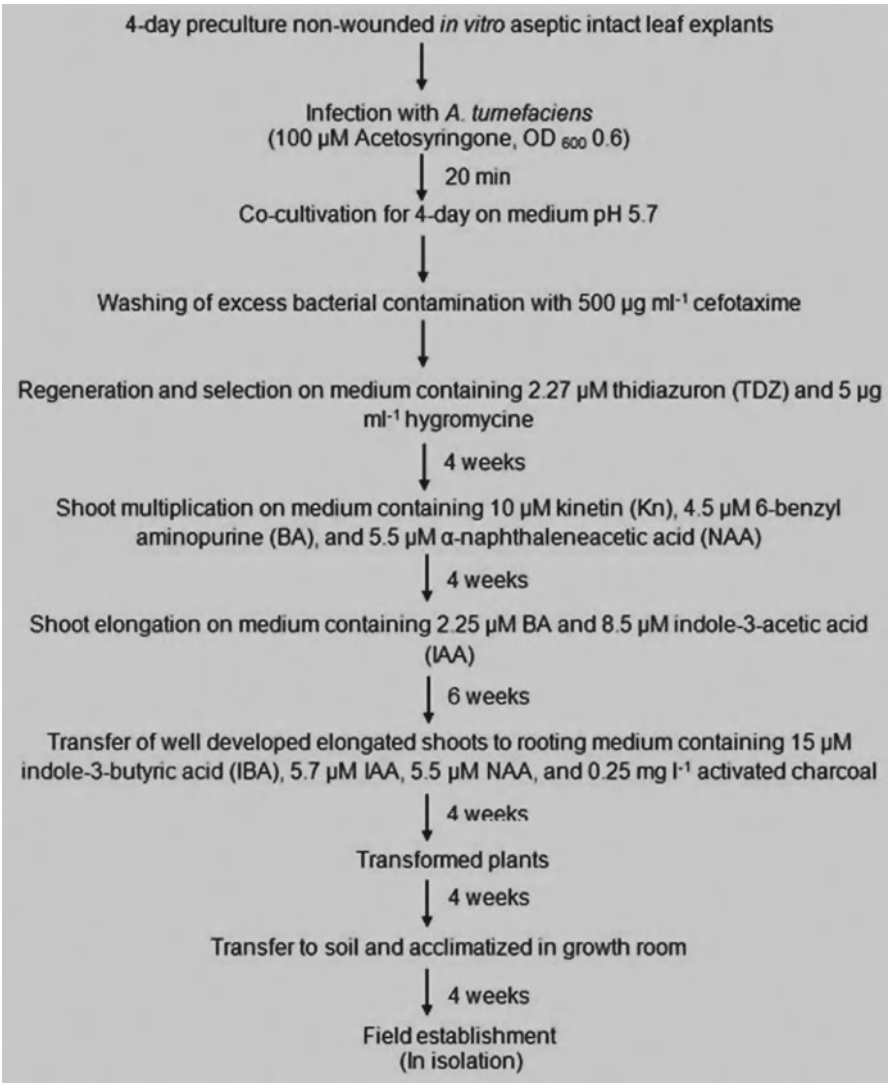

Fig. 28.1 Schematic representation of the protocol for *A. tumefaciens* – mediated transformation and regeneration of *J. curcas* using leaf explants (Source: Kumar et al. 2010b)

type and duration of osmotic pre-treatment (Purkayastha et al. 2010). The highest frequency of transient expression was achieved when explants were bombarded at 1,100 psi with a target distance 9 cm with 1 µm gold microparticles. Joshi et al. (2011) also reported genetic transformation in *J. curcas* through microprojectile bombardment with 44.7% transformation efficiency. Decotyledonated embryos isolated from mature seeds were pre-cultured and elongated embryonic axis after 5 days of culture was subjected to particle bombardment and, it was observed that microcarrier size, helium pressure and target distance had significant influence on transformation

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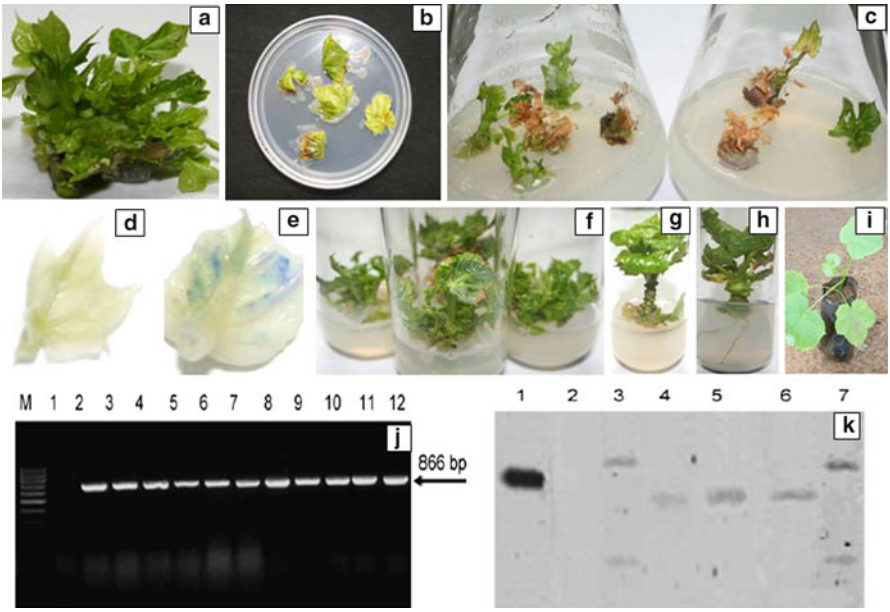

Fig. 28.2 Regeneration and transformation of *J. curcas* using leaf explants: (**a**) direct induction of adventitious shoots from leaf explants not subjected to *A. tumefaciens* treatment and cultivated on hygromycin-free medium (control); (**b**) *A. tumefaciens* infected leaf explants on co-cultivation medium; (**c**) selection of regenerated adventitious shoots on hygromycin containing medium; (**d**) untransformed leaf explants (control); (**e**) *blue* GUS spots in transformed leaf explants; (**f**) multiplication of hygromycin resistant regenerated adventitious shoots; (**g**) elongation of transformed shoots; (**h**) rooted transformed plant; (**i**) acclimatized transformed plant; (**j**) PCR analysis for detection of 866 bp of the *DREB2A* gene in transgenic plants. *Lanes*: *M* molecular size marker (100 bp ladder), 1 negative control (non-transgenic plant), 2 positive control (plasmid DNA), 3–12 putative transgenic plants; (**k**) DNA gel blot hybridization analysis of transgenic plants. *Lanes*: 1 plasmid DNA, 2 non-transgenic plant, 3–7 transgenic plants. Genomic DNA was digested with *Hin*d III and hybridized to the S-DREB2A probe (Source: Kumar et al. 2010b)

efficiency. Among different variables evaluated, microcarrier size of 1 μm, helium pressure 1,100 and 1,350 psi with a target distance of 9 and 12 cm, respectively were found optimum for *GUS* expression and survival of putative transformants.

Genes Identified in J. curcas

Preliminary studies on genetic transformation revealed susceptibility of seedling and leaf explants of *J. curcas* to *Agrobacterium*-mediated transformation (Li et al. 2006, 2008b; He et al. 2009; Hui Zhu et al. 2010; Kumar et al. 2010b; Mazumdar et al. 2010; Pan et al. 2010; Zong et al. 2010) but inheritance and stable transmission of the introduced gene in the progeny has not been demonstrated. As on date, there are no reports of transgenics in *J. curcas* harboring agronomically desirable traits.

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Table 28.1 Influence of various parameters on efficiency of transformation (%) in leaf explants of *J. curcas*

Parameters	Variable	Transformation efficiency (%) (Mean ± SE)
Preculture (days)	0	2.2 ± 0.2a
	1	3.2 ± 0.4b
	2	4.1 ± 0.5d
	3	4.6 ± 0.5d
	4	18.3 ± 1i
	5	8.5 ± 0.9g
	6	7.4 ± 0.8f
	7	7.1 ± 0.5f
Bacterial growth phase (OD)	0.3	2.1 ± 0.2a
	0.4	5.6 ± 0.5e
	0.5	7.1 ± 0.5f
	0.6	21.5 ± 1.9j
	0.8	10 ± 1.1h
	1.0	5.5 ± 0.9e
Infection duration (min)	10	11 ± 1.2h
	20	26.7 ± 1.9k
	30	11.9 ± 2h
Methods of wounding	Glass beads	3.6 ± 0.3c
	Hand pricking	2.8 ± 0.2b
	Non-wounding	29 ± 0.4l
Co-cultivation period (days)	0	2.4 ± 0.1ab
	1	3.4 ± 0.3bc
	2	7.6 ± 0.7f
	3	12.3 ± 1.2h
	4	22.1 ± 1.2j
	5	8.8 ± 0.8 g
	6	7.8 ± 0.7f
	7	6.1 ± 0.7e
pH of co-cultivation medium	5.2	3.3 ± 0.3b
	5.4	4.2 ± 0.4d
	5.6	7.1 ± 0.7f
	5.7	19.5 ± 0.9i
	5.8	7.1 ± 0.7f
	6.0	7 ± 0.7f
Acetosyringone (μM)	0	3.1 ± 0.1b
	50	4.3 ± 0.4d
	100	19.9 ± 0.9i
	200	8.8 ± 0.8g
	400	8.7 ± 0.8g

Source: Kumar et al. 2010b

Oil content of seeds can be increased by manipulation of the expression levels of key enzymes in the triacylglycerol biosynthetic pathway such as acyltransferases, disruption of homeobox genes like *GLABRA2*, and over expression of transcription

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factors and fatty acid profiles could be altered by manipulation of enzymes in the fatty acid biosynthesis (FAB) pathway. For understanding the function of the key genes involved in fatty acid metabolism and oil content, the method of virus induced gene silencing was adopted to silence more than 15 genes (Ye et al. 2009). Attempts are being made in few laboratories (Sichuan University, China; TLL, Singapore) for cloning and characterization of candidate genes involved in fatty acid metabolism, oil content enhancement and drought, such as, betaine aldehyde dehydrogenase gene, *JcBD1* for adaptation to environmental stresses like drought, heat and salt (Zhang et al. 2008), β-keto-acyl carrier protein (Li et al. 2008a), stearoyl-ACP desaturase (Tong et al. 2006, 2007), DRE-binding ERF3 gene (Tang et al. 2007), the plasma membrane intrinsic protein (*JcPIP*2) encoding aquaporin involved in drought response (Zhang et al. 2007), *JcERF* (showing enhanced resistance to salt and frost) and curcin gene (Lin et al. 2003a, b; Luo et al. 2006, 2009; Huang et al. 2008), Δ6-fatty acid desaturase gene, ω6-fatty acid desaturase gene, ω3-fatty acid desaturase gene, diacylglycerol acyltransferase (DGAT) gene and long chain acyl coenzyme A synthetase (LACS) (Chen et al. 2007; Ye et al. 2009). The genes, *Jchmgr* and *Jcggpps* in the toxic metabolic pathway of biosynthesis were cloned and characterized (Lin et al. 2009, 2010). Qin et al. (2011) reported that a cDNA clone arf1 isolated from *J. curcas* endosperm cDNA library which encodes a small GTP-binding protein has significant homology to ADP- ribosylation factor (ARF) in plants and the expression was observed in flowers, root, stem and leaves and the accumulation of arf1 transcripts was different under various environmental stresses. Gua et al. (2011) isolated full length genes of Acetyl-CoA carboxylase (ACCase) and reported that these genes are temporally and spatially expressed in leaves and endosperm of *J. curcas* plants which are regulated by plant development and environmental factors. Costa et al. (2010) have generated 13,249 ESTs from developing and germinating *J. curcas* seeds and identified ESTs coding for proteins that may be involved in the toxicity of *Jatropha* seeds. Eswaran et al. (2010) obtained 32 full length sequences from *J. curcas* genes including the genes involved in fatty acid biosynthesis, desaturation of fatty acids and hydrolysis of fatty acids from acyl-ACP. Gomes et al. (2010) sequenced a total of 2,200 clones and obtained a set of 931 non redundant sequences from *J. curcas* fruits at different maturity stages and reported that their expression was higher in fruits as compared to leaves. Life Technologies Corporation, USA (Nasdaq: LIFE), a provider of innovative life science solutions and SG Biofuels, Inc., a bioenergy crop company, have announced the completion of the genome sequence to 100x coverage, using the SOLiD 4.0 System by Life Technologies. Synthetic Genomics Inc. (SGI) and Asiatic Centre for Genome Technology (ACGT) also announced the completion of *Jatropha* genome project. Sato et al. (2011) published the sequence and made it available in international databases (DDBJ/GenBank/EMBL). Curcin-deficient transgenic *Jatropha* plants were developed using RNAi technology and are currently under evaluation (Ye et al. 2009; Yin et al. 2012). Similarly, transgenic *Jatropha* plants with increased levels of oleic acid have been developed by downregulating the expression of *JcFAD2-1* gene in a seed specific manner through RNA interference technology (Qu et al. 2012).

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Gressel (2008) and Sujatha et al. (2008) have enumerated strategies for genetic improvement of *J. curcas* through biotechnological interventions for modified plant architecture, non-shattering, low toxic genotypes, dwarfness, suppressed branching and improved quality. These studies have long term implications in genetic engineering of *J. curcas* for traits of interest.

Conclusions

Although *J. curcas* is well known for its wide adaptability and plethora of uses, the full potential is far from being realized due to low and inconsistent yields and non availability of superior genotypes for multiplication and use in large-scale planting which necessitates the need for improvement of the species. Identification of genotypes capable of ensuring both profitable yield and wide adaptability is very much essential. The reported protocols for genetic transformation need to be refined to take advantage of the great potential. Genetic transformation and gene transfer is particularly important for traits for which variability is unavailable in the cultivated species.

The small genome size, chromosome number, ease of vegetative manipulation and transformation are favorable features for the use of biotechnological tools for *J. curcas* improvement. Efforts in basic research on oil biosynthesis pathways and its applications in improving the oil composition and yield and reducing the phorbol esters content in the deoiled cake need to be intensified (Tong et al. 2006; Zhang et al. 2007, 2008). Molecular genetic tools may allow genetic improvement; however, one needs to be cautious regarding the stability of integration and expression of foreign gene/genes when taking a transgenic approach in perennial plants like *J. curcas.*

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Chapter 29
Improvement of *J. curcas* Oil by Genetic Transformation

Jian Ye, Yan Hong, Jing Qu, and Chunming Wang

Introduction

The US Department of Energy estimates the global demand for energy will increase by approximately 35% by 2030 compared to 2005. Over the next 20 years, most of this energy growth will come from the emerging markets of China, India, Africa, South and Central Americas. Despite significant gains in efficiency with non-renewable energy, governments of these emerging economies are keen to develop biofuel so as to reduce crude oil consumption and dependence on crude oil importation. Another main reason concerning increasing interests for biofuels is the depletion of world crude oil reserves, increasing prices and the global warming concerns, which has greatly stimulated the search for alternative renewable fuel sources. At present, bioethanol and biodiesel from plants are rapidly emerging as sustainable alternatives for a significant proportion of fossil fuels. Plant *fatty acids* (FA) can be further processed into jet biofuel, which has been successfully tested by several airlines. The aviation industry has been criticized for its high level of *green house gas* (GHG) emissions that is 649,000,000 tons annually, which represents 2–3% of the total. Consequently, the European Union determined to extend *Emission Trading Scheme* (ETS) to international aviation from 2012 and now requires airlines flying to Europe to compensate for greenhouse gas emissions according to the EU ETS. The annual fuel consumption of aviation worldwide is estimated to 260 billion litres. Many

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airlines are contemplating jet biofuel as a future option. China is projecting to use 12 million tons jet biofuel by 2020, which would account for 30% of the country's consumption. The market value of jet biofuels will exceed 120 billion yuan ($19 billion) by 2020. The increasing demand for oilseed FAs, however, is an additional pressure on food production by increasing the competition between bioenergy crops and food crops for arable land. One way to meet this challenge is to use oilseed crops that can grow on marginal land (Carroll and Somerville 2008).

Oil palm has been thought to be a good candidate for biofuel because of its high oil yield per hectare. However, there are several hurdles to its wide use for biofuel. First, it is a multi-purpose oil used mainly for food and many other industries. Second, its GHG lifecycle is not favourable, which makes difficult to qualify oil palm biodiesel as a renewable fuel (Environmental Protection Agency, USA, 2011). Third, because of its high content of saturated FAs, palm oil biodiesel has poor flow property at low temperature; a feature that is not suitable for cold climates.

Jatropha curcas L. (hereafter referred to as Jatropha) is recognized as a promising new energy crop due to the high content of oil in its seeds, tolerance to drought and ability to thrive in poor soil. Furthermore, the reduction of GHG emission for generating 1 gigajoule energy can be at least 40% and up to 107% with respect to fossil diesel from three independent investigations in South America, Europe and Asia (Bailis and Baka 2010; Almeida et al. 2011; Kumar et al. 2012). It is, however, a non-domesticated plant with poor and unpredictable seed productivity in large scale plantation (Sanderson 2009). Traditional breeding has been used to increase its productivity through improvement of traits such as branching pattern, total flower number and female flower ratio (Gao et al. 2010). However, the lack of sufficient genetic variability in *J. curcas* renders selective breeding more difficult and thereby increases the demand for approaches using direct molecular manipulations, such as gene functional analyses and genetic transformation (King et al. 2009; Ye et al. 2009; Yi et al. 2010). Genetic transformation will be very useful to introduce the traits that are difficult or impossible to manage through traditional breeding.

Although several methods of genetic transformation have been described for Jatropha (Li et al. 2008), improvements of agronomic traits and seed oil quality by transgenic technology was just reported by our group (Lu et al. 2011; Qu et al. 2012). One reason might be that the reported transformation method is still at an early stage of development and relatively inefficient (Lu et al. 2011). Another possible reason is the difficulty in selecting a good candidate gene and manipulating it precisely in order to produce the desired trait.

The fuel properties of a biodiesel are highly dependent on its FA composition. At present, the oil composition from Jatropha or other candidate oilseeds, such as oil palm and rapeseed, is still not optimized for biodiesel purposes. The biodiesel from Jatropha has reasonable performance at low temperatures, but suffers low oxidative stability because of its high concentration (>45%) of polyunsaturated FAs. Improving the fuel characteristics of biodiesel can be achieved by altering the FA composition. In this review, we describe the use of biotechnological strategies to improve FA composition with the aim to upgrade Jatropha to the level of a *premier* biofuel plant.

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The Fuel Properties of an Ideal Biodiesel

For Higher Oleic Acid and Lower Saturated Fatty Acid Levels in Jatropha Oil

Plant FAs or oils are composed of various *triacylglycerols* (TAGs), molecules that consist of three FA chains (usually 18 or 16 carbons long). Because of its high viscosity, Jatropha neat oil is not suitable for direct use as engine fuel. Neat oil used as a fuel often results in operational problems, such as carbon deposits, oil ring sticking as well as thickening and gelling of lubricating oil due to contamination by the vegetable oil (Akbar et al. 2009). The conversion of TAGs from Jatropha oil or most other plant oils into *FA methyl esters* (FAME) is necessary to decrease their viscosity to levels comparable to fossil diesel in order to minimize problems due to incomplete combustion (Durrett et al. 2008). The fuel properties of FAMEs are highly dependent on the composition of FA mixture before conversion. Major plant oils are mostly composed of five common FAs: palmitate (16:0), stearate (18:0), oleate (18:1), linoleate (18:2) and linolenate (18:3). One disadvantage of Jatropha oil as a diesel feedstock is the high level of polyunsaturated FA (mainly linoleic acid, 30–50%), which negatively impacts on biodiesel quality because FA desaturation decreases biodiesel stability and reduces the *cetane number* (CN). Another disadvantage of Jatropha biodiesel is its poor fluidity at low temperature due to its high level of saturated FA (including palmitate and stearate, 20%). Improving the characteristics of Jatropha biodiesel requires the decrease of saturated FA (for better fluidity at low temperature), the increase of oxidative stability and the decrease of unsaturated and polyunsaturated FAs in order to reduce *nitrogen oxide* (NO_x) emission. Two key properties for the characterization of fuel fluidity at low temperature are *cloud temperature* (CP) and *pour temperature* (PP), which in case of Jatropha's FAMEs are 10 °C and 6 °C, respectively (Rashid et al. 2010). These values are much larger than those of biodiesel from soybeans whose CP and PP values are 0 °C and−2 °C, respectively. This large difference is mainly due to the high level of polyunsaturated FA of soybean oil. In comparison, No. 2 diesel, which is the most used in automobile transport, has CP and PP values around −16 and −27 °C, respectively. Taking these factors together, we may find there are contradictory requirements between flowing characteristics at low temperature on one side, and oxidative stability, lower NO_x emissions and higher cetane number, on the other side. One would also conclude there is no perfect FA composition that can satisfy all these fuel properties at the same time. In fact, a very good compromise can be reached by considering a fuel with high levels of mono-unsaturated FAs, such as oleate or palmitoleate ($16:1\Delta^9$), and low levels of saturated as well as polyunsaturated FAs. Among all the esters of unsaturated FA, oleate esters are the most stable and 49 times more stable compared with esters of linoleate (Frankel 1998). The oxidation of polyunsaturated esters of linoleate results in the initial accumulation of hydroperoxides, which eventually polymerize forming insoluble sediments that may be responsible for filter plug-

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ging, injector fouling and other loss of engine performance and safety (Durrett et al. 2008). Furthermore, it has been proved that engines operating on high ratio of polyunsaturated soybean biodiesel produce higher NO_x emissions compared with fossil diesel (Tat et al. 2007; Graef et al. 2009). By contrast, the biodiesel from genetically modified soybean for high oleic content demonstrated improved fuel characteristics resulting in an increase of cetane number, a much better oxidative stability and a lower rate of NO_x emission (Tat et al. 2007; Graef et al. 2009).

Therefore, biodiesel from vegetable oil with higher oleate (>70%) and lower saturated FA (<10%) is expected to have ideal fuel qualities. According to data of biodiesel produced from high oleic and low palmitic soybeans, CP and PP are lowered to 4 °C and −9 °C, respectively (Tat et al. 2007; Graef et al. 2009).

Inter-Specific Crossing and Selective Breeding for the Improvement of Jatropha Biodiesel Properties

So far, the composition in major FAs is affected by environmental factors, but only slightly varies within *J. curcas*; it is as follows: oleic acid (18:1; 34.3–50%), linoleic acid (18:2; 29.0–44.2%), palmitic acid (16:0; 14.1–18%) and stearic acid (18:0; 3.7–10%). The low range of variation may also be associated with low level of genetic diversity in *J. curcas* and may rely on improvement through intra-species breeding (Yi et al. 2010). Efforts were made to bring in more variation for FA composition by inter-species breeding between *J. curcas* and the other species of the genus, such as *J. integerrima* and *J. gossypiifolia*, two species with much lower saturated FA content (Fig. 29.1 and Basha and Sujatha 2009). Based on our own observations (Liu et al. 2011), crosses of *J. curcas* with *J. integerrima* is feasible and reciprocal hybridizations of this pair are possible (Basha and Sujatha 2009). Significantly lower proportion of saturated FAs (13.6% compared to 24.6% for *J. curcas* parent) has been found in BC_1F_1 seeds of crossing population between *J. curcas* and *J. integerrima*. Some seeds of BC_1F_1 trees are bigger than seeds from the *J. curcas* mother tree (Fig. 29.1a). FA compositions in hybrid progenies were all intermediary to those of parents (Fig. 29.1b). However, according to our experience on inter-specific crosses in the genus *Jatropha* (Liu et al. 2011), the scope of increasing the proportion of oleic acid to 70% of total FAs by genetic transfer through interspecific hybridization is not attainable because the highest level of oleic acid observed in the progeny of these crosses was 52.95% (Basha and Sujatha 2009). Furthermore, the level of oleic acid in *J. curcas* seems to be the highest in the genus *Jatropha,* which prevents further increase of oleic acid proportion by this approach. All these data suggested that another different strategy need to be developed to reach the goal of 70% oleic acid in Jatropha's oil.

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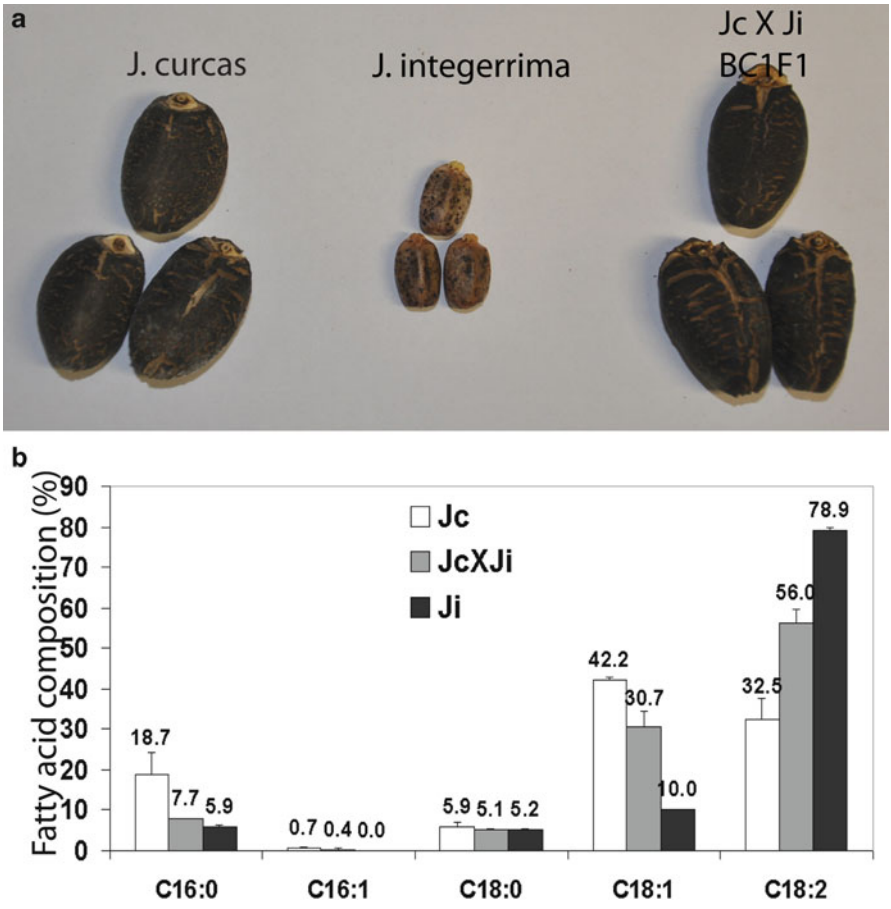

Fig. 29.1 Seed characters (**a**) and oil fatty acid profile (**b**) of *J. curcas* (*left*), *J. integerrima* (*middle*) and seed from a BC_1F_1 hybrid (JcXJi) of *J. curcas* (Jc) and *J. integerrima* (Ji)

Genetically Modifying Properties of Jatropha Oil for Better Biofuel

Improved Transformation of Jatropha

The method of plant transformation by shoot regeneration from cotyledon discs treated with *Agrobacterium tumefaciens* is still at an early development stage (Li et al. 2008; Lu et al. 2011). We have made some modifications to previous protocols and obtained higher transformation frequency using the binary vector pCAMBIA1300-35 S:GFP including a gene for the *green fluorescent protein* (GFP) under the control of cauliflower mosaic virus (CaMV) 35 S promoter (Fig. 29.2) as well as using the constructs described by Mao et al. (2010). With our protocol at least 25

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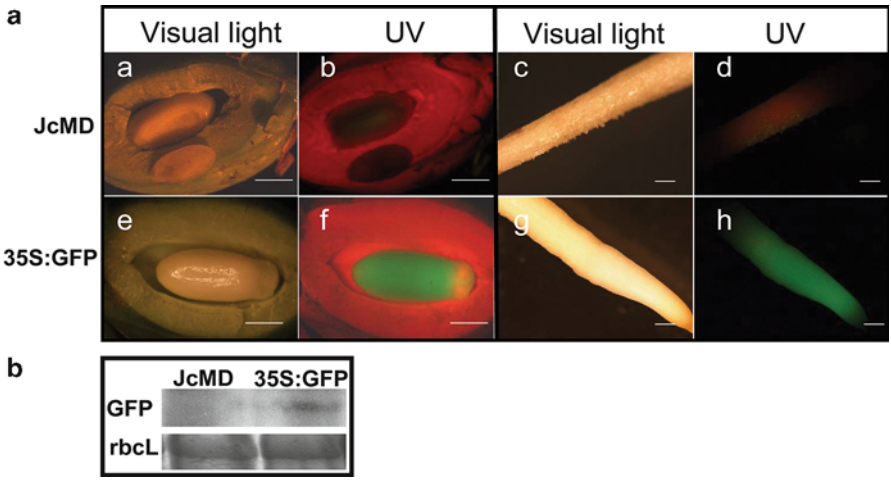

Fig. 29.2 Transgenic Jatropha expressing a GFP marker gene. (**a**) No green fluorescence signal in JcMD control developing seeds (*a* and *b*) and roots (*c* and *d*). Green fluorescence signal can be detected on developing seeds (*e* and *f*) and roots (*g* and *h*) of T_1 GFP transgenic plant (*e*–*h*). Size bar: 10 mm. (**b**) Western blot analysis of GFP expression in leaves. T_1 GFP transgenic plant leaves were analyzed by western blot. *Top panel*: GFP protein band detected with anti-GFP antibody. *Bottom panel*: large subunit of the ribulose-bisphosphate carboxylase (rbcL) band stained with coomassie brilliant blue, which serves as a loading control

transgenic lines can be generated from 100 Jatropha cotyledon explants, giving a transformation frequency ≥25%.

Functional Characterization of Genes Controlling Fatty Acid Composition in Jatropha Through Virus-Induced Gene Silencing

The next step of genetic improvement of Jatropha for oil quality would be an improved understanding of the genes involved in regulation of FA and TAG biosynthesis in Jatropha. An important strategy for that purpose would be to overproduce or suppress the interested gene(s) by stable transformation. For plants with long life cycle such as Jatropha, transgenic technology is unsuitable for routine testing of gene function because it is time consuming and laborious. *Virus-induced gene silencing* (VIGS) offers an attractive alternative as it allows the investigation of gene functions without plant transformation (Ye et al. 2009). Although Jatropha was not known to be a host of *tobacco rattle virus* (TRV), we found that this virus was able to infect Jatropha; accordingly, we used the TRV system for VIGS in Jatropha. Based on the phenotypes and fatty acid profiles, we proposed a working model with five major functional genes in the pathway of FA biosynthesis in Jatropha (Fig. 29.3). The functions of many genes involved in FA biosynthesis have been identified in the model plant *Arabidopsis*. By VIGS, we have confirmed the function of several such

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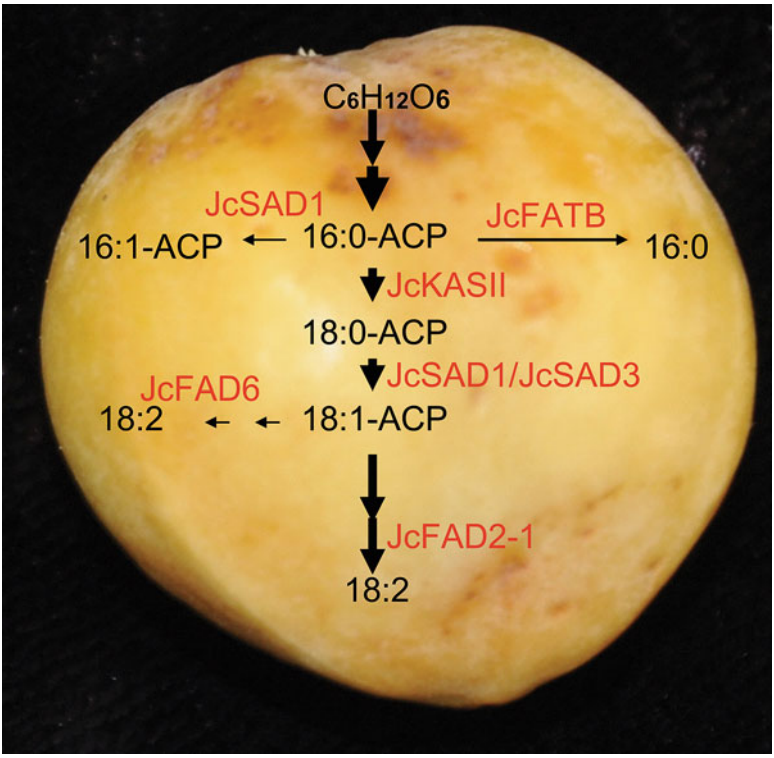

Fig. 29.3 A model for fatty acid biosynthetic pathway in Jatropha. This model is based on results obtained with *Arabidopsis* and Jatropha plants. The sites of action of 6 Jatropha genes (*JcKASII*, *JcFATB*, *JcSAD1*, *JcSAD3*, *JcFAD2-1* and *JcFAD6*) were placed in this pathway through VIGS of various candidate genes involved in fatty acid biosynthesis

genes from *Arabidopsis* also applied to Jatropha and this information should be helpful for modifying oil composition in Jatropha by transgenic methods. For example, the gene of *fatty acid desaturase* (FAD) *FAD2-1* is the major gene, instead of *FAD2-2* or *FAD6*, for the control of oleic acid content. Among *acyl carrier protein* (ACP), both *palmitoyl acyl-ACP thioesterase B* (*FATB*) and *stearoyl-ACP desaturase* (*SAD1*) are also important for the content in saturated FAs. Manipulation of these genes for improving biodiesel properties are discussed below.

Genetic Strategies for Improving Oxidative Stability of Jatropha Oil

The amount of linoleate in common Jatropha oil is roughly 30–50% of the total FAs, which greatly reduces oxidative stability of the oil, leading to rancidity and decreased shelf-life of products. Even low concentrations of polyunsaturated fatty esters have a disproportionately large effect on the oxidative stability of biodiesel. It is known

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that biodiesel with high monounsaturated FA content (oleate) has excellent characteristics with respect to ignition quality, NO_x emissions and fuel stability. It is ideal for biodiesel to substitute linoleic acid with oleic acid. This is because FA desaturation decreases biodiesel stability and affects CN. CN value is perhaps the most important factor for biodiesel since it is a determinant parameter for the ignition quality of diesel and is also negatively correlated with NO_x emissions.

FAD2 Gene

FAD2, the enzyme 1-acyl-2-oleoyl-sn-glycero-3-phosphocholine delta 12-desaturase, is the key enzyme responsible for the production of linoleic acid with oleic acid as the substrate in plants. We identified three putative *delta-12 fatty acid desaturase* genes in *Jatropha* (*JcFAD2s*) through genome-wide analysis (Qu et al. 2012) and the function of several key genes from Jatropha that regulate FA chain length and saturation levels (Ye et al. 2009). Among these *FAD2* genes, *FAD2-1* of Jatropha (*JcFAD2-1*) is of particular interest as it mediates the conversion of oleic acid to linoleic acid. The expression of *FAD2-1* is high in seeds and weaker in vegetative tissues, while *FAD2-2* is highly expressed in seeds and not detectable in leaves. The expression pattern of these two *FAD2* genes in Jatropha is very similar with those of other members of Euphorbiaceae, i.e., the genes *FAD2* and *FAH12* (fatty acid hydroxylase) in castor bean (Lu et al. 2006), *FAD2* and *FADX* in tung tree (Dyer et al. 2002).

Cre-lox Mediated Antibiotic Selection Marker-Free Transformation

Our primary objective was to produce transgenic Jatropha plants with a high oleic acid content in seeds. Considering possible large scale commercial planting of such transgenic Jatropha, an additional objective was to have transgenic plants free of antibiotic selection marker. To accomplish the first objective, we used *RNA interference* (RNAi) to silence the expression of *JcFAD2-1*. To generate marker-free plants, our strategy was to transform and regenerate transgenic plants using the conventional transformation process with *hygromycin phosphotransferase* (*HPT*) gene as the selectable marker; subsequently, we removed the marker from the host plant genome by chemically regulated site specific DNA excision. We used a chemical inducible Cre-lox-mediated site-specific recombination system, which has been shown to work in *Arabidopsis* (Zuo et al. 2001; Guo et al. 2003). Because the *FAD2* genes play roles in environmental adaptation of plant vegetative growth (Scherder and Fehr 2008), it is more desirable to specifically change the oleic acid content in seeds only. To this end, we replaced the G10-90 promoter in the pX7 vector with the soybean (*Glycine max*) seed storage protein *7 S* gene promoter, which displays seed-specific expression (Schuler et al. 1982; Guo et al. 2003). The XVE and Cre-lox marker free vector with *HPT* as a selection marker were chosen to construct the marker free system (Fig. 29.4a). The three transcription units of recombinant transcription factor XVE, *HPT* gene and *CRE-intron* fusion gene were flanked by two *loxP*

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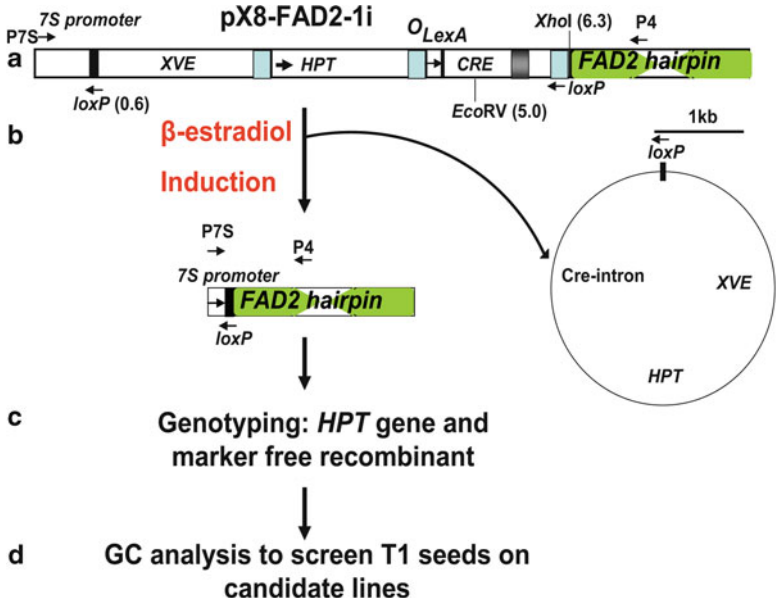

Fig. 29.4 Outline for the development of marker-free and seed specific transgenic Jatropha with high oleic acid content. (**a**) Schematic diagram of the silencing cassette and β-estradiol-induced Cre/loxP-mediated DNA recombination vector for *FAD2-1* suppression. (**b**) β-estradiol-induced DNA recombination would remove all XVE, *HPT* and *CRE-intron* components leading to the activation of the downstream *JcFAD2-1* hairpin structure by the *7 S* promoter. (**c**) Genotyping with *HPT* gene, P7S and FAD2-1 specific primers to tag putative recombinants. (**d**) Gas chromatography (GC) to screen T_1 seeds on candidate lines with marker free recombinant

sites so that β-estradiol-induced DNA recombination would remove all these components, leading to the activation of the downstream *JcFAD2-1* hairpin structure by the *7 S* promoter (Fig. 29.4b), which precisely controls the knock down effect only on targeting the seed organ. The new vector with the soybean *7 S* promoter was named pX8-FAD2-1i.

We used the transformation procedure described before to obtain hygromycin-resistant shoots. Small shoots were then transferred to β-estradiol induction medium without hygromycin to induce marker excision. Following 2 weeks induction, shoots were subsequently returned to the regeneration medium without hygromycin. Other procedures were similar to normal transgenic process (Mao et al. 2010).

Obtaining Jatropha Oil with High Oleic Acid Content by Genetic Engineering

We generated thirty X8-FAD2-1i RNAi primary transformation (T_0) lines that were confirmed by genotyping analysis using *polymerase chain reaction* (PCR) to be

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marker-free or partial marker-free and to have the expected sequence size (Fig. 29.4c, Qu et al. 2012). The T_1 progeny seeds were harvested from T_0 plants and tested for down regulation of *FAD2-1*. Quantitative PCR analysis showed that some seeds from T_1 progeny of T_0 line X8#34 contained only 0.7% *JcFAD2-1* transcript compared to the 35 S: GFP control in the endosperm (Qu et al. 2012). Further quantitative RT-PCR data showed that this engineered *FAD2-1* suppression was gene-specific as it had no effect on the expression of *FAD2-2* (the ortholog of *FAD2-1*) in endosperms of this transgenic line. The soybean *7 S* promoter is seed specific for the expression of a gene inserted downstream in soybean (Schuler et al. 1988). No significant change of *FAD2-1* transcript levels could be observed in vegetative organs, such as leaves (Qu et al. 2012), which proved that *7 S* promoter precisely controls *FAD2-1* silencing only in the seed (the target organ).

In *Arabidopsis*, *FAD2* encodes desaturases responsible for the introduction of a second double (Δ^{12}) bond in oleate. We hypothesized that RNAi-mediated silencing of *FAD2*-1 should block the conversion of 18:1 to 18:2 fatty acids. Indeed, *gas chromatography* (GC) showed a much higher oleic acid phenotype in T_1 endosperm of lines X8#34, X8#230 and X8#110 with 77.4%, 75.4% and 76.2% oleic acid of total FAs, respectively (Figs. 29.4d, 29.5, 29.6 and Qu et al. 2012). The linoleic acid levels were reduced to less than 5% of total FAs in these lines. In lines X8#34, X8#230 and X8#110, almost all the unsaturated FAs were due to oleic acid. Moreover, the stearic acid level was slightly reduced in transgenic plants (5.7–5.8%) compared to the control (7.7%). No marked difference could be found either for composition in C16 FAs or for total oil content when comparing endosperms of X8-FAD2-1i lines and those of JcMD control plants (Qu et al. 2012). Similarly, no significant difference of FA profile could be found comparing leaves of transgenic X8-FAD2-1i lines and JcMD controls, which is consistent with a specific alteration of gene expression in endosperm (Qu et al. 2012). These data confirmed seed-specific high oleic acid accumulation in transgenic lines. In addition, no significant differences could be found between transgenic lines with high or normal content of oleic acid and untransformed JcMD control Jatropha plants for common agronomic traits, such as plant height, seed number, seed weight and oil content, observed under greenhouse conditions (Qu et al. 2012). These preliminary data collected from T_0 (Qu et al. 2012) and T_1 plants (Fig. 29.7) under greenhouse condition indicated that a genetic modification to promote high seed oleic acid content does not show negative side effects on other agronomic traits in Jatropha.

Biodiesel Properties of Jatropha Transgenes with High Oleic Acid Content

As occurred with other crops (Tat et al. 2007; Graef et al. 2009), we expected the higher level of oleic acid in transgenic lines to confer desirable properties to biodiesel. The CN value is the most important factor for biodiesel. This number is a

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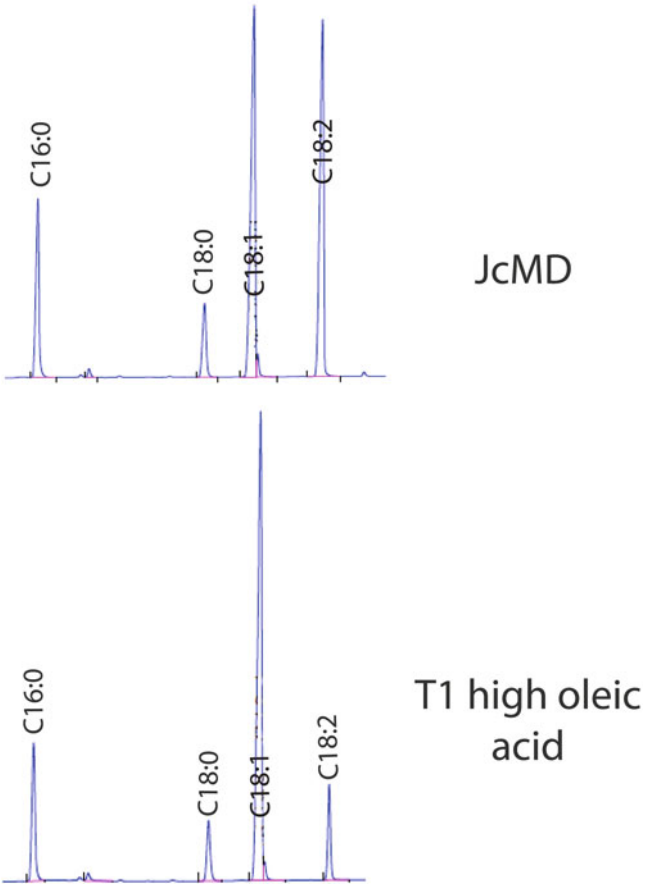

Fig. 29.5 Gas chromatography analysis of fatty acid methyl esters isolated from endosperm of untransformed Jatropha (JcMD) plant (*upper panel*) and from T_1 seeds of X8#34 (*lower panel*), a genetically modified line of Jatropha with high oleic acid content. C16:0, C18:0, C18:1 and C18:2 are for palmitic, stearic, oleic and linoleic acids, respectively

determinant parameter for the ignition quality of diesel fuels and also negatively correlated with NO_x emissions. We deduced the theoretical CN of Jatropha biodiesel to be 52.6 and increased it to more than 60 in transgenic lines overproducing oleic acid (X8#34, X8#230 and X8#110). The CN of high oleate acid lines is similar to the required CN for conventional premium diesel fuels (60) in Europe. The expected NO_x emission of Jatropha biodiesel with high oleic acid content is also lower as suggested by the experimental data from soybean biodiesel with a high oleic acid content (Tat et al. 2007; Graef et al. 2009).

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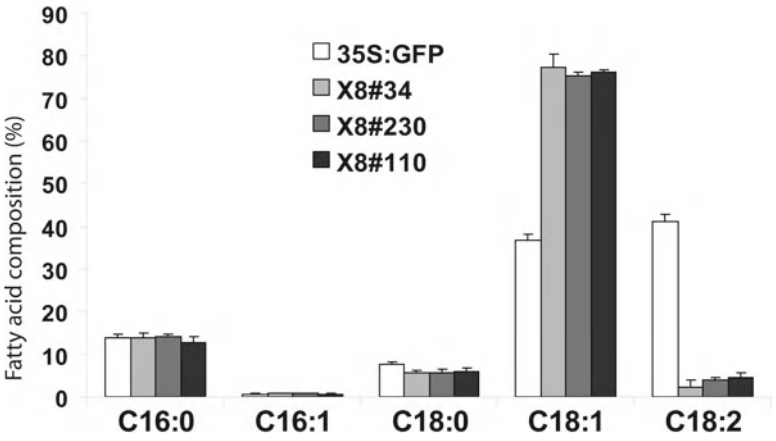

Fig. 29.6 Fatty acid profile of Jatropha oil. Analysis of fatty acid composition in T_1 endosperms of lines with high oleic acid content: X8#34, X8#230, X8#110 and 35 S:GFP (CK-)

Oxidative stability is another key issue for biodiesel. Neat Jatropha biodiesel exhibits an oxidation stability of 3.95 h (Sarina et al. 2009). As noted before, methyl esters of linoleate are 49 times more reactive to oxygen than oleate esters because they contain a *bis*-allylic methylene group between the two double bonds that easily oxidizes (Frankel 1998). Therefore, the oxidation stability of Jatropha biodiesel with high oleic acid content can be dramatically increased.

However, since the change in the proportion of saturated FAs in the Jatropha oil with high oleic acid content only affects the ratio of one double bond to the total FA content, the flow properties at low temperature, CP and PP of biodiesel derived from this oil might be at a level similar to the control biodiesel.

Genetic Strategies for Further Improvements Toward Flow Properties of Jatropha Oil at Low Temperatures

FATB removes the acyl-ACP from 16:0-ACP thereby terminating FA elongation. This enzyme has been shown to be important for the synthesis of saturated FAs, such as 16:0 and 18:0 (Jones et al. 1995). The functional properties of Jatropha *FATB* were described by Wu et al. (2009) and Ye et al. (2009). When *FATB* is down-regulated, a larger amount of FA can further elongate to chains of *18 carbons* (C18), and consequently, both the level of oleate and the proportion of total unsaturated FA can be increased. Biodiesel derived from such transgenic seeds should have improved flow properties at low temperature, CP and PP because of their increased level in unsaturated FAs.

Stearoyl-acyl carrier protein (SAD) catalyzes the first desaturation step in FA biosynthesis by converting stearoyl-ACP to oleoyl-ACP. Therefore, SAD plays

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Fig. 29.7 Phenotype of a genetically modified (GM) Jatropha with high oleic acid content (T_1 high oleic acid) compared to the control (JcMD). The transgenic plant (*right*) showed no noticeable difference of vegetative growth, flowering or seed yield compared with the untransformed individual (JcMD) of Jatropha (*left*)

important roles in determining FA chain length and the ratio of saturated to unsaturated FAs (Shanklin and Somerville 1991). At least three putative Jatropha *SAD* genes in a Jatropha seed cDNA library were identified. *SAD1* is highly expressed in seeds at 5–7 weeks after fertilization, when Jatropha triglycerides mainly accumulate in the endosperm. All data so far support that SAD1 is the key enzyme responsible for converting not only 18:0-ACP, but also 16:0-ACP to oleic acid. Therefore, it should be possible to generate transgenic Jatropha with even higher oleic acid (>75%) and lower saturated FA (C16:0 and C18) contents in seeds by specifically up regulating the expression of *SAD1* in seeds. Combining the *SAD1* up regulation together with down regulation of *FAD2-1* by RNAi, both oleic acid level and unsaturated FA content will be further increased; therefore flow properties at low temperatures and oxidative stability will be further improved.

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Other Genes Useful to Improve Fuel Properties

KASII encoding the β-ketoacyl-acyl carrier protein synthase II is responsible for the elongation of 16:0-ACP to 18:0-ACP (Carlsson et al. 2002). Both VIGS and transgenic *Arabidopsis* data proved that *KASII* is one key gene controlling the ratio of C16 FAs to C18 FAs (Ye et al. 2009; Wei et al. 2012). Palmitic acid (16:0) is 14.1–18% of total FAs in Jatropha oil. The over expression of *KASII* cDNA under the CaMV 35 S promoter in *Arabidopsis* resulted in decreasing C16 FAs and increasing C18 FAs in leaves and seeds (Wei et al. 2012). Combining *KASII* over expression in Jatropha together with down expression of *FAD2-1* by RNAi technology, both oleic acid level and unsaturated FA content will be even further increased and flow properties at low temperatures as well as oxidative stability will be further enhanced.

Fatty acid oils are generally stored in spherical intracellular organelles referred to as oil body that are covered by oleosins. Oleosins are the major constituents in oil body and therefore affect plant oil traits. Seeds with high oil content have more oleosin than those with low oil content. *Quantitative trait locus* (QTL) and *expression QTL* (eQTL) analyses were applied to identify genetic factors that are relevant to seed oil traits in Jatropha. Interestingly, the eQTL of OleIII, qOleIII-5, was detected on *linkage group* (LG) 5 with a percentage of *phenotypic variation explained* (PVE) of 11.7% and overlapped with QTLs controlling stearic and oleic acids, implying a cis- or trans-element for the *OleIII* affecting FA compositions (Liu et al. 2011; Popluechai et al. 2011). An over expression of this *OleIII* should also lead to higher stearic and oleic acid contents that would, in turn, also increase the oxidative stability and flow properties at low temperatures.

Besides oleosin genes, composite interval mapping identified 18 QTLs underlying the oil traits. A highly significant QTL, *qC18:1–1*, was detected at one end of LG1 with a *logarithm of odd* (LOD) of 18.4 and a PVE of 36.0%. Interestingly, *qC18:1–1* overlapped with *qC18:2–1*, which controls oleic and linoleic acid compositions. Among the significant QTLs controlling total oil content, *qOilC-4* was mapped on LG4 with a LOD of 5.0 and a PVE of 11.1%. Gene identification and isolation in chromosome regions of the major QTLs will be beneficial for genetic improvement not only by transgenic modification, but also by marker assisted selection (Liu et al. 2011).

Conclusions

With the availability of effective and robust genetic information technology and good understanding of genes involved in fatty acid biosynthesis and regulation network, we are able to perform genetic modifications to change fatty acid profile for ideal Jatropha biodiesel. We identified several key genes, such as *FAD2-1*, *oleosin*, *FATB*, *SAD1* and *KASII* to engineer the control of FA composition, desaturation and size through the exploration of data from genome, gene expression, gene silencing and QTL analysis. We set up an improved Jatropha transformation method along with a chemical inducible Cre-lox system to produce marker-free

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Jatropha transgenes. Using this combined technological platform together with hairpin RNA interference technology, we obtained marker-free transgenic plants with seed-specific *JcFAD2-1* suppression, which enhanced the FA composition of transgenic Jatropha oil from 37% oleic acid and 41% polyunsaturated FAs to more than 78% oleic acid and less than 3% polyunsaturated FAs. The changes of FA composition should lead to better Jatropha biodiesel properties. These data represent the first successful genetic modification of key agronomic traits in Jatropha and the marker-free system applied here should provide relief to public concerns for environmental issues about genetically modified plants. More biotechnological improvements concerning agronomic properties, such as oil yield, stress tolerance and oil quality are expected to come soon not only for Jatropha itself, but also for related biofuel crops such as castor bean.

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Chapter 30
Genome Structure of *Jatropha curcas* L.

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manipulation and sequencing in the last three decades has enabled us to analyze sequence information over the entire genomes of complex living organisms in a relatively short period of time. For higher plants, a highly accurate sequence of a whole genome of *Arabidopsis thaliana* was reported in 2000 (Arabidopsis Genome Initiative 2000) that has proven the value of sequence information in plant genetics and genomics for the first time. Since then, nucleotide sequences of whole genomes, including draft sequences, which are cost-effective but less accurate, have been pub-

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lished for a number of plant species, including rice (International Rice Genome Sequencing Project 2005), poplar (Tuskan et al. 2006), grapevine (The French–Italian Public Consortium for Grapevine Genome Characterization 2007), and *Lotus japonicus* (Sato et al. 2008). The widespread availability of new-generation sequencers has significantly accelerated this trend.

Jatropha curcas L. is an important oilseed crop with great potential for the production of biodiesel fuel, but molecular breeding of this plant has just been inaugurated. In order to accelerate the process of breeding, an understanding of the genetic systems by investigation of the genome structure and function is prerequisite. The size (410 Mb) and the base composition of the genome of *J. curcas* have been reported, and karyotypes of the species were characterized in 2008 (Carvalho et al. 2008), but generally, knowledge of the genome structure of *J. curcas* has been quite limited.

Since 2010, studies providing large quantities of information on the gene and genome structures have been published one after another. ESTs derived from developing seeds (Gomes et al. 2010; Natarajan et al. 2010) and from developing and germinating endosperm (Costa et al. 2010) were established by conventional Sanger sequencing. In addition, transcriptome analyses using new-generation sequencers have been carried out for leaves and calluses (Sato et al. 2011), mixtures of roots, mature leaves, flowers, developing seeds, and embryos (Natarajan and Parani 2011), and three different stages of developing seeds (King et al. 2011). EST-derived SSR markers have been developed by pyrosequencing the mRNAs (Yadav et al. 2011). And finally, the draft sequence of the whole genome of *J. curcas* was determined by a combination of conventional and new sequencing technologies (Sato et al. 2011), and further upgraded by the addition of new data in 2012 (Hirakawa et al. 2012).

In this chapter, the latest status of large-scale analyses of the genes and genome of *J. curcas* will be reviewed, and their characteristics will be summarized.

Genome and Transcriptome Sequence Analyses

Genome Sequencing

The draft sequence of the whole genome of *J. curcas* was published on-line at the end of 2010 (Sato et al. 2011), and then the sequences and annotations were further upgraded by the addition of new data and released in 2012 (Hirakawa et al. 2012). The strategy used was a combination of the conventional Sanger method and new-generation multiplex sequencing methods.

As a first step, 1,025,000 reads of Sanger sequencing and the 2,312,828 reads of pyrosequencing by Roche-454 sequencers were collected, and independently assembled. The resulting contigs and singlets as well as the 53,000 BAC end sequences were subjected to assembly. Independently, a total of 182 million (M) reads collected by paired-end sequencing with the Illumina GAII sequencer were

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Table 30.1 Assemble statistics of *J. curcas* genome sequences (JAT_r4.5)

Features	Frequency
Total length of sequence elements	297,661,187
Total number of sequence elements	39,277
Average length of sequence elements	7,579
Maximum length of sequence elements	277,264
N50 length of sequence elements	15,950
G+C content (%)	33.7

assembled into 569,576 contigs (total length: 75,539,079 bp). Then, the resulting contig sequences were mapped onto those generated previously to correct sequence errors by insertion and/or deletion. For scaffolding, 695,928 paired-end reads by the GS FLX sequencer were used. In parallel, 991,050 reads of cDNA sequences by pyrosequencing were collected from leaf and callus tissues and assembled, and 21,225 unigene sets consisting of 13,610 contigs and 7,615 singlets were used for scaffolding. In addition, unigenes generated from 26,447 ESTs registered in public DNA databases (http://www.ncbi.nlm.nih.gov/dbEST/) at the time were also used for scaffolding. As a result of the scaffolding, 15,300 scaffolds, the total length of which was 129,291,074 bp, were generated, and were designated as JcS followed by sequential numbers. The final draft genomic sequence of *J. curcas* determined in the first phase was 285,858,490 bp long, approximately 70% of the genome size estimated by flow cytometry (Carvalho et al. 2008); the sequence consisted of 120,586 contigs (276,710,623 bp total) and 29,831 singlets (9,147,867 bp total) and was released as JAT_r3.0.

In the second phase, the accuracy of the sequences in JAT_r3.0 was significantly improved by the addition of data equivalent to 83 times the genome size by Illumina GAII sequencers, followed by intensive information analyses. The accumulated data was subjected to *de novo* assembly, resulting in genomic sequences of 221,111,674 bp in length and consisting of 107,255 scaffolds. These sequences were then further assembled with those in JAT_r3.0. The statistics of the assembly, designated as JAT_r4.5, are shown in Table 30.1. The total length of the obtained sequences was 297,661,187 bp, consisting of 28,665 *super contigs* (SCs) and 10,612 *unassembled contigs* (UCs). The maximum length of the sequences was 277,264 bp, and the N50 was 15,950 bp. The G+C content (guanine+cytosine) was 33.7%. The resulting SCs and UCs were designated Jcr4S and Jcr4U, respectively, followed by a five-digit number.

In parallel with the draft sequencing described above, 17 BAC clones were subjected to high-quality sequencing by the Sanger method, followed by manual finishing. A total of 1.36 Mb of accurate sequences was obtained, and these sequences were manually annotated and subjected to gene prediction to investigate the gene structures in detail.

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Transcriptome Sequencing

A large number of segmental cDNA sequences generated by Roche/454 GS FLX sequencers have been accumulated in the public NCBI SRA (*Sequence Read Archive*) database: 534,137 reads and 456,913 reads derived from leaves and calluses, respectively (DRX000446 and DRX000447), 383,937 reads from a mixture of five major tissues (SRX035761), 195,692 reads from seeds (SRX011411), and 2,210 reads from leaves (SRX020243). In addition, 46,842 EST sequences generated by Sanger sequencing are also available in the public DNA databases. These include 12,084 ESTs derived using a normalized cDNA library from developing seeds (Natarajan et al. 2010), and 13,249 ESTs obtained using non-normalized cDNA libraries from developing and germinating endosperm (Costa et al. 2010).

The structure of gene transcripts can be investigated by creating *tentative consensus sequences* (TCs) from the cDNA and EST sequences. The above cDNA/EST sequences were retrieved from the databases and were subjected to assembly. As a result, a total of 19,454 independent TCs were obtained. The average length of the TCs was 969 bp and the average G+C content was 41.3%. Of these TCs, 19,435 (99.9%) had matched genomic sequences in JAT_r4.5, indicating a high degree of genomic coverage of the sequences in JAT_r4.5.

Assignment of Protein-Encoding Genes

As the result of gene prediction by an *ab initio* gene prediction program, AUGUSTUS (Stanke et al. 2004), a total of 50,313 genes were deduced in the JAT_r4.5 sequences. After comparison among the transcriptome sequences, predicted genes in JAT_r3.0 and those in JAT_r4.5, a total of 30,203 genes, as well as 17,575 transposon-related genes and 2,124 putative pseudogenes, were finally assigned to the genome of *J. curcas*. Thus, the number of assigned potential protein coding genes in JAT_r4.5 is comparable with that in other diploid eudicot species, such *A. thaliana* (29,084 in TAIR10), *L. japonicas* (30,799) and tomato (34,727).

Complete gene structures could be predicted for 25,433 genes, while only partial structures were predicted for 4,770 genes, mainly due to truncation at the ends of contigs. The structural features of the genes with complete structures were close to those of the manually annotated genes on the 17 BAC clones whose sequences had been accurately determined (Sato et al. 2011), indicating that the accuracy of the genomic sequences in JAT_r4.5 was sufficiently high. The features of the genes in *J. curcas* as well as those in *A. thaliana* are summarized in Table 30.2.

Assignment of RNA-Encoding Genes

In the JAT_r4.5 sequences, a total of 690 putative genes for transfer RNAs were identified in the *J. curcas* genomic sequences by a combination of computer

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Table 30.2 Features of the deduced protein-encoding genes on the *J. curcas* completed BAC clones, *J. curcas* genome (JAT_r4.5) and *A. thaliana* genome

Features	*J. curcas* completed BAC[a]	*J. curcas* genome (JAT_r4.5)	*A. thaliana*
Gene length (bp) including introns	273–9,806 (3,064)	303–32,994 (2,337)	22–30,734 (1,856)
Product length (amino acids)	90–1,700 (443)	100–4,807 (368)	6–5,336 (402)
Genes with introns	121 (83%)	20,820 (82%)	20,572 (75%)
Number of introns/gene	0–21 (4.8)	0–63 (4.0)	0–77 (4.0)
Coding exon length (bp)	3–3,264 (227)	3–4,820 (221)	1–7,713 (238)
Intron length (bp)	18–3,514 (356)	27–6,309 (306)	2–10,234 (158)
GC content of exons	42%	43%	44%
GC content of introns	32%	32%	32%

[a]17 completed BAC clones

prediction and similarity searches. While 91 of these were likely to be pseudogenes, the remaining 599 could code for intact tRNAs with 54 species of anticodons, which are sufficient for translation of all the amino acids. By referring to the list of *A. thaliana* snRNAs (Wang and Brendel 2004), 67 genes for snRNAs, some of which were likely to form clusters, were assigned in the genome of *J. curcas*.

Characteristics of Protein-Encoding Genes

Components of Protein-Encoding Genes in the J. curcas Genome

A similarity search of the translated amino acid sequences of the 30,203 potential protein-encoding genes against the TrEMBL database as a protein sequence library (Bairoch and Apweiler 1996) indicated that 22,088 (73.1%) genes had significant sequence similarity to those in the above database. Based on the sequence similarity and domain features assigned by the InterProScan program, the predicted protein-encoding genes were classified into plant GO slim categories, and compared to the classification of genes in castor bean (*Ricinus communis*) (31,221 genes) (Chan et al. 2010), which belongs to the same family as *J. curcas*, and those in *A. thaliana* (35,386 genes in TAIR10) (Fig. 30.1). The results indicated that the proportions of genes in each category in *J. curcas* were similar to those in the corresponding categories in *A. thaliana*.

A total of 2,402 genes in JAT_r4.5 could be assigned to the metabolic pathways in the KEGG database. Comparison of the assigned genes with those in *R. communis* and *A. thaliana* showed that 19 pathways, including “galactose metabolism” in carbohydrate metabolism, “biosynthesis of steroids” in lipid metabolism, “glycosylphosphatidylinositol (GPI) – anchor biosynthesis” in glycan biosynthesis and

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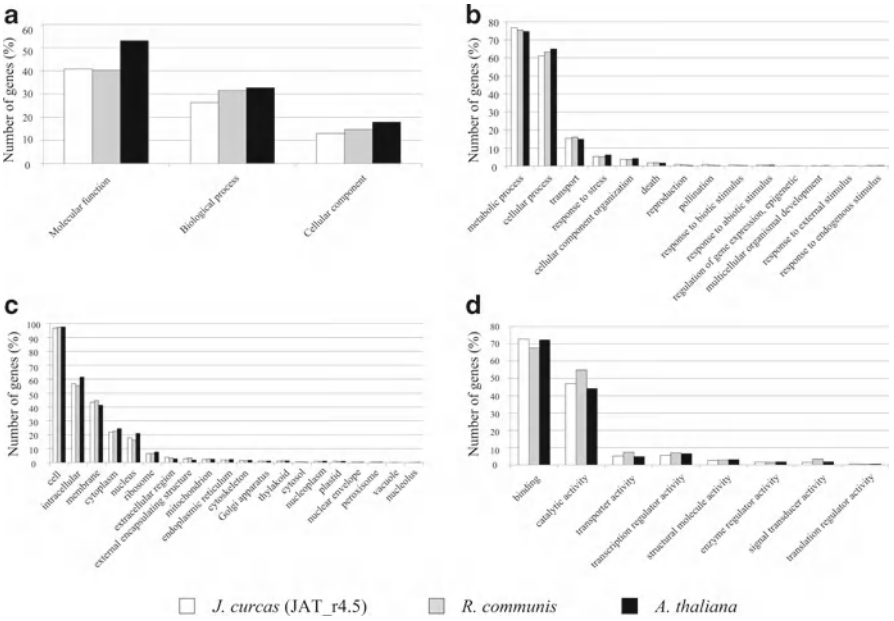

Fig. 30.1 GO category classification. The percentages of genes classified into each GO slim category in *J. curcas* (JAT_r4.5), *R. communis* and *A. thaliana* are respectively shown with *white*, *gray* and *black bars*. (**a**) GO terms; (**b**) biological process; (**c**) cellular component; and (**d**) molecular function

metabolism, and "retinol metabolism" in metabolism of cofactors and vitamins, contained enzyme(s) only found in *J. curcas*.

Genes Involved in Synthesis of Triacylglycerols

Although it is widely considered a potential feedstock of biodiesel, considerable effort will be required in the breeding of *J. curcas* before it can become an industrially beneficial plant species. In the efforts to improve oil accumulation in its seeds by genetic modification, the genes involved in the biosynthetic pathway of triacylglycerols (TAGs) are of great interest. In addition, the sequence information of the genes for TAG synthesis is useful for the development of marker assisted breeding, since some of the QTLs related to oil quality have been mapped to these genes (Gomes et al. 2010; Wang et al. 2011). Some of the TAG-synthesis genes have already been cloned from *J. curcas* (Tong et al. 2006; Ye et al. 2009; Gu et al. 2011). Recently, a collection of ESTs from developing and germinating *J. curcas* seeds was reported (Costa et al. 2010; Chen et al. 2011).

The *J. curcas* genome appears to contain no obvious gene duplication in this category, and basically one gene for each enzyme isoform was found in the genome. One gene model for a recently identified soluble type of DGAT (Saha et al. 2006) also existed in the *J. curcas genome*. To improve the quality of *J. curcas oil* for

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biodiesel, its fatty acid composition could be modified by altering the expression of some of those genes. For example, genetic modification to suppress the expression of the genes encoding Δ-9 and Δ-12 desaturases, i.e., Jcr4S01370.20 (or AAY86086.1 (1)) for FAB2 (SAD1) and Jcr4S09407.30 for FAD2, will result in transgenic *J. curcas* that can produce more saturated oil superior in oxidative stability.

Genes Responsible for the Toxicity of J. curcas

The wild-type *J. curcas* plant is able to protect itself against insects and pathogenic microorganisms by synthesizing toxic compounds and proteins (Devappa et al. 2010), which could prevent commercial development of *J. curcas* (Pradhan et al. 2011).

An example of toxic compounds is phorbol esters (Li et al. 2010), which have been shown to have activity to promote tumorigenesis (Haas et al. 2002). Various methods of detoxifying *J. curcas* have been tested, especially methods to detoxify the meal of seeds after oil extraction (Joshi et al. 2011; Xiao et al. 2011), since those residual meals (seed cakes) contain quality proteins at a high percentage comparable to soybean meal (Achten et al. 2008). Therefore, the suppression of any genes involved in the biosynthetic pathway of phorbol ester is one of the targets of genetic modification of *J. curcas*. To our knowledge, genes involved in the biosynthesis of phorbol esters have not been reported in *J. curcas*, with the exception of the gene for geranylgeranyl diphosphate synthase (GGPPS) (Lin et al. 2010). Similarity searches have putatively identified eleven copies of genes for GGPPS, eight copies of genes for casbene synthase (CS), and one copy each of a gene for terpene hydroxylase (cytochrome P450-dpendent monooxygenase) and acyltransferase in the *J. curcas* genome. Nine putative genes encoding casbene synthase were identified in the BAC clones, JHL23C09 (one gene tentatively designated *JcCS*1), JHL22C18 (two genes, *JcCS*2 and *JcCS*3) and JHL17M24 (six genes *JcCS*4, 5, 6, 7, 8 and 9). While *JcCS*2 in JHL22C18 appears to be a pseudogene containing several stop codons, the six intact genes are tandemly aligned in JHL17M24, suggesting enhanced activity of the gene due to the gene dosage effect. From phylogenetic analysis, it was suggested that continuous duplication of the original *JcCS* gene occurred recently. These CS genes as well as 102 putative terpenoid synthase genes, which form clusters at 6 loci in the genome, were most closely related to those of *R. communis*.

An example of toxic proteins in *J. curcas* is curcin, a type I ribosome-inactivating protein (RIP). RIPs are commonly found among members of the Euphorbiaceae family. Although curcin in *J. curcas* is analogous to ricin, a type II RIP, in *R. communis,* the toxicity of curcin is significantly lower than that of ricin (Stirpe et al. 1976). Research on curcin has been extensive (Stripe et al. 1976), and has revealed that curcin exhibits antitumor activity (Lin et al. 2003; Luo et al. 2006). Curcin protein has also been shown to have activity against viral and fungal diseases by heterologous expression in tobacco, and in *J. curcas*, the expression of the curcin gene is induced by abiotic as well as biotic stresses in leaves (Qin et al. 2005, 2009; Huang et al. 2008). So far, three *J. curcas* genes encoding isoforms of curcin have

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been reported and deposited in public databases. The recently published *J. curcas* genome sequence (Sato et al. 2011) confirmed that the *J. curcas* genome contains these three curcin genes. In addition, the genome-wide survey for curcin genes identified at least two more genes with DNA sequences potentially encoding curcin-like proteins, suggesting that the *J. curcas* genome contains two more genes for curcin isoforms. Costa et al. (2010) also reported data from proteomic analysis of developing seeds, which indicated the existence of five isoforms of curcin.

Ethylene-Related Genes

It has been reported that ethylene regulates responses to biotic and abiotic stresses, flower development, fruit ripening, and senescence (Lin et al. 2009). Regulation of fruit ripening in *J. curcas* may help to improve the efficiency of seed harvest. A total of 18 genes in the *J. curcas* genome showing sequence similarity to ethylene-related genes in *A. thaliana,* including the key ethylene biosynthesis enzymes 1-aminocyclopropane-1-carboxylic acid (ACC) synthase and ACC oxidase, multiple ethylene receptors and signal transduction components. This suggests that the ethylene biosynthetic pathway, the signal transduction through ethylene receptors, the CTR-EIN2-EIN3 pathway and the transcriptional regulation of the ethylene response genes in *J. curcas* are similar to those in *A. thaliana*.

Disease Resistance Genes

Most disease resistance (R) genes are NBS-LRR (*nucleotide binding* site and *leucine-rich repeat*) proteins, which are further classified into two groups on the basis of the presence of *Toll and human interleukin receptors* (TIR) at their amino termini (Eitas and Dangl 2010). A total of 52 genes for TIR NBS-LRR proteins and 112 genes for non-TIR NBS-LRR proteins could be assigned in the genome of *J. curcas*. Structural analysis of the R genes on five BAC clones (JHL06P13, JHS03A10, JHL25H03, JHL25P11 and JMS10C05) revealed that two clones (JHS03A10 and JMS10C05) contain singletons of JcTIR-NBS-LRR1 and JcNBS-LRR9, while three clones (JHL06P13, JHL25H03 and JHL25P11) contain gene clusters as tandem repeats of R genes, JcNBS-LRR1 and JcNBS-LRR2, JcNBS-LRR3-5, and JcNBS-LRR6-8. The phylogenetic analysis of R genes including eight R genes in *J. curcas* demonstrated that JcNBS-LRR3-5 or JcNBS-LRR6 and JcNBS-LRR7 are closely related, suggesting that these gene clusters evolved recently by way of gene duplication. Interestingly, JcNBS-LRR1 and JcNBS-LRR2 belong to different clades, suggesting that the gene duplication did not take place recently and that these genes were conserved after the evolutionary diversification of *J. curcas*.

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MADS-Box Genes

It is widely known that MADS-box genes, typical homeotic genes encoding transcription factors, form a family and are involved in plant development (Alvarez-Buylla et al. 2000). A similarity search of the *J. curcas* genome for MIKC type II MADS-box genes using amino acid sequences of *PISTILLATA (PI)* (Goto and Meyerowitz 1994) in *A. thaliana* as a query identified 28 potential MADS-box genes (JcMADS01-28). The phylogenetic analysis classified these genes into several subfamilies.

SHORT VEGETATIVE PHASE (SVP) controls flowering time by negatively regulating the expression of a floral integrator, *FLOWERING LOCUS T(FT)* in response to ambient temperature changes *in A. thaliana* (Lee et al. 2007). There are five potential paralogs of *SVP* in *J. curcas*, while only a single copy and three copies of *SVP* genes were identified in *A. thaliana* and *Oryza sativa*, respectively (Hartmann et al. 2000; Lee et al. 2008). Considering that eight paralogs of *SVP* copies have been found in 57 MIKC type II MADS box genes of *Populus trichocarpa* (Leseberg et al. 2006), it can be speculated that frequent amplification and functional diversification of the *SVP* gene have taken place in woody plants.

Flowering-Related Genes

Flowering is closely related to the production of seeds, and thus the modification of flowering-related genes involved in organ identity could change the number or size of male and female organs or flowers. A similarity search of the *J. curcas* genomic sequences resulted in the identification of eight potential orthologs of flowering-related genes: five flowering regulators, *CONSTANS (CO)* (Putterill et al. 1995), *FLOWERING LOCUS D (FD)* (Abe et al. 2005), *FLOWERING LOCUS T (FT)* (Kobayashi et al. 1999), *LEAFY (LFY)* (Weigel et al. 1992) and *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1 (SOC1)* (Samach et al. 2000), designated as *JcCO, JcFD, JcFT, JcLFY and JcSOC1*, respectively, and three floral identity genes, *APETALA2 (AP2)* (Drews et al. 1991), *APETALA3 (AP3)* (Jack et al. 1992) and *PI*, designated as *JcAP2, JcAP3-1* and *JcPI*, respectively. Phylogenetic analysis indicated that all of these genes except *JcCO* are closely related to those in woody plants, such as *Betula pendula*, *Hevea brasiliensis*, *R. communis* and *Vitis vinifera*, while *JcCO* is not related to any flowering-related genes, implying that *JcCO* is not directly involved in flowering regulation. The presence of additional potential *CO* homologues in the *J. curcas* genome indicates the possibility that different components and pathways are involved in the response to light in *J. curcas.*

As flowering time and flower number are the major targets of molecular breeding, the orthologs of genes for flowering regulators and floral identity in *J. curcas* may draw attention for both fundamental and applied researches. One such example would be genetic modification for controlling the expression of *FT* ortholog, which is likely to

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play a universal role in regulating flowering time. Evidence of this comes from experiments showing that overexpression of *FT* orthologs causes early flowering in several plant species including tomato (Lifschitz et al. 2006), apple (Tränkner et al. 2010), rice (Izawa et al. 2002; Kojima et al. 2002) and wheat (Yan et al. 2006). Controlling the expression of *FT* ortholog in the favorite season or timing might lead to reduction of the cost to collect fruits by synchronizing blooming and bearing fruits.

Characteristic Genome Structures

Tandem Gene Duplication

Gene duplication is thought to be one of the most significant factors for adaptive evolution (Hanada et al. 2008). In *J. curcas*, it was found that approximately 12.3% of the potential protein-encoding genes formed tandem arrays of two or more family genes (Sato et al. 2011). These tandem arrays included genes for NBS-LRR proteins and cytochrome P450 proteins. There were 31 genes encoding the NBS-LRR disease resistance protein tandemly arrayed on 14 SCs, and 78 genes for the cytochrome P450 family protein tandemly arrayed on 32 SCs. Some gene families involved in secondary metabolism, especially lipid metabolism, were also found to form tandem arrays. It can be speculated that expansion of the genes in this functional category provides the variation and controls the efficiency of secondary metabolism pathways in *J. curcas*. Examples include genes for the alpha/beta-hydrolases superfamily protein (42 genes in 20 sites), 2-oxoglutarate and Fe(II)-dependent oxygenase superfamily protein (38 genes in 16 sites), UDP-glycosyltransferase superfamily protein (38 genes in 19 sites), NAD(P)-binding Rossmann-fold superfamily protein (32 genes on 12 sites), HXXXD-type acyl-transferase family protein (25 genes in 9 sites), lipid-transfer protein (24 genes in 8 sites), and GDSL-like lipase (17 genes in 8 sites). In addition, gene families related to membrane transport, including the ATP-binding cassette transporter protein family (31 genes in 15 sites), major facilitator protein superfamily (25 genes in 12 sites) and MATE efflux protein family (23 genes in 11 sites), were highly represented in the group of tandemly arrayed genes.

Repetitive Sequences

By analyzing the JAT_r4.5 sequences with the RECON and RepeatMasker programs, two subfamilies of the *J. curcas hAT*-type transposable element (class II), named *JcDT1* (*Jatropha curcas* DNA-type transposon 1) and *JcDT2*, were identified. The consensus sequence of *JcDT1* members was approximately 3.4 kb long with *terminal inverted repeats* (TIRs) of 12-bp long (TAGRCATGGCCA), and had 29 copies of a hexamer motif (AACCGG) in the subterminal region. It had an ORF that encodes a polypeptide of 722 amino acids long. The predicted amino acid sequence had high similarity with

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transposase (TPase) of *DART*, a subfamily of the rice *hAT*-type transposable element, and had the BED zinc finger as well as the *hAT* family dimerization domain like TPase of *DART* (Fujino et al. 2009). On the other hand, the consensus sequence of *JcDT2* members was approximately 3.3 kb long with TIRs of 11-bp long (TAGGCATGGCC), and had 20 copies of a hexamer motif (AACCGG) in the subterminal region. The homology between the consensus sequences of *JcDT1* and *JcDT2* was 58%. *JcDT2* had an ORF that encoded a polypeptide of 681 amino acids long. The predicted amino acid sequence had high similarity with TPase of rice *DART*, and had the BED zinc finger and the *hAT* family dimerization domain, as in *JcDT1*.

In the case of the *En/Spm*-type transposable element (class II), a subfamily named *JcDT3* was identified in the *J. curcas* genomic sequences. The consensus sequence of *JcDT3* members was approximately 9.4 kb long with TIRs of 17-bp long (CACTACAAAAAAAACGC), and had 23 copies of a decamer motif (TTGCGACCGA) in the subterminal region. It had a predicted coding sequences (CDS) that encodes a polypeptide of 1,097 amino acids long. The predicted amino acid sequence had high similarity with ORF2 of *TdcA1*, a subfamily of the carrot *En/Spm*-type transposable element (Itoh et al. 2003), as well as ORFs of other plant *En/Spm*-type transposable elements.

A subfamily of LINE (Long INterspersed Element), named *JcLINE1,* was also identified. LINE is a non-LTR retrotransposon that belongs to the class I transposable element. The consensus sequence of *JcLINE1* members showed that the length of *JcLINE1* was 5.7 kb or longer. The 3' terminal sequences of *JcLINE1* members were mostly $(A)_n$. The consensus sequence had two ORFs, named ORF1 and ORF2. ORF1 and ORF2 encode polypeptides of 476 and 1,302 amino acids, respectively. The predicted amino acid sequence of ORF2 had high similarity with ORF2 of *Arabidopsis* LINEs, *ATLN39* and *ATLN43* (Noma et al. 2000), and had domains of reverse transcriptase and endonuclease.

A subfamily of nonautonomous LTR retrotransposons, named *JcNLR1* (*Jatropha curcas* nonautonomous LTR retrotransposon 1), was also found. The consensus sequence of *JcNLR1* members had LTRs of ca. 3.6 kb on both sides, with an internal sequence of ca. 2.5 kb between them. The internal sequence had a putative *primer binding* site (PBS) complementary to the 3' end of $tRNA^{Met}$ (TGGTATCAGAGC) and a polypurine tract (PPT) immediately downstream of the 5' LTR and immediately upstream of the 3' LTR, respectively, but did not have ORFs longer than 300 bp. These facts indicate that *JcNLR1* is a nonautonomous LTR retrotransposon like the rice *Dasheng* (Jiang et al. 2002).

Databases

Detailed information on the *J. curcas* genome sequences, SCs and UCs, updated predicted genes, TCs and singleton ESTs are available at the web database for the *J. curcas* genome information at http://www.kazusa.or.jp/jatropha/. A BLAST search engine against the updated genome sequences, TCs and singleton ESTs of accumulated transcript sequences as well as nucleotide and amino acid sequences of predicted genes

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are available. A keyword tool for the search of codes and definitions concerning the IPR domains assigned on the predicted genes by the InterProScan program is also available.

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Chapter 31
Toward the Metabolomics of *Jatropha curcas*

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Introduction

Describing the metabolomics of any biological system is a tremendous challenge. Different from proteomics, for which the analysis can start from a genomic sequence, there is no reference to start for metabolomics. Comparative methods used to identify possible functions and activities for proteins, will fail for the understanding of roles played by many metabolites. Whilst there are some very common metabolites, many are unique and have specific functions such as those related to chemical defense.

Usually, the interest for metabolomic starts with growing investigation of the system on hand. The *Jatropha* species have a large record of use in popular medicine, and recently they became even more attractive due to the efforts invested in the production of biodiesel. Accordingly, some of the predominant metabolites have been characterized with their structures and roles (Zhang et al. 2009; Devappa et al. 2010a, c) in an effort towards a better understanding of the metabolomics and industrial crop recycling. Hereafter, we review the metabolites described in the literature (Table 31.1).

J. curcas

The species *J. curcas* is the most investigated member of the *Jatropha* genus. It is a member of the Euphorbiaceae family that can be found in tropical regions all over the world. It grows quickly in stony and dry soils, even without assistance (Ishii et al. 1987; Makkar et al. 1998; Gandhi et al. 1995; Makkar and Becker 1998). Traditionally it is used in popular medicine (Duke 1985), but it has also been cultivated as living fence

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Table 31.1 Metabolites in the *Jatropha* genus

Compounds	Origin	Activity	References
Diterpenes			
Palmarumycin CP1	*J. curcas*	NA	Ravindranath et al. 2004b
Palmarumycin JC1	*J. curcas*	NA	Ravindranath et al. 2004b
Palmarumycin JC2	*J. curcas*	NA	Ravindranath et al. 2004b
Integerrimene	*J. integerrima*	NA	Sutthivaiyakit et al. 2003
2-Epicaniojane	*J. integerrima*	NA	Sutthivaiyakit et al. 2003
Caniojane	*J. integerrima*	NA	Sutthivaiyakit et al. 2003
1,11-Bisepicaniojane	*J. integerrima*	NA	Sutthivaiyakit et al. 2003
Heudolotinone	*J. curcas*	NA	Das et al. 2003a
Faveline methyl ether	*J. phyllacanthus*	NA	Endo et al. 1991
Faveline	*J. phyllacanthus*	NA	Endo et al. 1991
Deoxofaveline	*J. phyllacanthus*	NA	Endo et al. 1991
16-Hydroxyphorbol	*J. podagrica*	NA	Kong et al. 1993
12-Deoxy-16-hydroxyphorbol	*J. curcas*	NA	Haas and Mittelbach 2002
Curcusone A	*J. curcas*	NA	Muangman et al. 2005
Curcusone B	*J. curcas*	Anti-metastatic	Muangman et al. 2005
Curcusone C	*J. curcas*	NA	Muangman et al. 2005
Curcusone D	*J. curcas*	NA	Muangman et al. 2005
2-Epijatrogrossidione	*J. grossidentata*	NA	Jakupovic et al. 1988
Citlalitrione	*J. gossypiifolia*	NA	Das and Venkataiah 1999
Jatrophatrione	*J. gossypiifolia*	Anti-tumor	Rahmani and Ismail 1990
	J. macrorhiza		Ojewole 1983
Jatrowedione	*J. weddeliana*	NA	Brum et al. 1998
Jatrogrossidione	*J. grossidentata*	NA	Schmeda-Hirschmann et al. 1992
Jatrophenone	*J. gossypiifolia*	Anti-bacterial	Ravindranath et al. 2003
(4*E*)-15-*O*-acetyl-15-epijatrogrossidentadione	*J. curcas*	NA	Ravindranath et al. 2004
(14*E*)-14-*O*-acetyl-5,6-epoxyjatrogrossidentadione	*J. curcas*	NA	Ravindranath et al. 2004
(4*E*)-15-Epijatrogrossidentadione	*J. curcas*	NA	Ravindranath et al. 2004

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Japodagrin	*J. podagrica*	Anti-bacterial	Alyelaagbe et al. 2007
(4*Z*)-Jatrogrossidentadione	*J. podagrica*	NA	Alyelaagbe et al. 2007
(4*Z*)-15-Epijatrogrossidentadione	*J. podagrica*	NA	Alyelaagbe et al. 2007
2-Hydroxyisojatrogrossidione	*J. podagrica*	NA	Alyelaagbe et al. 2007
2-Epihydroxyisojatrogrossidione	*J. podagrica*	NA	Alyelaagbe et al. 2007
Epiisojatrogrossidione	*J. curcas*	NA	Schmeda-Hirschmann et al. 1992
Japodagrol	*J. podagrica*	NA	Kong et al. 1993
Japodagrone	*J. podagrica*	Anti-bacterial	Alyelaagbe et al. 2007
Jatrophone	*J. elliptica*	Anti-leukemic	Kupchan et al. 1970
	J. gossypiifolia		Taylor et al. 1983
	J. isabellii		Calixto and Sant'Ana 1987
			Schmeda-Hirschmann et al. 1996
2α-Hydroxyjatrophone	*J. gossypiifolia*	Anti-leukemic	Taylor et al. 1983
2β-Hydroxyjatrophone	*J. gossypiifolia*	Anti-leukemic	Taylor et al. 1983
2β-Hydroxy-5,6-isojatrophone	*J. gossypiifolia*	Anti-leukemic	Taylor et al. 1983
3β-Acetoxy-12-methoxy-13-methylpodocarpa-8,11,13-trien-7-one	*J. curcas*	NA	Ravindranath et al. 2004
3β,12-Dihydroxy-13-methylpodocarpane-8,10,13-triene	*J. curcas*	NA	Ravindranath et al. 2004
3β,14α-Hydroxypimara- 7,9(11),15-triene- 12-one	*J. divaricata*	NA	Denton et al. 2001
ent-15(13 □ 8)abeo-8β(ethyl)-Pimarane	*J. divaricata*	NA	Denton et al. 2001
Spruceanol	*J. divaricata*	NA	Denton et al. 2001
Cleistanthol	*J. divaricata*	NA	Denton et al. 2001
12-*O*-methylspruceanone	*J. divaricata*	NA	Denton et al. 2001
ent-3β-Hydroxypimara-8(14),9,15-trien-12-one	*J. divaricata*	NA	Denton et al. 2001
Jatropha factor C1	*J. curcas*	NA	Haas et al. 2002
Jatropha factor C2	*J. curcas*	NA	Haas et al. 2002
Jatropha factor C4	*J. curcas*	NA	Haas et al. 2002
Jatropha factor C5	*J. curcas*	NA	Haas et al. 2002

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Table 31.1 (continued)

Compounds	Origin	Activity	References
Jatropha factor C3	*J. curcas*	NA	Haas et al. 2002
Jatropha factor C6	*J. curcas*	NA	Haas et al. 2002
Jatropholone A	*J. curcas*	NA	Kong et al. 1993
Jatropholone B	*J. curcas*	NA	Kong et al. 1993
Jatrophol	*J. curcas*	NA	Kong et al. 1993
Phyllacanthone	*J. phyllacanthus*	NA	de Lemos et al. 1991
Riolozatrione	*J. dioica*	NA	Kong et al. 1993
Sesquiterpenoids and triterpenes			
(1R,2R,5 S,6 S,7 S,10 S)-5-Epieudesm- 4(15)-ene-1R, 2a, 6R-triol	*J. neopauciflora*	NA	García and Delgado 2006
(1R,2R,5 S,6 S,7R,10 S)-Ax-4(15)-ene-1R,2a,7a-triol	*J. neopauciflora*	NA	García and Delgado 2006
Acetylaleuritolic acid	*J. macrorhiza*	NA	Torrance et al. 1977
	J. elliptica	NA	Viswanathan et al. 2004
3-Acetylaleuritolic	*J. weddeliana*	NA	Torrance et al. 1977
β-Amyrin cinnamate	*J. urens*	NA	Bhattacharyya and Barros 1986
Germanicol acetate	*J. urens*	NA	Bhattacharyya and Barros 1986
Friedelin	*J. curcas*	NA	Ravindranath et al. 2004
	J. maheshwarii	NA	Viswanathan et al. 2004
3α-hydroxyfriedelinol	*J. maheshwarii*	NA	Viswanathan et al. 2004
Taraxerol	*J. curcas*	NA	García and Delgado 2006
(Z)-3-O-coumaroyloleanolic acid	*J. curcas*	NA	Sant'Ana et al. 1993
Calenduladiol	*J. neopauciflora*	NA	García and Delgado 2006
(3a,16a)-16-Hydroxylup-20(29)-en –3-yl (E)-3-(4-hydroxyphenyl) prop- 2-enoate	*J. neopauciflora*	NA	García and Delgado 2006
(2a,13a,14b,20 S)- 2,24,25- Trihydroxylanost-7-en- 3-one	*J. gossypiifolia*	NA	Tinto et al. 1992

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(13a,14b,20 S)- 2,24,25- Trihydroxylanosta- 1,7-dien- 3-one	*J. gossypiifolia*	NA	Tinto et al. 1992
3βb-hexanoyllupeol	*J. vitifdius*	NA	Carmelo 1979
3β-O-cinnamoyl lupeol	*J. phyllacanthus*	NA	de Lemos et al. 1991
3β-O-dihydrocinnamoyl lupeol	*J. phyllacanthus*	NA	de Lemos et al. 1991
Epilupeol acetate	*J. avotinfolia*	NA	Carmelo 1979
Lignanes and coumarins			
Gadain	*J. gossypiifolia*	NA	Banerji et al. 1984
Jatrophan	*J. gossypiifolia*	NA	Banerji et al. 1984
Propacin	*J. gossypiifolia*	NA	Das and Venkataiah 2001
	J. glandulifera	NA	Parthasarathy and Saradhi 1984
Prasanthaline	*J. curcas*	NA	Chatterjee et al. 1988
Gossypifan	*J. gossypiifolia*	NA	Das and Das 1995
Jatrodiene	*J. gossypiifolia*	NA	Das et al. 1996a
Gossypiline	*J. gossypiifolia*	NA	Das 1998
Arylnaphthalene	*J. gossypiifolia*	NA	Das and Banerji 1988
Tomentin	*J. curcas*	NA	Calixto and Sant'Ana 1987
	J. glandulifera	NA	Parthasarathy and Saradhi 1984
Scopoletin	*J. gossypiifolia*	NA	Das and Kashinatham 1997
Fraxetin (7,8-dihydroxy-6-methoxycoumarin)	*J. glandulifera*	Analgesic	Parthasarathy and Saradhi 1984
	J. ciliata		Okuyama et al. 1996
	J. gossypiifolia		Das and Kashinatham 1997
Gossypidien	*J. gossypiifolia*	NA	Das and Anjani 1999
Cleomiscosin A	*J. gossypiifolia*	NA	Das et al. 2003
Jatrorins A	*J. gossypiifolia*	NA	Carmelo 1979
Jatrorins B	*J. gossypiifolia*	NA	Carmelo 1979
Jatrocins A	*J. gossypiifolia*	NA	Das et al. 2003
Jatrocins B	*J. gossypiifolia*	NA	Das et al. 2003
5-Hydroxy-6,7-dimethoxycoumarin	*J. gossypiifolia*	NA	Kong et al. 1996

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Table 31.1 (continued)

Compounds	Origin	Activity	References
6-Methoxy-7-hydroxycoumarin		NA	Kong et al. 1996
2,3,7-Trimethoxy-8-*O*-β-d-glucoside ellagic acid	*J. curcas*	NA	Carmelo 1979
2,3-Bis(hydroxymethyl) -6,7- (methylenedioxy) -1- (3′,4′-dimethoxyphenyl) naphthalene	*J. gossypiifolia*	NA	Das and Banerji 1988
Multifidanol	*J. multifida*	NA	Kanth et al. 2011
Multifidenol	*J. multifida*	NA	Kanth et al. 2011
9β, 13α-Dihydroxyisabellione	*J. isabelli*	Cytotoxic	Pertino et al. 2007a
15-*O*-acetyl japodagrone	*J. multifida*	NA	Das et al. 2009b
Isojatrogrossidion	*J. grossidentata*	NA	Sutthivaiyakit et al. 2003 Das et al. 2008
(4*E*)-Jatrogrosidentadione acetate	*J. multifida*	NA	Sutthivaiyakit et al. 2003 Das et al. 2008
(4*E*)-Jatrogrosidentadione	*J. multifida*	NA	Sutthivaiyakit et al. 2003
2α -Hydroxyjatropholone	*J. integerrima*		Sutthivaiyakit et al. 2009
2β -Hydroxyjatropholone	*J. integerrima*		Sutthivaiyakit et al. 2009
Jatropherol	*J. curcas*		Jing et al. 2005
Curculathyrane A	*J. curcas*	NA	Naengchomnong et al. 1986
Curculathyrane B	*J. curcas*	NA	Naengchomnong et al. 1986
Multifolone	*J. multifida*	NA	Das, Ravikanth et al. 2009b
Multifidone	*J. multifida*	Cytotoxic	Das, Reddy et al. 2009c
Multidione	*J. multifida*	NA	Das et al. 2009b
Jatrophalactam	*J. curcas*	Cytotoxic	Wang et al. 2009
Jaherin	*J. zeyheri*		Dekker et al. 1987
Flavonoids			
Vitexin	*J. gossypiifolia* *J. heywii*	NA	Sankara Subramanian et al. 1971 Lakshmi and Venkata 1975
Isovitexin		NA	

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Apigenin	*J. gossypiifolia*	NA	Sankara Subramanian et al. 1971
Flavonoid glycoside I	*J. curcas*	NA	Khafagy et al. 1977
Flavonoid glycoside II	*J. curcas*	NA	Khafagy et al. 1977
Isoorientin	*J. ciliata*	Anxiolytic	Okuyama et al. 1996
Orientin	*J. ciliata*	Anxiolytic	Okuyama et al. 1996
Nobiletin	*J. curcas*	NA	Kong et al. 1993
Luteolin (3′,4′,5,7-tetrahydroxyflavone)	*J. unicostata*	NA	Franke et al. 2004
Alkaloids			
Tetramethylpyrazine	*J. podagrica*	NA	Ojewole 1983
Jatropham	*J. macrorhiza*	NA	Khafagy et al. 1977
Alkaloid A	*J. gossypiifolia*	NA	Ahmad et al. 1992
Alkaloid B	*J. gossypiifolia*	NA	Ahmad et al. 1992
Alkaloid C	*J. gossypiifolia*	NA	Ahmad et al. 1992
5-Hydroxypyrrolidin-2-one	*J. curcas*	NA	Staubmann et al. 1999
Pyrimidine-2,4-dione	*J. curcas*	NA	Staubmann et al. 1999
Cyclic peptides			
Labaditin	*J. multifida*	Anti-bacterial	Kosasi et al. 1989 Barbosa et al. 2011
Curacycline A	*J. curcas*	NA	Beukelman et al. 1995
Curacycline B	*J. curcas*	NA	Auvin et al. 1997
Podacycline A	*J. podagrica*	NA	Kroes et al. 1996
Podacycline B	*J. podagrica*	NA	Kroes et al. 1996
Cyclogossine A	*J. gossypiifolia*	NA	Horsten et al. 1996
Cyclogossine B	*J. gossypiifolia*	NA	Auvin-Guette et al. 1997
Mahafacyclin A	*J. mahafalensis*	NA	Baraguey et al. 2000
Mahafacyclin B	*J. mahafalensis*	Anti-malarial	Baraguey et al. 2001
Cheralierin A	*J. chevalieri*	NA	Baraguey et al. 1998
Cheralierin B	*J. chevalieri*	NA	Baraguey et al. 1998
Cheralierin C	*J. chevalieri*	NA	Baraguey et al. 1998

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Table 31.1 (continued)

Compounds	Origin	Activity	References
Pohlianin A	*J. pohliana*	NA	Auvin-Guette et al. 1999
Pohlianin B	*J. pohliana*	NA	Auvin-Guette et al. 1999
Pohlianin C	*J. pohliana*	Anti-malarial	Auvin-Guette et al. 1999
Phytosterols			
β-Sitosterol	*J. maheshwarii*	Cholesterol reducing	Viswanathan et al. 2004
	J. maheshwarii	NA	Viswanathan et al. 2004
5α-Stigmasta-3,6-diene	*J. curcas*	NA	Carmelo 1979
Campesterol	*J. unicostata*	NA	Franke et al. 2004
Campest-4-ene-3,6-dione	*J. unicostata*	NA	Franke et al. 2004
Campest-4-en-3-one	*J. unicostata*	NA	Franke et al. 2004
Stigmasta-4,22-dien-3-one	*J. unicostata*	NA	Franke et al. 2004
Stigmast-4-en-3-one	*J. unicostata*	NA	Franke et al. 2004
Stigmasta –4,22- diene –3,6- dione	*J. unicostata*	NA	Franke et al. 2004
Stigmast-4-ene-3,6-dione	*J. unicostata*	NA	Franke et al. 2004
Stigmasterol	*J. unicostata*	NA	Franke et al. 2004
Stigmastanol	*J. unicostata*	NA	Franke et al. 2004
Others			
(*Z*)-1-Cyano-3-β-d-(glucopyranosyloxy)-1-methylprop-1-ene	*J. multifida*	NA	van den Berg et al. 1995
4-Butyl-2-chloro-5-formyl-1*H*-imidazole	*J. curcas*	NA	Das et al. 2005
Tetradecyl (*E*)-ferulate	*J. gossypiifolia*	NA	Okuyama et al. 1996
Ferulic acid	*J. gossypiifolia*	Anti-oxidant	Okuyama et al. 1996
3,3-Dimethylacrylyshikonin	*J. glandulifera*	NA	Mujumdar and Misar 2004
Acetylshikonin	*J. glandulifera*	Anti-tumor	Mujumdar and Misar 2004
Multifidol	*J. multifida*	NA	Van der Sluis and Labadie 1989
Multifidol glucoside	*J. multifida*	NA	Van der Sluis and Labadie 1989

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(Jones and Miller 1992), green manure (Sherchan et al. 1989), erosion control, as raw material for soap and paint production (Scavone et al. 1980; Gandhi et al. 1995; Gübitz et al. 1999) and for use as a pesticide (Solsoloy 1995).

The seeds and the oil have been shown to be toxic to mice (Adam 1974; Li et al. 2010), rats (Corvera and García-Sáinz 1984; Liberalino and Bambirra 1988), calves, sheep, goats (Adam and Magzoub 1975; Ahmed and Adam 1979a, b) and chicken (el Badwi et al. 1992). In popular medicine, seeds and oil extracted from them are commonly used as laxatives, in treatment of skin disorders, hydropsis, paralysis and rheumatism (Scavone et al. 1980; Adolf et al. 1984; Gandhi et al. 1995). In-spite of the use in popular medicine, the seeds are highly toxic. The ingestion of seeds or their oil can result in serious poisoning (Mampane et al. 1987). More recently several activities have been documented: antimalaria (Makkar et al. 1997; Kaou et al. 2008), anti-inflammatory (Staubmann et al. 1997; Aiyelaagbe 2001), anti-bacterial (Ravindranath et al. 2004b), anti-coagulant (Osoniyi and Onajobi 2003), anti-diarrheal (Mujumdar et al. 2000), anti-viral (HIV) (Auvin et al. 1997), anti-tumor (based on curcin) (Lin et al. 2003a, b), anti-tumor (based on diterpenes) (Kong et al. 1993), anti-leukemic (Khafagy et al. 1977), anti-metastatic (Muangman et al. 2005), cutaneous wound healing (Nath and Dutta 1991; Esimone et al. 2008), pregnancy-terminating (Goonasekera et al. 1995), insecticidal (Jing et al. 2005), mosquito biting deterrency (tested against *Aedes aegypti*) (Cantrell et al. 2011), inhibition of proliferation of human T-cells (Beukelman et al. 1995), molluscicidal (Liu et al. 1997; Rug et al. 1997) and fungicidal (Nwosu and Okafor 1995). In total, 39 active compounds have been identified from *J. curcas* (Thomas et al. 2008; Zhang et al. 2009).

The toxicity was originally assigned to *curcin*, present abundantly in the seeds. Independent investigations showed that the effect is caused by the phorbol esters found in seeds and oil. Actually, curcin was found in comparable amounts in both types of *J. curcas*: the ones considered poisonous and the ones not. The effect of protein synthesis inhibition by curcin was shown to be about 10,000 times lesser than that of *ricin* or *abrin*. In oral toxicity tests performed on rabbits, rats and mice, the oil showed similar effects as tung-oil extracted from the seeds of *Aleurites fordii Hemsley*, which is known to be toxic due to phorbol esters.

In the 1990s, *J. curcas* was investigated as a possible source of biodiesel (Foidl and Eder 1997), and different methods of oil extraction were tested (Foidl et al. 1996; Winkler et al. 1997). However, toxicity remains to be addressed (Makkar and Becker 1998; Aregheore et al. 1998; Makkar et al. 1998; Haas and Mittelbach 2000; Zhang et al. 2009; Li et al. 2010; Devappa et al. 2010b; Pradhan et al. 2012; Roach et al. 2012).

The increasing interest on biodiesel over the last decade has generated a huge demand of oil, and the interest for *J. curcas* has renewed (Becker and Makkar 2008; Kumar and Sharma 2008). Several health related activities were revisited (Mujumdar et al. 2000; Aiyelaagbe 2001; Lin et al. 2003b; Osoniyi and Onajobi 2003; Kaou et al. 2008; Thomas et al. 2008; Esimone et al. 2008), including other alternative applications (Verma et al. 2011; Monteiro et al. 2011; Silva et al. 2012; Nithiyanantham et al. 2012; Devappa et al. 2012), such as insecticides and cake detoxification methods (Haas and Mittelbach 2000; Joshi et al. 2011; Das et al. 2011; Ceasar and Ignacimuthu 2011; Prasad et al. 2012).

Recently the genome of *J. curcas* was sequenced and the genes for the synthesis of triacylglycerols, phorbol esters and curcin were identified (Sato et al. 2011) as well as a first metabolomics analysis of stress reactions (Debnath et al. 2011).

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Other Species of the *Jatropha* Genus

J. gossypiifolia

J. gossypiifolia grows naturally in almost the entire tropical area worldwide (Burkill and Dalziel 1997). It is native of Brazil, and was naturalized in many parts of India, growing on nearly all types of soils within its range, common in waste lands, road sides, poorly tended agricultural fields and river overflow areas (Kakade et al. 2008). Besides *J. curcas,* it is the most investigated species of the *Jatropha* genus.

J. gossypiifolia is reported to be beneficial to dyscrasia, anemia, vertigo and dysphonia. It is an antibiotic, insecticidal, used in toothache and acts as blood purifier (Baleé 1994). The leaves are employed to cure carbuncles, eczema and itches and also act as a purgative. A decoction of the leaves is useful for stomach ache, venereal disease and as blood purifier (Banerji et al. 1993).

The leaves, either in decoction or boiled like spinach, serve as a purgative remedy for dry belly ache. Extracts of the plant are used as a purgative, emetic and to treat headache, diarrhea, venereal disease, skin sores, mouth sores and cancer (Burkill and Dalziel 1997). The seeds are used as a purgative; its oil is similar to castor oil. The use of seeds in herbal medicine is not advised because of their high toxicity level (Mampane et al. 1987). The seed oil is used as an emetic, purgative and stimulant. It is also applied for ulcers and leprosy and is beneficial in adenites and worm infestation (Banerji et al. 1993). The roots are recommended for leprosy and as an antidote for snake bites (Kirtikar 1918).

In many countries, *J. gossypiifolia* is used as a medicinal plant. In India, it is used to treat diarrhea (Padhy 2006) and the roots are employed to treat dysentery (Dabur et al. 2007). In Trinidad, the decoction of *J. gossypiifolia* is used to treat wounds and to reduce pain. In ethnoveterinary, it has been used by hunters to treat snake bites, scorpion stings, for injuries and manage of their dogs (Lans et al. 2001). In ethnomedicine the use to treat snatch wounds, sores and swellings is reported (Lans 2007). In Ghana, decoction of leaves of *J. gossypiifolia*, *Combretum ghaselensis* and the whole part of *Ocimun canum* are used to treat malaria (Asase et al. 2005). In Ekiti, Nigeria, *J. gossypiifolia* is cultivated to serve as boundary plant, for erosion control and healing of mouth cancer (Kayode 2008). In Southern Nigeria, the extract from fresh leaves applied with crushed leaves is routinely used by herbalists and local people to stop bleeding skin and nose bleeding (Oduola et al. 2005a, b, 2007). In Suriname, the fruits are used as a purgative (van Andel et al. 2008).

A total of 36 compounds were isolated and characterized from *J. gossypiifolia* (Sankara Subramanian et al. 1971; Taylor et al. 1983; Banerji et al. 1984; Das and Banerji 1988; Das and Das 1995; Das et al. 1996a, b, 1999, 2003a, b; Das and Kashinatham 1997; Das 1998; Das and Anjani 1999; Das and Venkataiah 1999, 2001; Ravindranath et al. 2003b; Pertino et al. 2007c) and a series of activities have been described: anti-tumor (Taylor et al. 1983; Pertino et al. 2007b; Devappa et al. 2010b), anti-leukemic (Taylor et al. 1983), cytotoxic (Taylor et al. 1983; Pertino et al. 2007b; Devappa et al. 2010b), molluscicidal (Taylor et al. 1983; dos Santos and Sant'Ana 1999; Pertino et al. 2007b; Devappa et al. 2010b), leishmanicidal

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(Taylor et al. 1983; Pertino et al. 2007b; Devappa et al. 2010b), gastroprotective (Taylor et al. 1983; Pertino et al. 2007b; Devappa et al. 2010b), anti-bacterial (Ravindranath et al. 2003a) and anti-microbial (Dabur et al. 2007).

J. multifida

Fifteen compounds were isolated and characterized from *J. multifida* (Van der Sluis and Labadie 1989; Kosasi et al. 1989; van den Berg et al. 1995; Osoniyi and Onajobi 2003; Das et al. 2008, 2009a, b, c, 2010; Kanth et al. 2011). Popular medicine uses *J. multifida* for the treatment of infections, and anti-microbial activity has been confirmed (Aiyelaagbe and Oguntuase 2008). Furthermore, other activities have been reported: anti-infection (Hart et al. 1989), anti-bacterial (Kosasi et al. 1989; Aiyelaagbe 2001), antibody-supplementary (Matsuse et al. 1999).

J. integerrima

Whilst there is no popular medicine use known for *J. integerrima* (Sutthivaiyakit et al. 2003), 18 different mostly unique compounds have been isolated from the extracts (Mongkolvisut et al. 2006; Sutthivaiyakit et al. 2009). Two of the compounds isolated are reported to have anti-malarial and anti-tuberculosis activities (Sutthivaiyakit et al. 2009).

J. unicostata

Twelve compounds were isolated and characterized from *J. unicostata* (Franke et al. 2004), but no activity has been reported.

J. elliptica

Ten compounds have been isolated from *J. elliptica*, one of them showed a series of activities: molluscicidal (dos Santos and Sant'Ana 1999), antiprotozoal (Schmeda-Hirschmann et al. 1996), antileishmanial (Chan-Bacab and Peña-Rodríguez 2001), anti-leukemic (Kupchan et al. 1970, Lillehau et al 1973), interaction with sRNA from *Escherichia coli* (D'Alagni et al. 1983), relaxation effect of induced uterine contraction (Calixto and Sant'Ana 1990), inhibition of insulin release (Menezes et al. 1992), relaxant action in rat portal vein (Silva et al. 1995), inhibition of lymphocytes activation (de Moraes et al. 1996), inhibition of glutamate binding (in rat cortex membrane) (Martini et al. 2000), and anti-bacterial (Marquez et al. 2005; de Lima et al. 2006).

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J. podagrica

Ten compounds have been isolated from *J. podagrica*, with reported activities: anti-bacterial (Alyelaagbe et al. 2007; Schmeda-Hirschmann et al. 1992; Aiyelaagbe and Gloer 2008), antifungal (Schmeda-Hirschmann et al. 1992) and anti-tumor (Bamidele Sanni et al. 1988; Kong et al. 1993; Dahiya 2008). Several activities have been reported for the extracts, such as neuromuscular-blocking and hypotensive (Ojewole and Odebiyi 1980), anti-bronchoconstrictor and anti-arrhythmic (Ojewole 1983) and hemodynamic (Dai and Bache 1985). In popular medicine the latex of *J. podagrica* is used for the treatment of infected wounds, skin infections and scabies (Kosasi et al. 1989; Kroes et al. 1996).

J. divaricata

Six compounds were isolated and characterized from *J. divaricata* (Denton et al. 2001), but no activity has been reported.

J. glandulifera

Five compounds were isolated and characterized from *J. glandulifera* (Parthasarathy and Saradhi 1984). A strong anti-bacterial activity has been reported for the latex of *J. glandulifera*, but the active compounds have not been characterized (Rajasekaran and Tishya 2012).

J. phyllacanthus

Five compounds were isolated and characterized from *J. phyllacanthus* (Endo et al. 1991; de Lemos et al. 1991), but no activity has been reported.

J. maheshwarii

Four compounds were isolated and characterized from the extracts of *J. maheshwarii*. Popular medicine uses the extract of *J. maheshwarii* in skin diseases and toothaches. These activities were confirmed in tests against 12 human pathogenic bacteria and 3 fungal strains (Viswanathan et al. 2004).

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J. neopauciflora

Four compounds were isolated and characterized from the extracts of *J. neopauciflora* (García and Delgado 2006). Only moderate cytotoxic activity was found for one of them and no application in popular medicine is known.

J. macrorhiza

A total of three compounds were isolated and characterized from *J. macrorhiza* (Kupchan et al. 1970; Torrance et al. 1977; Ojewole 1983). One compound was identified specifically as anti-leukemic (Kupchan et al. 1970; Torrance et al. 1977), another one as anti-tumor (Ojewole 1983).

J. pohliana

Three compounds were isolated and characterized from *J. pohliana* and all three compounds showed anti-malarial activity (Auvin-Guette et al. 1999).

J. chevalieri

Three compounds have been isolated from *J. chevalieri*, one of them showing anti-malarial activity (Baraguey et al. 1998).

J. ciliata

Three compounds were isolated and characterized from *J. ciliata*. Two of them were identified as anxiolytic components and the third as an analgesic (Okuyama et al. 1996).

J. grossidentata

Two compounds were isolated and characterized in *J. grossidentata* (Rahmani and Ismail 1990), but several activities were reported for its extracts, such as antiplasmodial and cytotoxic (Sutthivaiyakit et al. 2003), leishmanicidal and trypanocidal

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(Schmeda-Hirschmann et al. 1992, 1996), anti-bacterial and antifungal (Schmeda-Hirschmann et al. 1992; Alyelaagbe et al. 2007) and anti-tumor (Ojewole 1983). However, no active principle identification has been described for these activities. In popular medicine the treatment of haemorrhages (Schmeda-Hirschmann 1993) is a common application.

J. dioica

Two compounds were isolated and characterized from *J. dioica* (Watson et al. 1982; Kong et al. 1993) and one compound from the root extract was confirmed as having anti-bacterial activity according to reports from popular medicine (Watson et al. 1982).

J. weddeliana

Two compounds were isolated and characterized from *J. weddeliana* (Brum et al. 1998, 2001), but no activity has been reported.

J. urens

Two compounds were isolated and characterized from *J. urens* (Bhattacharyya and Barros 1986), but no activity has been reported.

J. heynei

Two compounds were isolated and characterized from *J. heywii* (Lakshmi and Venkata 1975), compounds that have already been isolated from *J. gossypiifolia* (Subramanian et al. 1971).

J. isabellii

Only one compound was isolated and characterized from *J. isabellii*, it has also been found in *J. gossypiifolia* and *J. elliptica* (Schmeda-Hirschmann et al. 1996). It has been reported to have anti-leukemic and antinasopharyngeal carcinoma activity (Kupchan et al. 1970).

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J. zeyheri

Only one compound was isolated and characterized from *J. zeyheri*; it exhibits a strong antimicrobial and antifungal activity (Dekker et al. 1987).

J. vitifdius

Only one compound was isolated and characterized from *J. vitifdius* (Carmelo 1979), but no activity has been reported.

J. avotinfolia

One compound was isolated and characterized from *J. avotinfolia* (Carmelo 1979), but no activity has been reported.

J. tanjorensis

No compound from *J. tanjorensis* has been characterized yet, but antianaemic effect of the leaves has been reported for rats (Ornoregie and Osagie 2007). Popular medicine has reported anti-malarial and antihypertensive effects (Orhue et al. 2008). The leaves are part of the normal diet of the Edo people from Nigeria, but no specific effects have been associated to this diet (Mensah et al. 2008).

Conclusions

In total, 164 compounds have been identified in different members of the *Jatropha* genus. Due to their concentrations they can all be assumed to be at the end of the metabolic chain, accumulated with mostly unknown properties. Almost all of the diterpenes identified are specific to *Jatropha* as well as the cyclic peptides, the majority of the lignans and coumarins; most activities associated to these compounds remain unknown. Many of the other compounds, like the phytosterols and flavonoids, are common to other genera and have been investigated for more time, but also lack information about activity or probably have no activity. In general, no intermediate metabolite has been described, making it impossible to really assess the metabolomics of *Jatropha* spp.

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There are two major metabolomic databases available today: Kyoto Encyclopedia of Genes and Genomes (KEGG) and PlantMetabolomics. KEGG contains general information about the biosynthetic pathways of flavonoids, diterpenoids, triterpenoids, sesquiterpenoids, steroids and to a certain extent other alkaloids. With this information at hand, combined with the genome sequence (Sato et al. 2011), the identification of many pathways involved in production of these metabolites might become possible. PlantMetabolomics comprises 1,800 metabolites from *Arabidopsis thaliana* (Laibach 1943), the model plant (Rensink and Buell 2004; Coelho et al. 2007; Platt et al. 2010) for biological studies, and has recently been made available online (Bais et al. 2010). Although it might not contain the compounds that are specific to *Jatropha* spp., some compounds like the phytosterols can be found there. By comparing the biological synthesis and function in *A. thaliana* it might be possible to understand the biochemistry of these metabolites in *Jatropha* as well. By combining all the biological pathways identified *via* the characterized metabolites, a directed guess can be made about the other involved metabolites, which can in turn be searched for. At least, the part of the metabolomics common to a large proportion of plants can be accessed in this way. Guessing for the more specific part will need further experimental evidence; new methods for metabolomics (Hirai et al. 2010) might help in improving the current situation.

The newly sparked interest for biodiesel fuels has lead to a lot of research on *J. curcas*, but there is still a long way to go. Most investigations are focused on analyzing or improving the process of oil production and cake detoxification. However, some efforts have already been invested towards understanding Jatropha's metabolomics.

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Index

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