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REVIEW ARTICLE

Jatropha curcas: a review on biotechnological status and challenges

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Abstract Plant tissue culture and molecular biology techniques are powerful tools of biotechnology that can complement conventional breeding, expedite crop improvement and meet the demand for availability of uniform clones in large numbers. *Jatropha curcas* Linn., a non-edible, eco-friendly, non-toxic, biodegradable fuel-producing plant has attracted worldwide attention as an alternate sustainable energy source for the future. This review presents a consolidated account of biotechnological interventions made in *J. curcas* over the decades and focuses on contemporary information and trends of future research.

Keywords Crop improvement · In vitro plant regeneration · *Jatropha curcas* · Molecular markers · Organogenesis · Somatic embryogenesis

Introduction

The utilization of energy crops as a source of renewable fuels is a concept with great relevance to current ecological and economic issues at both national and global scales. This non-conventional source of energy will help in removing regional imbalance in energy use by making energy available in a decentralized manner. Oil-yielding crops produce different types of oil, called vegetable oils, and we have long experience in using some of the vegetable oils directly as biofuel. In the recent past, biofuels derived from plant species has been a major renewable source of energy. The interest in biofuels as an alternative to fossil fuels is mainly driven by concerns over the increase in crude oil prices, depleting reserves of petrofuels, global warming and increase in demand of transportation fuel in developing economies. Further, political unrest in countries where crude oil reserves are highest is another cause of concern. The production of biofuels will lower nations' dependence on foreign oil supplies, and will reduce emissions of greenhouse gases. Biofuel plants are treated as high value commercial crops of great importance and play a vital role in the economy of producing countries.

Biomass as a source of renewable energy is fundamental for the development and sustenance of civilization. In view of growing interest in renewable energy sources, liquid bioenergy production from vegetable oils has been viewed as one of the possible options to reduce greenhouse gas (GHG) emissions. The past decade has ushered in an unprecedented interest in technologies to produce biofuels and make them cost-competitive with conventional petroleum-derived fuels. Global awareness of the climatic changes linked to a gradual increase in CO_2 emission in the atmosphere is creating a relative impact on the societies of developed and developing countries. The world is searching for an alternative to fossil fuels to reduce CO_2 emission. Production of biofuels using natural raw materials is not new, but what is new is the plant biotechnological intervention for quantitative and qualitative biofuel improvement. Scientists are engaged in developing biofuels from systems of

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microbes like *Escherichia coli*, *Saccharomyces cerevisiae* (Kalscheuer et al. 2004, 2006), third generation biofuels from microalgae like *Botryococcus braunii*, *Chlorella* sp., *Dunaliella tertiolecta*, *Gracilaria*, *Pleurochrysis carterae*, *Sargassum* etc. (Becker 1994; Cristi 2007; Miao and Wu 2004; Qin 2005), edible crop plants or the first generation biofuels like corn, wheat, maize, soybean, sunflower, safflower, sugarcane, *Brassica*, palm oil, etc. (Pinto et al. 2005; Filho et al. 1992; Demirbas 2006; Cahoon 2003; Edem 2002; Mittelbach and Remschmidt 2004; Gunstone 2004; Isigigur et al. 1994), and non-edible crop plants or the second generation biofuels which encompass plants like *Miscanthus*, *Panicum virgatum* or switchgrass, *Hevea brasiliensis* or rubber seeds, *Calotropis gigantia* or ark, *Euphorbia tirucalli* or sher, *Boswellia ovalifololata*, *Jatropha curcas* or ratanjyot, *Pongamia pinnata* or karanj, and *Calophyllum inophyllum* or nagchampa (Shay 1993; Ma and Hanna 1999; Ramadhas et al. 2004a, b; Demirbas 2003; Azam et al. 2005; Karmee and Chadha 2005; Adholeya and Singh 2006; Lohia 2006; Mandal and Mithra 2006). It is evident from a worldwide literature study that food crops comprise about 30–40% of the biofuel production. This automatically reflects in the countries' food price inflation economy, whereas second generation biofuels can reduce competition with food production, require less agronomic (fertilizer, plowing and pesticide) inputs, and have a lower environmental impact than first generation biofuels. Therefore, plants producing non-edible oil may be the material of choice for production of biofuel. As India is deficient in edible oil production, focus is now shifting from edible to non-edible oil crops.

Why *Jatropha curcas*?

Jatropha curcas has spread beyond its original distribution because of its hardiness, ease of conventional propagation, drought endurance, low seed cost, short gestation period, rapid growth, adoption to wide agro-climatic conditions, bushy/shrubby nature and multiple uses of different plant parts (Jones and Miller 1992; Francis et al. 2005; Kumar and Sharma 2008). *J. curcas* is also reported to partially exhibit viviparous germination (Deore and Johnson 2008a). In addition, *J. curcas* as an alternate source of energy crop has gained importance on the basis of its relatively high seed oil content (30–40%) and lipid composition similar to that of fossil fuel, while it does not compete with edible oil supplies (Jha et al. 2007; Deore and Johnson 2008b). Moreover, the plant can be easily cultivated on marginal land and thus does not put pressure on fertile agricultural land or natural ecosystems. Currently, feed stock for biodiesel production is derived from rape seeds and oil palms. Many developing nations cannot afford to use edible oil supplies as a source of biodiesel which directly contributes to escalation of food prices.

Taxonomic status and geographical distribution

The genus *Jatropha* belongs to tribe Joannesieae of Crotonoideae in the Euphorbiaceae family and contains approximately 175 known species native to South America and widely distributed in South and Central America and now almost pantropical. Dehgan and Webster (1979) revised the subdivision made by Pax (1910) and now distinguish two subgenera (*Curcas* and *Jatropha*) of the genus *Jatropha*, with 10 sections and 10 subsections to accommodate the Old and New World species. They postulated the physic nut {*J. curcas* L. [sect. Curcas (Adans.) Griseb., subg. Curcas (Adans.) Pax]} to be the most primitive form of the *Jatropha* genus which forms artificial and natural hybrid complexes readily and poses a problem to the genetic fidelity (Prabakaran and Sujatha 1999). Species in other sections evolved from the physic nut or another ancestral form, with changes in growth habit and flower structures.

Reasons for review

For several reasons, both technical and economical, the full potential of *J. curcas* is far from being realized. Apart from agronomic, socioeconomic and institutional constraints, planned crop improvement programs with genetically uniform germplasm are lacking globally. Earlier research programs involving large-scale plantations launched in Brazil, Nicaragua, South Africa and India indicated that the crop productivity is far too low to be commercialized. In extreme cases, the plantations failed to produce fruits. There is limited information available on genetics and agronomy of *Jatropha*. There is a lack of bench mark descriptors and information on genetic variability, effects of the environment, and genotype environment interaction (Jongschaap et al. 2007). Furthermore, there is a need for integration of the available scattered knowledge and experience with crop performance of different *J. curcas* provenances in different environments and biotechnological management interventions. Keeping this in view, an attempt is made to review available literature on biotechnological progress and genetic improvement of *J. curcas.*

The disadvantages of conventional propagation in *J. curcas*

Jatropha is a seed-bearing plant and can produce 1–2 kg of seed per plant/year when the plant is 2–3 years old. The production amount may increase with increasing age of the plant. The edaphic factors also play a role in the rate of

seed production. However, the main disadvantage of in vivo cultivation of *J. curcas* is that it produces an uncertain yield of nuts during a short period of time (once or twice a year) and may not produce optimal yields for several years, which makes it unsustainable. The plant is cross-pollinated and thus the seeds are of unknown genetic potential. Trees propagated by cuttings show a lower longevity and possess a lower drought and disease resistance than those propagated from seeds. Trees produced from cuttings often produce pseudo-taproots as reported by Sujatha et al. (2005). Seed set has been reported to be low in vegetatively propagated plants (Sujatha et al. 2005). Cuttings often result in high-density *Jatropha* plantings more susceptible to insect and disease infestation. Large-scale cultivation of *J. curcas* remains the single most important issue that will ultimately decide success. Low and unpredictable yields are reported from established plantations.

Problems of seed cultivation

- Heterozygosity.
- Genetic uniformity in planting materials is difficult.
- Practices are seasonal and seed-borne diseases may be transmitted to the seedling.
- Cannot ensure quality in terms of oil content because the plant is heterozygous.

In vitro plant regeneration systems in J. curcas

In vitro culture is one of the key tools of plant biotechnology that exploits the totipotent nature of plant cells, a concept proposed by Haberlandt (1902) and unequivocally demonstrated for the first time in *Daucas carota* cell culture by Steward (1958). In vitro culture exploits flexibility of plant cells and, depending on inherent and induced competence of plant cells, systems of plant regeneration are categorized as direct and indirect. Almost all types of explant tissues are now used as regeneration systems through direct and indirect methods. The following review will discuss direct and indirect methods of plant regeneration in *J. curcas* using in vitro regeneration protocols for both direct and callus-mediated shoot regeneration, somatic embryogenesis using shoot and nodal meristems, cotyledonary and normal leaf, hypocotyls, and epicotyls segments, as well as mature and immature zygotic embryos.

Direct plant regeneration in *J. curcas*

Several reports are available on direct regeneration from leaf (Sujatha and Mukta 1996; Sujatha et al. 2005; Deore and Johnson 2008b; Khurana-Kaul et al. 2010; Reddy et al. 2008; Divakara et al. 2010), petiole (Sujatha and Mukta 1996; Kumar and Reddy 2010; Kumar et al. 2011), nodal (Datta et al. 2007), axillary nodes (Sujatha et al. 2005), shoot tips (Rajore and Batra 2005; Purkayastha et al. 2010), hypocotyl (Sujatha and Mukta 1996; Kaewpoo and Te-chato 2010), epicotyl (Qin et al. 2004; Kaewpoo and Te-chato 2010), and cotyledons (Kumar et al. 2010a) (Table 1). Direct plant regeneration without an intervening callus phase (adventitious shoot bud formation) is a more reliable method for multiplication or clonal propagation. The plants produced by the direct organogenesis method may exhibit greater genetic stability than those produced via callus-mediated organogenesis (D'Amato 1975).

Murashige and Skoog's medium (1962) was the most preferred medium to initiate and improve the response in in vitro cultures, but B5 medium (Gamborg et al. 1968) was used by Warakagoda and Subasinghe (2009). For direct regeneration, the most commonly used growth regulators are TDZ, BAP, IBA, and NAA. The growth regulators were either used alone or in combination at varying concentrations for shoot bud initiation or direct regeneration. The most responding explant in *J. curcas* was found to be cotyledonary leaf tissue. Sujatha and Mukta (1996) worked on *J. curcas* to study the morphogenesis and plant regeneration from tissues. Shoot buds from the axillary nodes and leaf segments of non-toxic *J. curcas* were cultured on MS + 2.3–46.5 μM Kn, 2.2–44.4 μM BA and 2.3–45.4 μM TDZ individually, and the leaf segments were cultured on MS + 8.9 μM BA + 2.5 μM IBA to induce adventitious shoots (Sujatha et al. 2005). Direct shoot bud regeneration using a combination of 22.19 μM BAP and 4.90 μM IBA was reported by Sujatha et al. (2005). For further differentiation, reduced concentrations of BAP and IBA were used. The authors noticed differences in responses among genotypes which they attributed to endogenous concentration of hormones. Rajore and Batra (2005) cultured shoot tips of *J. curcas* on MS + 8.87 μM BAP and 2.85 μM IAA along with adenine sulphate, glutamine and activated charcoal. The regenerated shoots were rooted on half-MS + 2.46–24.60 μM IBA. The high frequency rooting was observed on MS medium supplemented with 14.70 μM IBA. The survival rate of acclimatized plants was 60–70%. In contrast, Khurana-Kaul et al. (2010) reported shoot bud induction accompanied by callus formation if a combination of IBA and BAP was used. Thepsamran et al. (2007) reported multiple shoot bud induction from axillary buds. The BAP and IBA combination provided the best shoot bud induction of 6.0 (approx.) shoots per explant. When in vitro epicotyl explants from *J. curcas* were cultured on medium containing IBA and BAP, adventitious shoot buds were directly induced (Qin et al. 2004). Low concentrations of IBA in combination with 2.22 μM BAP induced the

Table 1 Tissue culture studies in *Jatropha curcas*

Type of explant	Mode of regeneration	PGRs + medium	References
Node	Direct organogenesis	BA (22.2 μM); Kn (2.3 μM) + IBA (0.5 μM) + Adenine sulphate (27.8–55.6 μM)	Datta et al. (2007)
Axillary node and leaf sections	Direct adventitious multiple shoot bud regeneration,	TDZ (2.3–4.5 μM), BA (4.4–44.4 μM), IBA (4.9 μM)	Sujatha et al. (2005)
Hypocotyl, petiole and leaf disc	Regeneration via adventitious shoots, rooted in vitro	BA (0.44–2.22 μM) + IBA (0.49–4.9 μM)	Sujatha and Mukta (1996)
Shoot tip	Organogenesis, shooting rooted in vitro	BAP (2.0 mg/l), IAA (0.5 mg/l), Adenine sulphate (25 mg/l), Glutamine (100 mg/l)	Rajore and Batra (2005)
Petiole	Direct induction of shoot buds	TDZ (2.27 μM), Kn (10 μM), BAP (4.5 μM), NAA (5.5 μM)	Kumar and Reddy (2010)
Leaf disc	Adventitious shoot buds	TDZ (2.27 μM), BAP (2.22 μM), IBA (0.49 μM)	Deore and Johnson (2008b)
Axillary node	Direct shoot regeneration	BA (3.0 mg/l) + IBA (1.0 mg/l) + Adenine sulphate (25 mg/l) + Glutamine (50 mg/l) + L-arginine (15 mg/l) + Citric acid (25 mg/l)	Shrivastava and Banerjee (2008)
Immature embryo	Indirect organogenesis (callus mediated)	BA (1 mg/l) + Kn (0.5 mg/l) + IBA (0.25 mg/l) + 500 mg/l PVP + 30 mg/l citric acid	Varshney and Johnson (2010)
Cotyledonary leaf	Direct shoot regeneration	TDZ (9.08 μM), Kn (10 μM), BAP (4.5 μM), NAA (5.5 μM)	Kumar et al. (2010a, b)
Nodal segments	Multiple shoot formation	BAP (1.5 mg/l), Kn (0.5 mg/l), IAA (0.1 mg/l)	Kalimuthu et al. (2007)
Cotyledon explant	Somatic embryo induction	BAP (2.0 mg/l)	
Epicotyl	Direct organogenesis and shoot differentiation from callus	BA (0.2–0.7 mg/l) + IBA (0.1–1.0 mg/l)	Qin et al. (2004)
Shoot apices	Shoot bud induction with callus formation at the base	BAP (2.5 μM), GA_3 (0.5 μM)	Purkayastha et al. (2010)
Leaf segments	Somatic embryogenesis	Kn (2.3–9.3 μM), IBA (0.5–4.9 μM), Adenine sulphate (13.6 μM)	Jha et al. (2007)
Epicotyls and hypocotyls	Direct and indirect organogenesis	TDZ (0.25 mg/l), Kn (0.5 mg/l), IBA (0.25 mg/l)	Kaewpoo and Te-chato (2010)
Leaf explants	Regeneration of somatic embryos	MS salts and B5 vitamins + BA (3.0 mg/l) + IAA (1.0 mg/l)	Sardana et al. (2000)
Leaf and hypocotyls explants	Callus and suspension culture	2,4-D (0.5 mg/l) + 2% coconut milk	Soomro and Memon (2007)
Axillary bud	Multiple shoot induction	BAP (2.22 μM) + IBA (0.49 μM)	Thepsamran et al. (2007)

highest regeneration frequency (Qin et al. 2004), whereas shoot regeneration from callus required the combination of 2.46 μM IBA and 0.44 μM BAP (Qin et al. 2004). IBA in combination withof TDZ produced adventitious shoot buds directly on the cultured leaf surface. Deore and Johnson (2008b) reported high frequency induction of shoot buds in leaf disc cultures without any intervening callus phase. TDZ in the induction medium has greater influence on the induction of adventitious shoot buds whereas, if BAP alone was used in absence of TDZ, only callus formation occurred rather than shoot buds (Fig. 2f). The petiole is a somatic tissue and plants raised from the petiole are more resistant to genetic variation (Pierik 1991). In petiole explants, Sujatha and Mukta (1996) claimed direct shoot organogenesis by using 2.22 μM BAP in combination with 4.90 μM IBA as observed by Khurana-Kaul et al. (2010) in cultures derived from leaf explants. The role of TDZ in inducing high frequency multiplication was first reported by Deore and Johnson (2008b) (Fig. 2f–h). Subsequent investigations (Kumar and Reddy 2010; Kumar et al. 2011; Khurana-Kaul et al. 2010; Khemkladngoen et al. 2011) proved that the concentration of TDZ significantly influenced the response of shoot bud induction. In addition to role of TDZ, Kumar et al. (2011) reported influence of orientation of the explant as another important factor for shoot bud formation. The percentage of induction of shoot

buds and the number of induced shoot buds per explant were higher when the explant was cultured in the horizontal position as compared to vertically positioned petioles. In some laboratories, axillary shoot buds or the nodal portion of the stem was used as an explant source (Datta et al. 2007). Kalimuthu et al. (2007), reported shoot bud induction from nodal explants on MS medium fortified with 22.19 μM BAP, 2.32 μM Kn and 0.57 μM IAA. Shrivastava and Banerjee (2008) reported that when a combination of auxin (4.90 μM IBA) with cytokinin (13.31 μM BAP) along with growth additives (adenine sulphate, glutamine and L-arginine) was used shoot regeneration from axillary nodes was observed. But Datta et al. (2007) found that MS medium supplemented with 22.19 μM BAP and 108.58 μM adenine sulphate would be most suitable for the highest (30.8 ± 5.48) axillary shoot bud proliferation from nodal explants (Fig. 1a, b). Nodal meristems are an important source tissue of micropropagation, and plants raised from these are comparatively more resistant to genetic variation (Pierik 1991). It is now well established that regenerated plantlets derived from organized shoot meristems are less susceptible to genetic variation than those regenerated from disorganized callus tissues (Pierik 1991). Production of genetically uniform propagules is important for commercially exploited plants and also essential for germplasm conservation. The problem of in vitro root induction was well investigated by Datta et al. (2007). For rooting, MS + 4.90 μM IBA were used and subsequent transfer to MS basal medium and later acclimatized which deservedly elaborates a laboratory to land protocol of in vitro plant regeneration from nodal explant in *J. curcas* (Datta et al. 2007) (Fig. 1c, d). A report on shoot tip culture (Rajore and Batra 2005) demonstrated that MS medium incorporated with BAP and IAA along with 100 mg/l glutamine and 135.72 μM adenine sulphate gave the best response for shoot proliferation. The other explant sources, epicotyl, hypocotyls and cotyledonary leaves, from germinating seedlings were successfully used to induce direct shoot bud regeneration in the combination of IBA and BAP (Sujatha and Mukta 1996; Qin et al. 2004). In contrast, Khemkladngoen et al. (2011) and Kumar et al. (2010a) found that TDZ had a higher impact on direct regeneration of shoot buds in comparison to BAP, while Khurana-Kaul et al. (2010) proved that TDZ treatment resulted in more than a doubling of the rate of shoot bud induction than BAP. In conclusion, it is obvious that BAP with or without IBA is playing a very important role for direct shoot bud induction. TDZ had a higher impact on the induction of direct shoot buds in comparison to BAP. For further shoot bud multiplication, a reduced concentration of auxin (IBA) was found to be effective. The high concentrations of auxins are generally inhibitory to morphogenesis, and the use of an appropriate auxin–cytokinin ratio is essential to obtain proper shoots and root primordia (Kalimuthu et al. 2007).

Indirect plant regeneration in *J. curcas*

Organogenesis

Organogenesis is a complex phenomenon involving the de novo formation of organs (shoots or roots). Shoots can be derived either through differentiation of non-meristematic tissues known as adventitious shoot formation or through pre-existing meristematic tissues known as axillary shoot formation. Both the approaches require synergistic interaction of physical and chemical factors. A successful plant regeneration protocol requires appropriate choice of explant, age of the explant, definite media formulations, specific growth regulators, genotype, source of carbohydrate, gelling agent, and other physical factors including light regime, temperature, humidity, etc. Plant hormones play a crucial role in controlling the way in which plants grow and develop. They regulate the speed of growth of the individual parts and integrate these parts to produce the plants. Both auxins and cytokinins are synergistically required to induce cell division and growth in plant tissue cultures. Studies on whole plant and excised tissues of *J. curcas* have demonstrated the existence of antagonistic and additive interactions between these two types of plant hormones. Shoot regeneration in *J. curcas* is also highly influenced by the media formulations containing plant hormones and other growth regulators. Sucrose (3%, w/w) is commonly used as a source of carbohydrate. Genotype is one of the main factors that influence the organogenic response of cultures in different plant species. Among the different factors affecting *J. curcas* regeneration, the genotypic dependence is ranked quite high. Growth and morphogenesis of the plant tissue under in vitro conditions are largely governed by the appropriate choice of the explant. Analogous to this, *J. curcas* regeneration is also dependent on the age and type of the explant involved. Different explants including cotyledons, epicotyls, hypocotyls, leaves, shoot tips, petioles, zygotic embryos, and embryonal leaves have been employed for indirect plant regeneration in *J. curcas* (Table 1).

There are few reports available on regeneration of *J. curcas* through an intermediary callus phase. The explants tested for morphogenic capacity were immature cotyledons and embryos (Varshney and Johnson 2010) (Fig. 2a), and mature cotyledonary leaf (Sujatha and Mukta 1996; Khurana-Kaul et al. 2010). Varshney and Johnson (2010) reported plant regeneration from immature embryo- and cotyledon-derived cultures and found that morphogenic callus induction and subsequent plant regeneration

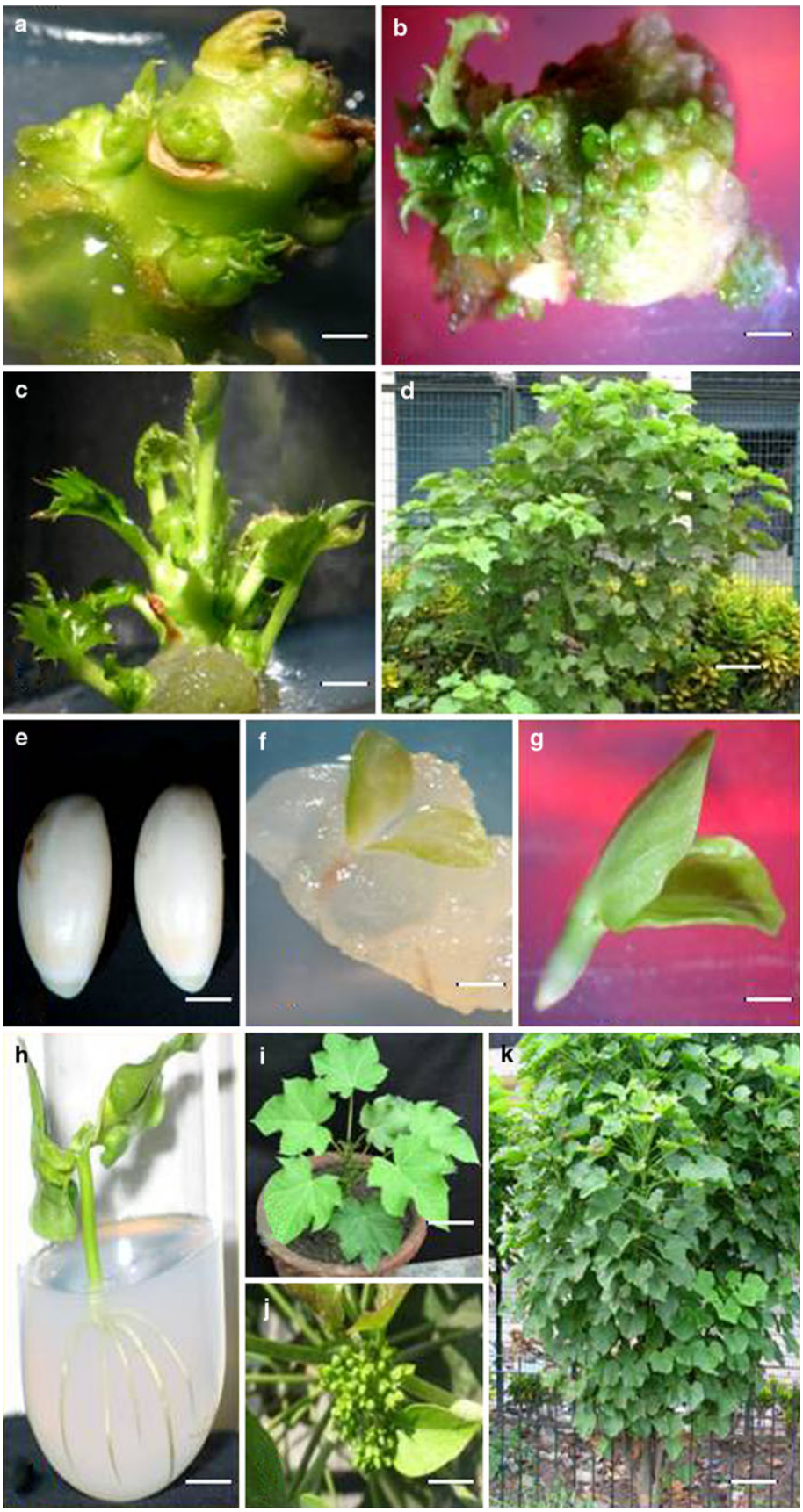

Fig. 1 **a** Axillary bud break and initiation of shoot multiplication (*bar* 0.5 cm). **b** Multiplication of shoot buds after in MS 3% + 22.2 μM BA and 55.6 μM ADS (*bar* 0.5 cm). **c** Differential responses of shoot elongation in different types of media (*bar* 0.5 cm). **d** 23- to 26-month-old micropropagated plant of *Jatropha curcas* growing in the experimental garden of Presidency University (formerly Presidency College) (*bar* 10.0 cm). **e** Immature fruits of donor plant (*bar* 2.54 cm). **f** Germination of zygotic embryo (*bar* 0.5 cm). **g** 7-day-old excised embryo in culture with distinct bipolarity (*bar* 2.54 cm). **h** Complete developed plantlet with healthy root system prior to hardening (*bar* 1.0 cm). **i** 4- to 6-month old zygotic embryo culture grown plant of *J. curcas* (*bar* 5.0 cm). **j** Flowering twig of 2-year-old field-grown excised zygotic embryo-derived plants of *J. curcas* (*bar* 2.54 cm). **k** 2-year-old field-grown excised zygotic embryo derived plants (*bar* 10.0 cm). Source: modified from Datta et al. (2007); Mukherjee and Jha (2009)

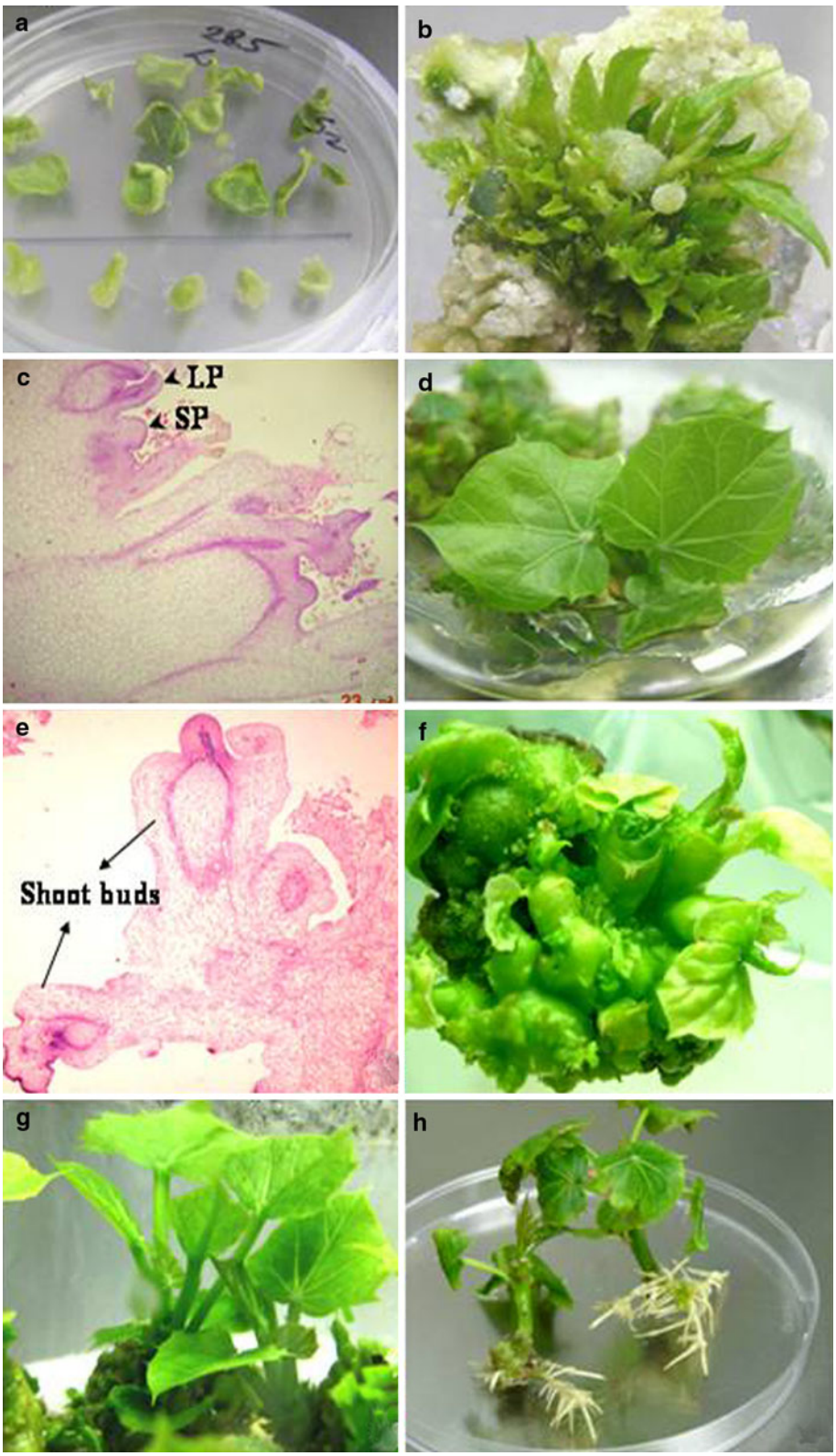

Fig. 2 Direct plant regeneration and callus-mediated morphogenesis from various explants of *J. curcas*. **a** Induction of callus from immature embryos. **b** Morphogenic callus showing induction of multiple shoot buds from immature embryos. **c** Histological evidence of multiplication of pre-existing shoot buds in immature embryo cultures. **d** Elongation of regenerated plantlets. **e** Histological evidence of organogenesis from immature cotyledon cultures. **f** Adventitious shoot bud induction from leaf disc cultures. **g** Elongation of multiple shoot buds from leaf disc cultures. **h** In vitro root induction in regenerated plants. *SP* Shoot primordium, *LP* leaf primordium. Source: modified from Deore and Johnson (2008b); Varshney and Johnson (2010)

was dependent on several factors like age of explant, size of the explant, and combinations of various auxins and cytokinins, in addition to growth additives (L-proline, silver nitrate and copper sulphate). The best response is obtained when the culture initiation was on the medium containing IBA in combination with BAP and $CuSO_4$. Other growth additives did not have significant impact on morphogenic callus formation. Thus, the addition of a combination of

growth adjuvants to the culture medium appeared to be critical for obtaining high regeneration rates. Similarly, significant improvements in shoot bud induction and multiplication media were also observed by Khurana-Kaul et al. (2010). For the regeneration of the morphogenic callus, the regeneration medium strategy is the addition of a lower concentration of the auxin in combination with cytokinin and GA_3. Stem segments were regenerated by Singh et al. (2010). They tested TDZ, BAP or Kn alone or in combination and found that MS medium fortified with BAP gave the best response in terms of maximum number of shoot buds. Induction of callus and suspension cultures has also been reported using hypocotyl explants (Soomro and Memon 2007).

Histological evidence of morphogenesis in *J. curcas*

Histology is widely used in plant tissue culture since it is a useful approach in the study of plant morphogenesis. Different histological methods have contributed significantly to the understanding of in vitro culture system (Kothari and Varshney 1998). There were limited reports of histological studies in *J. curcas* (Kalimuthu et al. 2007; Khurana-Kaul et al. 2010) and in *J. integerrima* (Sujatha et al. 2000) which confirmed the process of morphogenesis. However, there has been no systematic and detailed study on the histological events of growth and morphogenesis in vitro. Varshney et al. (2011) presented a detailed study on histological events of growth and morphogenesis from immature cotyledon and embryonal axis cultures of *J. curcas* (Fig. 2c, e). They found that the process of shoot morphogenesis from immature cotyledon was found to be through adventitious shoot morphogenesis (Fig. 2b) while two types of plant regeneration were observed in immature embryonal axis-derived cultures—one type of shoot regeneration was via organogenesis and the second type was through multiplication of the pre-existing meristems (Fig. 2b, f). It is, however, interesting to note that under similar experimental conditions the process of organogenesis varied from explant to explant. The authors attributed the difference in regeneration response to pre-disposed genetic conditions.

Embryogenesis (somatic and zygotic) in *J. curcas*

Somatic embryogenesis is one of the principal pathways of plant regeneration in vitro and is primarily a process of conversion of a single genetically stable vegetative cell to a bipolar structure (George et al. 2008). These cells are by nature genetically stable and less prone to mutational changes. Indeed, there is a strong selection in favor of genetically normal cells during somatic embryo development. Consequently, plants derived from somatic embryogenesis are primarily non-chimeric and truly clonal populations. Much progress has been made since the prediction of plant cell totipotency by Haberlandt in the early 1900s, but the journey towards unearthing underlying events of somatic embryogenesis was actually initiated in the late 1960s. Somatic embryogenesis is the developmental process by which somatic cells other than a gamete under suitable induction undergo restructuring to generate embryogenic cells. These cells then go through a series of morphological and biochemical changes that result in the formation of somatic or non-zygotic embryos capable of regenerating plants. (Komamine et al. 2005; Schmidt et al. 1997; Yang and Zhang 2010). Somatic embryogenesis represents a unique developmental pathway that includes a number of characteristic events: dedifferentiation of cells, activation of cell division, and reprogramming of their physiology, metabolism, and gene expression patterns. This is not an artificial phenomenon, and is known in nature as a form of apomixes called adventitious embryony, first described by Strasburger (1878). Although Steward (1958) and Reinert (1958) are generally given credit for providing the first descriptions of somatic embryogenesis, such credit might more properly belong to Levine (1947), who reported the recovery of carrot "seedlings" from tissues exposed to low levels of α-napthaleneacetic acid, via a process whose description sounds very much like somatic embryogenesis.

Given that embryogenic systems are capable of producing substantial numbers of propagules for a number of commercially important species, it might be expected that field testing of somatic embryo-derived plants would be well underway. Until somatic embryos can be consistently fortified to the point where they resemble zygotic embryos with regard to robustness and vigor, there is little point in deploying them in the field. However, the ability of somatic embryo-derived plantlets to survive and grow in the field is not the only aspect of their performance which is of consequence, as there are likely to be some behaviors associated with their origin that are not apparent until after field establishment. Of these behaviors, one which is of the most concern is the appearance of unexpected variation among regenerants, which may either be epigenetic or heritable (somaclonal). Although it is generally believed that plants regenerated via somatic embryogenesis would be less likely to display within-clone variation than plants regenerated via other in vitro routes.

Somatic embryogenesis has been documented in some species of the family Euphorbiaceae (Michaux-Ferriere et al. 1992; Raemakers et al. 2000; Stamp and Henshaw 1982, 1987; Stamp 1987; Szabados et al. 1987; Cabral et al. 1992; Raemakers et al. 1993a, b, 1999; Carron and Enjalric 1982; Carron et al. 1985, 1995), but not so prominent in any of the species of *Jatropha.* Somatic embryogenesis has been reported for the first time in

J. curcas by Jha et al. (2007). Our literature review revealed that there was no complete protocol of somatic embryogenesis in *J. curcas* prior to the works of Jha et al. (2007) (Fig. 3a–h). Kalimuthu et al. (2007) reported the induction of somatic embryos on the surface of cotyledonary explants on medium containing 8.87 μM BAP. However, the inadequate documentation of the embryo development and deployment in the field makes the protocol nonviable. Sardana et al. (2000) describes a two-step protocol of somatic embryogenesis using MS salts and B5 vitamins supplemented with 13.31 μM BA + 5.71 μM IAA. It has been proposed that plant growth regulators (PGRs) and stresses play a central role in mediating the signal transduction cascade leading to the reprogramming of gene expression, followed by a series of cell divisions that induce either unorganized callus growth or polarized

Fig. 3 **a**, **b** Embryogenic calli (*bar* 1.0 mm). **c**, **d** Bipolar somatic embryo showing distinct bipolarity (*bar* 0.2 cm). **e** Repetitive/secondary embryogenesis (*bar* 0.2 cm). **f** Initiation to cotyledonary stages of somatic embryogenesis (14 weeks of culture) (*bar* 0.2 cm). **g** 2-month-old plant of *J. curcas* in field (*bar* 2.54 cm). **h** 2- to 3-year-old small-scale plantation of somatic embryo-derived plants of *Jatropha curcas.* All the plants flowered almost throughout the year with comprehensive fruit and seed yield. Source: modified from Jha et al. (2007)

growth leading to somatic embryogenesis (Dudits et al. 1991; De Jong et al. 1993). Jha et al. (2007) reported that the type and concentration of the plant growth regulators were the strong determining factors for induction of somatic embryogenesis in *J. curcas*. Leaf pieces were used as the primary explants. Development of nodular, creamish, embryogenic calli was observed on MS medium supplemented with 9.3 μM Kn (Fig. 3a). The highest frequency (80%) of globular somatic embryos (58.5 ± 12.7) of callus was recorded in the combination of 2.3 μM Kn and 1.0 μM IBA (Fig. 3a, b). Activation of auxin responses may be a key event in cellular adaptation and genetic, metabolic, and physiological reprogramming, leading to the embryogenic competence of somatic plant cells. Earlier studies have reported that the continued presence of an auxin promotes the completion of the globular stage during embryogenesis (Lo Schiavo 1989; Litz and Gray 1995). Jha et al. (2007) also supported that IBA promoted the completion of the globular stage of the embryos. It has also been noted that transition of the globular stage to the heart-shaped stage embryos and their further development requires either a low level of auxin or its complete absence (Liu et al. 1993). The exposure to auxin shock serves as a trigger, inducing cell division in epidermal cells and promoting their differentiation into somatic embryos. Addition of 13.6 μM adenine sulphate along with 2.32 μM Kn and 1.0 μM IBA led to the highest mean number of mature somatic embryos (24.0 ± 6.8) (Fig. 3d, f). Adenine sulphate is known to enhance the efficiency of maturation of somatic embryos, which is a critical step in somatic embryogenesis (Das et al. 1993; Martin 2003). An interesting observation was the induction of a low frequency of secondary embryogenesis (Fig. 3e). Jha et al. (2007) also developed a small plantation using the somatic embryo germplasm (Fig. 3). An important parameter of early flowering in somatic embryo-derived regenerants of *J. curcas* has also been observed. A small plantation of about 50–60 plants has been developed and they are observed to flower almost throughout the year (Fig. 3h). The clonal fidelity of the plants has been checked by chromosomal study and AFLP marker analysis (Fig. 4d; Tables 2 and 3) (Mukherjee and Jha, personal communication).

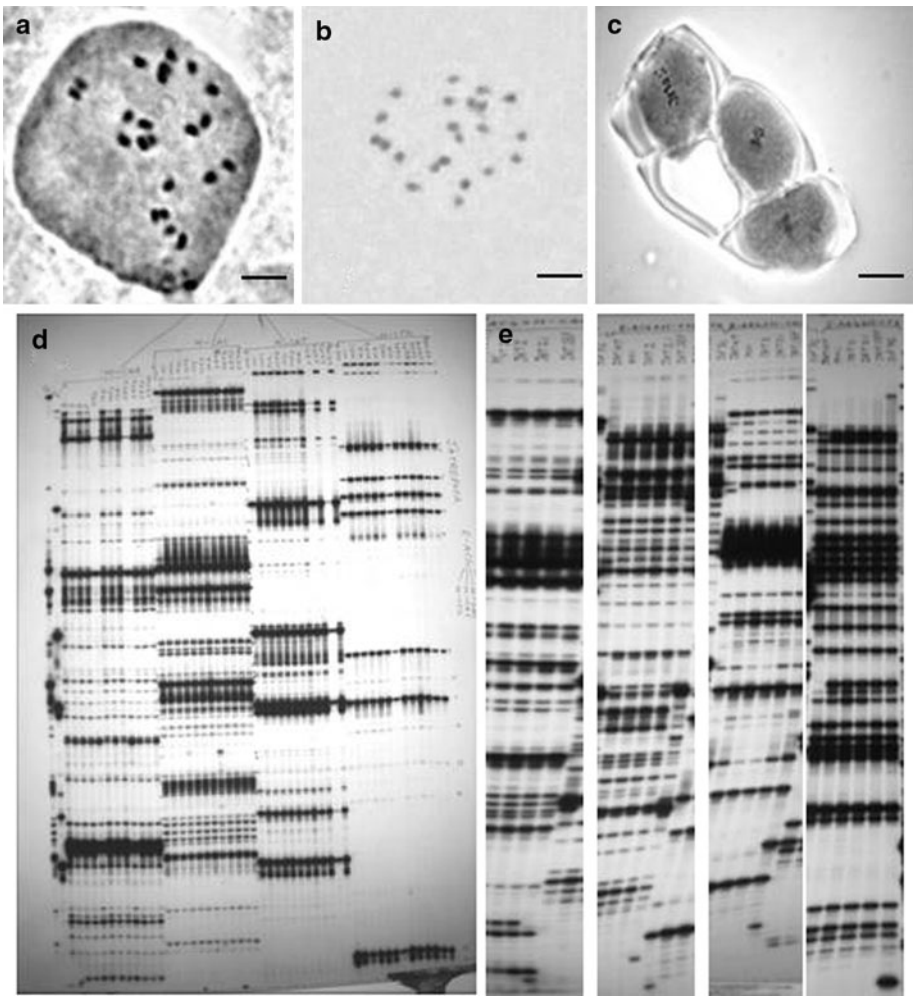

Fig. 4 **a** Orcein-stained mitotic metaphase plate of in vitro grown plants of *J. curcas* showing a stable diploid $2n = 22$ small chromosomes (*bar* 1.0 μm). **b** Giemsa-stained mitotic metaphase plate of in vitro grown plants of *J. curcas* showing a stable diploid $2n = 22$ small chromosomes (*bar* 1.0 μm). **c** Meiotic first metaphase (*bar* 2.0 μm). **d** AFLP fingerprint of the donor, micropropagated and somatic embryo plants. **e** AFLP fingerprint of the donor and excised zygotic embryo plants. Source: Mukherjee and Jha (personal communication)

Table 2 Cytological work is rather meager in *J. curcas* in view of the size of the group

Species	Chromosome number		References[a]
	G	S	
1. *Jatropha capensis* Sond.	–	22	Dehgan (1984)
2. *J. cardiophylla* Arg.	–	22	Missouri Botanical Garden (2003)
3. *J. cathartica* Teran & Berland	–	22	Missouri Botanical Garden (2003)
4. *J. cordata* Arg.	–	22	Missouri Botanical Garden (2003)
5. *J. curcas* L.	11, –	22, 44	Darlington and Wylie (1965), Missouri Botanical Garden (2003), Moore (1973), Samboonsarn (1983), Soontornchainaksaeng and Chaiyasut (1999)
6. *J. glandulifera* Roxb.	–	22	Sinpatananon and Hongthongdaeng (1993)
7. *J. gossypilfolia* L.	11	22	Darlington and Wylie (1965), Missouri Botanical Garden (2003), Moore (1973), Samboonsarn (1983), Soontornchainaksaeng and Chaiyasut (1999)
8. *J. heterophylla* Heyne	–	44	Missouri Botanical Garden (2003)
9. *J. integerrima* Jacq.	–	22	Missouri Botanical Garden (2003)
'Red flower'	11	22	Soontornchainaksaeng and Chaiyasut (1999)
'Pink flower'	11	22	
10. *J. macrorhiza* Benth.	–	22	Missouri Botanical Garden (2003)
11. *J. multifida* L.	11	22	Darlington and Wylie (1965), Missouri Botanical Garden (2003), Soontornchainaksaeng and Chaiyasut (1999)
12. *J. panduraefolia* Andrews	11	22	Missouri Botanical Garden (2003)
13. *J. podagrica* Hook.	–	22	Moore (1973), Soontornchainaksaeng and Chaiyasut (1999)
14. In vitro regenerants of *J. curcas*	11	22	Mukherjee and Jha (personal communication)

[a] For references, see Soontornchainaksaeng and Jenjittikul (2003)

To date, all applied research focuses on somatic embryogenesis, and it is now considered as the gateway to many more technologies. Plant propagation by somatic embryogenesis not only helps to obtain a large number of plants year round but can also act as a powerful tool for genetic improvement of any plant species because of its single cell origin (Bhansali 1990). An understanding of embryogenic pathway initiation and origin of somatic embryos is critical to scientific and biotechnological applications. Combined with genetic engineering, micropropagation through somatic embryogenesis provides an efficient means of producing a large number of elite or transgenic plants (Jin et al. 2005; Li et al. 2006a, b), which has been incorporated into many breeding programmes. Somatic embryogenesis in *J. curcas* may also be a system for any future transformation and metabolic engineering studies. Plant transformation methods and gene silencing technology can effectively be used to evaluate and authenticate newly discovered endogenous genes to characterize their function in plants as well as genetically manipulate trait quality and productivity (Dandekar 2003).

Zygotic embryo culture has been proved as an important tool for conventional and modern agriculture. Regarding the genetic improvement of this primitive species, zygotic embryo culture will be the key source for future genetic engineering. This will definitely help to increase the net production of *Jatropha* seeds within conventional time span. Keeping in mind all the above information, an efficient protocol for zygotic embryo culture has been documented in *J. curcas* (Fig. 1e–k) (Mukherjee and Jha 2009). In conventional practice, dormancy of seeds and the slow growth of seedlings necessitate long breeding seasons. Dormant seeds fail to germinate under apparently suitable conditions of adequate moisture and oxygen supply and optimum temperature unless they are given the appropriate dormancy-breaking stimulus. Since dormancy mechanisms in most cases hinge on the inability of the enclosed embryo to grow, special interest attaches to the role of embryo culture method in overcoming this developmental block. Excised immature embryos of *J. curcas* when cultured on nutrient medium not only bypass the stage of dormancy but also undergo further embryogenic mode of development. The excised embryos of *J. curcas* start germination within 48 h and within 1–2 weeks transplantable seedlings are formed (Mukherjee and Jha 2009) (Fig. 2f–h). Thus, through zygotic embryo culture, it is possible to produce more than two generations in a year by reducing the breeding cycle.

Isolation and culture of *J. curcas* zygotic embryos prior to abortion may circumvent barriers to interspecific and intergeneric hybridization. There are sexual incompatibilities observed between different domesticated and wild species of *J. curcas* that hinder valuable exchange of genetic traits. These restrictions can be overcome by in

Table 3 Characterization of *Jatropha curcas* accessions using molecular markers

Species/accessions	Primers	Number	References
142	AFLP	–	DBT India (Sujatha et al. 2008)
5	RAPD	18	Ganesh et al. (2008)
22	RAPD	7	Ranade et al. (2008)
	DAMD	4	
13	RAPD	20	Gupta et al. (2008)
	ISSR	14	
7	RAPD	52	Pamidiamarri et al. (2008)
	AFLP	27	
20	RAPD	–	Reddy et al. (2007)
	AFLP	–	
43	RAPD	400	Basha and Sujatha (2007b)
	ISSR	100	
225	AFLP	–	Montes et al. (2008)
58	SSR	30	Sun et al. (2008)
	AFLP	7	
40	RAPD	43	Subramanyam et al. (2009)
40	RAPD	50	Boora and Dhollin (2010)
26	RAPD	55	Kumar et al. (2009)
48	AFLP	7	Tatikonda et al. (2009)
72	RAPD	100	Basha et al. (2009)
	ISSR	100	
	SCAR	2	
	SSR	17	
	Curcin-specific primers	3	
In vitro regenerated plants of *J. curcas*	AFLP	4	Mukherjee and Jha (personal communication)

vitro culture of zygotic embryos. There is insufficient information on zygotic embryo culture and its application to *Jatropha* genetic improvement.

Overcoming seed dormancy in these plants was also a major breakthrough. Recovery of large number of clonal plants within a short period of time may help to increase quality genetic stock development in *J. curcas*. The potential of zygotic embryo culture to transfer foreign genes in a single step method affords hope for significant advances in the genetic engineering of plants. The system/protocol can also help to generate variation among plants by treatment with different physical and chemical mutagens. The system may be explored in rescuing interspecific hybrids and transgenic research. Thus, in totality, the protocol paves the way for rapid multiplication and conservation of *J. curcas* and can serve as a support system for selective breeding and transgenic research for further genetic improvement of this crop.

Molecular assessment of *J. curcas*

Chromosomes, genomic DNA and organelle DNA's are important molecules used for assessment and improvement of plants. The potential benefit of molecular assessment in *J. curcas* is that it facilitates screening of plant varieties of superior quality, genetically uniform, and high oil yielding, and serves as an integrative part in crop improvement of the species.

Chromosomal analysis

Chromosome number is an important indicator for determining relationships between plant species. Chromosome numbers also represent the first dataset that leads to an understanding of the genetics of any species. *J. curcas* is a monoic plant with unisexual flowers, the male and female flowers being produced in the same raceme. In spite of its economic value, cytogenetic studies in the genus are limited (Table 2). Undoubtedly the difficult fixation and the small size of the chromosomes have been important factors in the neglect of the family cytologically. Of the 175 known species, chromosome analysis have been done only for approximately 36 *Jatropha* species plus several interspecific hybrids. Almost all the species are diploid, with $2n = 2x = 22$ chromosomes. Three species (*J. cuneata* Wiggins & Rollins, *J. dioica* Sesse and some populations

of *J. heterophylla* Heyne) are tetraploid (2n = 4x = 44) and *J. tirucalli* L. has 2n = 20 chromosomes (IPCN 2009; Fedorov 1969; Soontornchainaksaeng and Jenjittikul 2003; Carvalho et al. 2008). Most of the reported artificial interspecific hybrids are generally diploid, but two triploid hybrids (2n = 3x = 33) have been recovered from crosses between the diploid *J. curcas* × *J. cathartica* Teran & Berlan and *J. curcas* × *J. podagrica* Hook (Dehgan 1984). While *J. curcas* germplasm is being harvested all over the world with the purpose of crop improvement, genome and transcriptome information is being made available in public databases. Carvalho et al. (2008) quite lucidly documented the genome size and base composition and released the first karyogram of *J. curcas*. The flow cytometry indicates an average 2C value of 0.85 pg and an average base composition of 38.7% GC. They also concluded *J. curcas* to be an autotetraploid species. Very few reports on meiotic analysis point to a regular meiotic behavior. Soontornchainaksaeng and Jenjittikul (2003) documented the meiotic configurations of five species of *Jatropha* in Thailand. This review also documents mitotic and meiotic chromosomal analysis from in vitro grown culture regenerants of *J. curcas* (Fig. 4a–c). Chromosomal analysis were carried out using conventional and enzyme maceration techniques by Mukherjee and Jha (personal communication) for the first time to determine if any major genetic changes were detectable within a population of *J. curcas* plants regenerated through micropropagation and somatic embryogenesis system (Table 2; Fig. 4a, b). The chromosomes of *J. curcas* are very small (ranging between 1.091 and 2.471 μm). The chromosome number of 2n = 2x = 22 for *J. curcas* agrees with literature data for other populations of the species (IPCN 2009; Fedorov 1969; Soontornchainaksaeng and Jenjittikul 2003; Carvalho et al. 2008; Dahmer et al. 2009). Improved molecular cytogenetic techniques have not been applied extensively to determine phylogenetic relationship at the species and cultivar level. Molecular cytogenetical studies using techniques like multicolour FISH used by Witkowska et al. (2009) will further help in interspecific characterization of *J. curcas.*

Recent extensive research on plant chromosomes has moved the chromosome from a minor position in biological research to a position where many researchers consider the chromosome as one of the major targets of biological study, especially in the fields of biotechnology and life sciences. To sum up, chromosome number, size, morphology, preparation and staining techniques (Mukherjee and Jha, personal communication) the small genome size (Carvalho et al. 2008) and initial success in molecular cytogenetics (Witkowska et al. 2009) are favorable features for the entry of the new crop *J. curcas* in the biotechnology era.

Molecular markers in genetic diversity analysis and selection

The molecular markers can help in improvement of the plant species through marker-assisted breeding in a sustainable manner (Joshi et al. 2004; Canter et al. 2005; Kumar and Gupta 2008). DNA markers are reliable for informative polymorphisms as the genetic composition is unique for each species and is not affected by age, physiological conditions or environmental factors (Chan 2003). 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 establishment of molecular fingerprints for distinct and most divergent accessions using diversity analysis (Caetano-Anolles and Gresshoff 1997). DNA-based markers such as Random Amplified Polymorphism DNA (RAPD), Enhanced Random Amplified Polymorphism DNA (ERAPD), Simple Sequence Repeat (Simple Sequence Repeat), Sequence Characterized Amplified Region (SCAR), Amplified Fragment Length Polymorphism (AFLP) and Inter-Simple Sequence Repeat (ISSR) can be used to assist breeding through Marker Assisted Selection (MAS) to select prospective varieties from seedling stage (Da Camara Machado et al. 1997).

Assessment of genetic diversity using molecular markers is crucial for the efficient management and conservation of plant genetic resources in gene banks. There has been little analysis of the genetic variations within *J. curcas*. Isozyme markers were also used to determine the genetic relatedness of the members of the genus *Jatropha* and *Ricinus* sp. (Sujatha et al. 2008). RAPD markers were employed to confirm hybridity of interspecific hybrids between *J. curcas* and *J. integerrima* (Ganesh et al. 2008) and ISSR markers were used to determine inter-and intra-population variability in *J. curcas* (Basha and Sujatha 2007b; Senthil Kumar et al. 2009). Ranade et al. (2008) used RAPD and directed amplification of minisatellite-region DNA (DAMD) markers to characterize the genetic variation of *J. curcas* samples from India.

There is virtually no information with regard to the number of introductions and the genetic diversity of *J. curcas* populations grown in India. Considering the enormous demand of *J. curcas*, all future plantations should be done with quality planting materials.

The majority of the studies on *J. curcas* are confined to characterization of accessions available in India (Table 3), except Basha and Sujatha (2007a) having one non-toxic accession from Mexico, Montes et al. (2008) having accessions from 30 countries and Sun et al. (2008) having accessions from south China. Regardless of the number of accessions used, the robustness of the primer and number

of marker data points, all accessions from India clustered together. In general, diversity analysis with local germplasm revealed a narrow genetic base in India (Basha and Sujatha 2007a, b; Ganesh et al. 2008) and south China (Sun et al. 2008), indicating the need for widening the genetic base of *J. curcas* through introduction of accessions with broader geographical background and creation of variation through mutation and hybridization techniques. RAPD marker analysis in *J. curcas* showcases a varied dataset of narrow genetic base (Basha and Sujatha 2007b; Ganesh et al. 2008) to about 95–98% polymorphism (Pamidiamarri et al. 2008; Boora and Dhollin 2010) in Indian accessions. Moreover, Kumar et al. (2010b) detected around 30% polymorphism amongst 26 accessions of Rajasthan, and Subramanyam et al. (2009) detected 75% polymorphism amongst 40 accessions collected from different geographical regions of India using RAPD markers. In contrast to the above studies, AFLP-based molecular characterization of *J. curcas* accessions from Andhra Pradesh were found to be as diverse as these and were scattered in different groups, showing the occurrence of higher numbers of unique/rare fragments and with greater variation in percentage oil content (Tatikonda et al. 2009). The few studies on genotyping of *J. curcas* are based on assessing Indian wild and cultivated accessions. AFLP/RAPD on 20 Indian accessions has been widely studied (Reddy et al. 2007).

The genetic diversity in the natural population appears narrow since no remarkable morphological differences have been observed (Sujatha and Mukta 1996). In AFLP analysis, the lowest PP was found in *J. podagrica* followed by *J. curcas*. However, the highest GS reported was solely within the *J. curcas* germplasm. Therefore, the local and global results indicate a narrow genetic base. About 75% similarity is observed among the global *J. curcas* accessions. Incidentally, preliminary data suggest that *J. curcas* accessions exhibit monomorphism even with microsatellite markers (Drs. Gen Hua Yue and Hong Yan, Temasek Lifescience Laboratories, Singapore, personal communication) indicating very low genetic divergence. The reasons for the globally low genetic variability seen in *J. curcas* are not clear. A pronounced phenotypic plasticity is in itself a genotypic trait that allows the plant to respond to different environments through morphological and physiological changes for its survival (Richards et al. 2006). Genetic evaluation of the in vitro regenerated plants is also considered a priority. Molecular markers based on DNA sequence variation have become increasingly important for the evaluation and authentication of in vitro grown plants, and for the estimation of genetic diversity within them. AFLP fingerprinting proved stable clonal fidelity among the regenerants (Fig. 4d, e) (Mukherjee and Jha, Plant Biotechnology Laboratory, Presidency University, India, personal communication).

However, careful understanding of the phylogeny and use of adequate number of molecular markers are essential prerequisites for drawing valid inferences about the genetic affinities (Sujatha et al. 2008). However, Basha and Sujatha (2007a) characterized *Jatropha* species occurring in India, using nuclear and organelle-specific primers, and revealed high inter-specific genetic variation (98.5% polymorphism). Further characterization of both natural and artificially produced hybrids using chloroplast-specific markers revealed maternal inheritance of the markers (Basha and Sujatha 2007b; Basha et al. 2009). In support, genetic variation studies using RAPD, AFLP and combinatorial tubulin-based polymorphism (cTBP) indicating greater possibilities of improving *J. curcas* by inter-specific breeding (http://precedings.nature.com/documents/2782/version/1). Hence, molecular diversity estimates combined with the datasets on other agronomic traits will be very useful for selecting the appropriate accessions. The study of genetic diversity of *J. curcas* provides the proper guidelines for collection, conservation and characterization, thereby intensifying the possibility of widening the genetic base of its germplasm through hybridization among different genotypes as a step towards genetic improvement.

Transgenics

Genetic transformation for improving traits is another valuable method for the development of *J. curcas* variety. Recently, a new full-length cDNA of stearoyl-acyl carrier protein desaturase was obtained by RT-PCR and RACE techniques from developing seeds of *Jatropha*, and the gene was functionally expressed in *E. coli* (Tong et al. 2006). To understand the molecular mechanism of salt and drought tolerance, a new full-length cDNA-encoding aquaporin (*JcPIP2*) was isolated from seedlings of *J. curcas*, and the abundance of *JcPIP2* was induced by heavy drought stress. It was found that it played an important role in the rapid growth of *Jatropha* under dry conditions (Ying et al. 2007). Zhang et al. (2008) reported a novel betaine aldehyde dehydrogenase gene (*BADH*) named *JcBD1* (cloned by RT-PCR and RACE techniques from *J. curcas*) that is increasingly expressed in leaves undergoing different environmental stresses. The JcBD1 protein was functionally expressed in *Escherichia coli* and conferred its resistance to abiotic stresses like salt.

Genetic transformation of any crop species through genetic engineering techniques requires an efficient in vitro regeneration system which is rapid, reproducible, and applicable to a broad range of genotypes. Considering the potential of *J. curcas*, scientists have started genetic transformation studies of this species and some reports of its genetic transformation are now available. Li et al. (2008) established a highly efficient genetic transformation

procedure from Chinese accessions of *J. curcas* for the first time via *Agrobacterium tumefaciens* infection of cotyledon disc explants, and later Trivedi et al. (2009) established *Agrobacterium*-mediated genetic transformation from cotyledonary leaves from Indian accessions. Kumar et al. (2010b) established *Agrobacterium tumefaciens*-mediated genetic transformation using in vitro leaf explants. Purkayastha et al. (2010) used in vitro regenerated shoot apices for successful gene transfer through particle bombardment. These techniques help in genetic modification and subsequent in vitro multiplication of cultivars for various uses. Further, the technology helps in better understanding and subsequent improvement of the oil biosynthesis pathway, which could have positive implications in reducing the world's dependence on fossil reserves.

Future strategy for crop improvement

J. curcas is still in its infancy with respect to genetic improvement. Future research should be directed towards multifarious lines of efficient transformation protocols. Gene pyramiding is the latest trend which can also be applied to *J. curcas*. The application of metabolic engineering and RNAi technology may show their potential both in basic research and as tools of modern plant breeding. Designer crops will be able to produce valuable enzymes, proteins, and antibodies. Research and development should aim at metabolic engineering of the oil biosynthesis pathway and selective molecular markers for marker-aided selection. There is a possibility to increase the yield of oil from *Jatropha* seeds through transgenesis. The molecular study of development of the seed, endosperm and oil bodies of *Jatropha* will be an essential step toward transgenesis. The information on endosperm and oil development of *Jatropha* seeds will greatly facilitate the study of gene expressions that is involved in biosynthesis of storage lipids. Genetic approaches to investigating the regulation of oil content have so far met with limited success. The fatty acid composition of seed oil varies considerably both between species and within species, with fatty acids varying in both chain length and degrees of desaturation. The target would be to isolate the key genes which are involved in de novo fatty acid/lipid biosynthesis through molecular and genomic approaches. The aim should be to genetically engineer fatty acids (FA)/lipid biosynthesis in *Jatropha* seeds to increase oil yield and unsaturated FA in storage lipids. Altering the seed oil composition will aid in achieving the desired density, dynamic viscosity, heat capacity, boiling point and heat of combustion. To develop economically viable oilseed crops with modified fatty acid profiles, there is a requirement to manipulate the activity (or gene expression) of relevant key constituent steps in the synthetic or modification pathways, i.e. to carry out genetic metabolic engineering. This can be achieved either through up- or down-regulation of an introduced recombinant gene (transgenic), deletion of endogenous genes (mutagenesis), or by selection of appropriate combinations of the relevant naturally occurring alleles present in the gene pool. Therefore, metabolic engineering of the fatty acid biosynthetic pathway to produce a greater amount of medium chain fatty acids is indeed a feasible approach. The most notable point of research will be to produce transgenic *Jatropha* seeds with enhanced rates of oleic or linoleic acid. These can be obtained either by down-regulation or over-expression of a particular gene. Further modification can be done using genetic engineering to down-regulate conversion of oleic acid into linoleic acid and omega 3 fatty acid. Thus, new cultivars with the desired percentage of oleic acid and linoleic acid and omega 3 fatty acid can be produced. Enormous potential lies if this high-oleic oil variety can be bred with non-toxic species to raise cultivars that can be used for production of both edible oil and biodiesel. The future line of research targets the monitoring of detoxification of seed beans and oil, utilization of the residue de-oiled cake for fodder and manure, and conversion of non-edible to edible oil. Metabolic engineering and RNAi technology can be considered the most effective tools for further improvement to achieve the goal. Above all, these will be possible through an efficient in vitro plant regeneration system. Therefore, for genetic improvement, tissue culture protocols for the rapid propagation and regeneration of selected genotypes of *J. curcas* are highly desirable.

Conclusion

The small genome size, chromosome number, ease of vegetative manipulation and transformation are favorable features for the use of molecular tools for *J. curcas* improvement. *J. curcas* can be improved through assessment of variation in wild source and selection of superior/elite genotypes and application of mutation, alien gene transfer through inter-specific hybridization and biotechnological interventions to bring about the change in the desired traits. Considering the plant's enormous potential, a large amount of quality planting material is required for future use. Several research groups have been working on the improvement of *Jatropha* through conventional selection or breeding programs but the biomass productivity has been rather low compared to other oil crops. Therefore, if investigations of its genetic diversity and its yield potential can be covered by adequate scientific research, the problem of low yield can be overcome. Application of

biotechnology will expedite this process. Importantly, biomass and oil yield can be improved by exploiting genetic engineering focusing on the optimization of enzymes involved in oil biosynthesis and biomass production. However, a prerequisite for the implementation of genetic manipulation of *Jatropha* is the molecular characterization using marker-assisted selection and establishment of a robust and efficient plant regeneration procedure. The review has focused on most recent developments in tissue culture protocols for *J. curcas* and present strategies of crop improvement by genetic engineering. Improvement programmes of *J. curcas* by modern methods of agrobiotechnology are of interest worldwide. Therefore, there is an immediate need to create superior genotypes for successful implementation of *J. curcas* cultivation as a sustainable source of biodiesel.

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