Review

Genetic diversity in the *Jatropha* genus and its potential application

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Abstract

Jatropha curcas L., a drought tolerant, monoecious perennial shrub, has gained attention in the tropics and sub-tropics during the past decade as a potential biodiesel crop. Adequate genetic diversity for key agronomic traits is of fundamental importance in crop improvement programmes particularly for crops such as *J. curcas*, which are in the early stages of domestication. In *J. curcas*, genetic diversity in local populations and worldwide collections has been estimated using both dominant and co-dominant molecular markers systems such as random amplified polymorphic DNA (RAPD), inter simple sequence repeats (ISSRs), sequence-characterized amplified region (SCAR), amplified fragment length polymorphism (AFLP), simple sequence repeats (SSRs) and single-nucleotide polymorphism (SNPs), etc. Assessment of genetic variation using molecular markers unequivocally established the existence of rich genetic diversity in the germplasm from Central and South American regions and narrow genetic base in populations from Asia and Africa. Establishment of phylogenetic relationships among *Jatropha* species using molecular markers has been limited to species naturalized and distributed in India. Research expansion over the past decade has indicated the availability of considerable genetic variation in the genus *Jatropha* for vegetative and floral traits linked to productivity, seed oil content, fatty acid profiles, toxicity (phorbol esters, curcin), etc. The diverse genetic sources identified in *J. curcas* germplasm and the compatible wild species need to be exploited for genetic improvement of the crop through conventional breeding and interspecific gene transfer, which could be further accelerated through marker-aided selection.

Keywords: *Jatropha curcas*, Genetic diversity, Molecular markers, Genome sequence

Review Methodology: The following were consulted for the primary literature review: CAB Abstracts, Agricola, Mendeley and ScienceDirect. Some of the articles were obtained using Google Scholar and Australian New Crops. The review presents an overview on the extent of genetic diversity available in *Jatropha curcas* germplasm and its wild allies as disclosed through different molecular marker systems and the role of genomics and marker-assisted breeding in genetic improvement of *J. curcas*.

Introduction

The genus *Jatropha* (Euphorbiaceae) 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 [1]. The genus is classified into two subgenera (*Curcas, Jatropha*), ten sections and ten subsections to accommodate the Old and New World species [2]. 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), Antillean, all Indian (except one) and two North American species. Most species in this genus are native to Central and South America, the diversity centre of *Jatropha* [3]. Several *Jatropha* species are cultivated for their ornamental leaves and flowers, while some are grown in the tropics for their economic uses. Since some species of this genus having significant economic

importance are regarded as potential biodiesel plants, the genus has created tremendous interest all over the world for the biodiesel use.

Jatropha curcas L. (2*n*=22) has been considered as one of the candidate crops for biodiesel production. This species is a perennial deciduous, multipurpose shrub widely distributed in Central and South America, Africa, India and South East Asia as a hedge crop and live fence for erosion control, soil conservation and protection from grazing animals. According to Heller [3], *J. curcas* is native to Central and South America, and spread to other tropical countries *via* the Cape Verde islands and Guinea Bissau. It is mainly evolved for xerophytic adaptation and is naturalized in the seasonally dry tropics, particularly in the southern hemisphere where there is no severe and prolonged frost. Several properties of *J. curcas* stemming from its drought hardiness, rapid growth, easy propagation and wide adaptation to soil conditions have resulted in the spread of *J. curcas* far beyond its original distribution [4–6]. Although an introduced crop in several countries, it has established itself in different agro-ecological regions in the drier regions because of its wide adaptability. *J. curcas* is also an ideal source for rehabilitation of degraded lands and rural development. In addition, its seeds contain 32–35% semi-drying oil which on transesterification is comparable to biodiesel of European (EN 14214) and American (ASTM D6751) standards [4, 7]. Biodiesel produced from *J. curcas* seed oil is biodegradable, renewable, non-toxic and environmentally superior to petroleum diesel [8, 9]. It can be used as a straight vegetable oil or transesterified into biodiesel for use in standard diesel engines. Owing to these properties *J. curcas* has attracted global attention to develop a sustainable alternative feedstock for biodiesel production on marginal lands [10, 11].

In addition to its biodiesel use, the by-products of *J. curcas* seeds and its residue can be used as organic fertilizer. Alternatively, seed cake can be pressed into pellets that can be used for direct combustion or converted into charcoal. In China, various components were tested for pharmaceutical and pesticide use [12]. Two products of anti-viral (against herpesvirus I and II) and antibacterial (*Staphylococcus aureus* and *Monilia albicans*) skin disinfectors utilizing leaf extracts are commercialized [13]. The other useful products from *J. curcas* are A2-Jetfuel kerosene, polyol biodegradable foam for use in packaging and insulation industry, paint from the bark and active carbon for use in exhaust pipe systems [14]. The costs and energy returns from *J. curcas* and its products are discussed and for realization of the full potential of the plant, adequate information need to be generated on the actual and potential markets for all its products [10, 15, 16].

Currently, *J. curcas* has been planted as a biodiesel crop in India, China, South America and Africa [17, 18]. However, it is uneconomical for both the grower and the oil producer to grow the crop only for use of its oil as a diesel substitute [10], because of use of wild and undomesticated material with unpredictable and varying yield patterns, lack of access to germplasm, non-availability of quality planting materials, low and inconsistent seed yields, low oil content, the presence of toxic and carcinogenic compounds, asynchronous flowering associated with non-synchronous fruit maturation, and pre-harvest sprouting of seed (vivipary) under humid conditions. Also, lack of well-developed technologies, government controlled policies, marketing infrastructure for oil and the by-products were some impediments for implementing the biofuels programme [19]. In spite of increasingly widespread interest in planting *J. curcas,* very little improved germplasm resources are available in practice. Furthermore, systematic breeding efforts are still in their infancy and currently agronomically elite cultivars of *J. curcas* are not available [20]. The objectives for genetic improvement of the crop should aim at development of:

- High-yielding varieties both in terms of seed yield and oil content.
- Early maturing and dwarf varieties for reducing the gestation period and also the cost of cultivation.
- Thin hull types to allow efficient extraction of oil from the seeds.
- Plants with modified plant architecture (short, compact types with good ramification) for effective light interception, more number of inflorescences and fruits, and amenability for mechanization.
- Plants with frequent fructification.
- Pistillate lines or genotypes with higher ratio of female to male flowers per inflorescence.
- Genotypes with tolerance to abiotic stresses (drought, frost, salinity, alkalinity, water logging, etc.) as the plantations are targeted at poor soils and harsh environments.
- Genotypes with inbuilt tolerance/resistance to insect pests and diseases.
- Varieties with modified seed oil quality (to suit different purposes).
- Toxin-free dual purpose genotypes for utilization of the seed cake as cattle feed and also for safety of workers involved in seed processing.
- Genotypes with high toxicity (pesticide use).

The key for success of any genetic improvement programme lies in the availability of genetic variability for desired traits [3]. Genetic resources through global 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. The fact that *J. curcas* has adapted itself to a wide range of edaphic and ecological conditions indicates the existence of considerable amount of genetic variability that needs to be exploited for

potential realization [21]. *J. curcas* is at an early stage of domestication, therefore identification and maintenance of a high level of genetic diversity are essential for the long-term success of the breeding programmes. During the past decade, significant advances in crop improvement programmes have been made in several crops through use of molecular markers [22–24]. However, in the case of *J. curcas*, most of the studies with molecular markers were confined to assessment of genetic diversity, and the need for development of molecular maps as a prelude for mapping agronomically desirable traits has just been realized. Against the background for the need for genetic improvement of *J. curcas*, this review presents the currently available information on use of different molecular marker systems for estimation of existing genetic diversity, and the role of genomics and marker-assisted breeding in genetic improvement of *J. curcas*.

Genetic Diversity of *J. curcas* using Molecular Markers

Molecular markers refer to assays that allow the detection of specific sequence differences between two or more individuals and have played a major role in the genetic characterization and improvement of many crop species. They have contributed to 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. It is essential to characterize germplasm not only for evaluation and conservation but also for their utilization in prebreeding and breeding programmes. Currently, genetic diversity studies in the genus *Jatropha* are focused on *J. curcas* and few wild species that are commonly distributed in India. Different types of single and multilocus molecular markers were employed for estimation of genetic diversity in the available germplasm (Table 1). Initial studies on estimation of genetic diversity were confined to populations from Asian region and during the past 2–3 years, and information has been generated on the extent of genetic variation in populations from Central and South-American and African regions.

Amplified fragment length polymorphism (AFLP) analysis

As the AFLP technique is reliable, allows high throughput and is cost-effective, AFLP markers were used by a number of researchers for investigation of the genetic diversity in *J. curcas* accessions from India, China, Brazil and Mexico. Pamidimarri *et al.* [45] reported low genetic variability among toxic *J. curcas* accessions from India and wide variability between toxic Indian accessions and a non-toxic Mexican accession using AFLP markers. In this study, the similarity between toxic and non-toxic genotypes was 83.5%. A broad genetic base of 48 *J. curcas* germplasm from six different states in India was reported by Tatikonda *et al.* [29]. Seven effective AFLP primer combinations generated a total of 770 fragments, of which 680 (88.0%) fragments were polymorphic. Sun *et al.* [44] estimated genetic variation in 58 *J. curcas* accessions from China and two from Malaysia using AFLP markers. The polymorphism was 14.3%, suggesting a lack of genetic variation of *J. curcas* accessions in China. Shen *et al.* [49] also reported a low polymorphism and variation pattern in 38 populations of *J. curcas* from different geographical areas in China. Zhang *et al.* [61] employed the AFLP technique to survey the genetic diversity of 240 samples from three Asian countries, two African countries and different geographical regions in China. Molecular polymorphism was 14.8%, suggesting that the germplasm of *J. curcas* has a narrow genetic diversity in China and Southeast Asia. Analysis of genetic relationships indicated that the origin of *J. curcas* in China may be from Southeast Asia.

Shen *et al.* [50] characterized the genetic variation among 63 populations of *J. curcas* from 10 countries in Asia, Africa and Mexico. The genetic diversity parameters of the 63 *J. curcas* Chinese populations were low, while the populations from Mexico displayed higher genetic diversity than others. Similarly, compared with the genetic diversity observed in collections from Africa, India and China, high genetic diversity in germplasm from Mexico and Central America has been reported by many researchers using AFLP markers [38,62,63]. Using genetic diversity evaluation and analysis of molecular variance (AMOVA) analysis, researchers explained the fact that *J. curcas* germplasm from Mexico and Central America harbours greater genetic diversity than in other parts of the world by reasoning that Mexico and central America (Mesoamerican region) may be a centre of origin and diversity of *J. curcas* [38, 64]. It is interesting to note that the divergent accessions from Mexico and Central America regions with high oil content and other characters were associated with productivity. These genetically divergent germplasm from Mexico and Central America regions may provide critical germplasm resources for future breeding and genetic improvement of *J. curcas* in practice.

Randomly amplified polymorphic DNA (RAPD) analysis

RAPD involves PCR amplification of genomic DNA using a single short oligonucleotide primer under low stringency conditions, which results in multiple amplification products from loci distributed throughout the genome. The technique is simple, rapid, inexpensive and applicable to any genome without any prior information regarding the genome of the plant. RAPD technique has been broadly applied in initial assessment of genetic diversity for *J. curcas* in last 10 years. Basha and Sujatha [43]

Table 1 Assessment of genetic diversity in the genus *Jatropha* using molecular markers

Markers employed	Germplasm	Result	Reference
Local populations			
Ten ISSR markers	Nine populations from five provinces of China	High level of genetic diversity	He *et al*. [25]
ISSR	*J. curcas* accessions from Southern Yunnan, China	–	Xiang *et al*. [26]
Seven RAPD and four DAMD primers	Eighteen *J. curcas* accessions from different regions	Usefulness of SPAR method for diversity assessment in *Jatropha* has been demonstrated	Ranade *et al*. [27]
Twenty RAPD and 14 ISSR primers	Thirteen *J. curcas* genotypes from different parts of India	Importance of both the markers in *J. curcas* genetic diversity assessment	Gupta *et al*. [28]
Seven AFLP primer combinations	Forty-eight *J. curcas* accessions from six different states of India	High genetic variability (88% polymorphism)	Tatikonda *et al*. [29]
Fifty-two RAPD and 18 AFLP primer combinations	Twenty-eight accessions from distinct geographical regions of India	Low genetic diversity among the accessions used	Pamidimarri *et al*. [30]
Twenty-six RAPD primers	Twenty-six accessions from Rajasthan, India	–	Kumar *et al*. [31]
ISSR primers	One hundred and twenty accessions from three regions in China	69% polymorphic	Ou *et al*. [32]
Thirty-six EST-SSR and 20 G-SSR markers from cassava	Forty-five accessions from distinct geographical regions of China	Intergroup genetic diversity was higher than the intragroup diversity index	Wen *et al*. [33]
Forty-four RAPD primers	Forty genotypes from five states in India	Wide genetic base	Ikbal Boora *et al*. [34]
Ninety-six RAPD and six SSR primers	One hundred and ninety-two *J. curcas* accessions from Brazil	Low genetic diversity	Rosado *et al*. [35]
Seven ISSR primers	Three hundred and thirty-two accessions from eight states in Brazil	High level of genetic differentiation	Grativol *et al*. [36]
Ten RAPD primers	Ten accessions from three states in India	High genetic variation	Subramanyam *et al*. [37]
Six AFLP primer combinations	Eighty-eight accessions from Chiapas, Mexico	High level of polymorphism and several rare fragments in a pistillate accession	Pecina-Quintero *et al*. [38]
Eight RAPD primers	Forty-eight accessions from Malaysia	High genetic variation	Rafii *et al*. [39]
Five RAPD and Twelve ISSR primers	Twenty-four populations from China	Limited genetic variation	Chen *et al*. [40]
Eight ISSR primers	Sixteen accessions from Malaysia	Low genetic variation	Noor Camellia *et al*. [41]
Local and exotic populations			
One hundred and twenty RAPD primers	One accession each of Indian toxic and Mexican non-toxic varieties	Reference fingerprints established for distinguishing the non-toxic variety from the toxic Indian cultivar	Sujatha *et al*. [42]
Four hundred RAPD and 100 ISSR markers	Forty-two *J. curcas* accessions from different locations of India along with a non-toxic genotype from Mexico	Modest level of genetic variation in Indian and wide variation between Indian and Mexican genotype	Basha and Sujatha [43]

Seventeen SSRs and seven AFLP primer combinations	Fifty-six Chinese and two Malaysian *J. curcas* accessions	Very low genetic variability (14.3% polymorphism)	Sun *et al*. [44]
Fifty-two RAPD, 56 AFLP and seven SSR markers	Seven *J. curcas* accessions (6 toxic + 1 non-toxic)	All the markers are effective in differentiating both the toxic and non-toxic accessions	Pamidimarri *et al*. [45]
Hundred RAPD, 100 ISSR and 17 SSR markers	Seventy-two *J. curcas* accessions collected from 13 countries	Rich diversity among Mexican genotypes and narrow genetic variation among accessions from different regions of the world	Basha *et al*. [46]
Ten RAPD, 32 AFLP primer pairs and two combinatorial tubulin-based polymorphism (cTBP)	Thirty-eight *J. curcas* accessions from 13 countries and six *Jatropha* species	Narrow genetic diversity in accessions from Thailand, Nigeria and India	Popluechai *et al*. [47]
Fifteen ISSR primers	Two hundred and twenty-four accessions including 219 from China and five from Myanmar	High genetic diversity in Chinese germplasm	Cai *et al*. [48]
Nine AFLP primer combinations	Thirty-eight *J. curcas* accessions including 37 from China and one from Indonesia	Low genetic diversity	Shen *et al*. [49]
Four AFLP primer combinations	Sixty-three populations from ten countries in Asia, Africa, Mexico	High genetic variation in populations from Mexico	Shen *et al*. [50]
Jatropha species			
Twenty-six RAPD primers	Five *J. curcas* accessions from Tamil Nadu and seven *Jatropha* species native to India	High genetic variability among the eight species (80.2% polymorphism)	Ganesh Ram *et al*. [51]
Thirty-three RAPD and 27 AFLP primer combinations	Seven *Jatropha* species native to India	High genetic variability among the species (97.7% by RAPD and 97.2% by AFLP)	Pamidimarri *et al*. [52]
Two ITS sequences encoding the 18, 5.8 and 26 s nuclear ribosomal RNA subunits	Seven *Jatropha* species along with a natural hybrid occurring in India	Usefulness of the nrDNA ITS sequences in phylogenic analysis of genus *Jatropha*	Pamidimarri *et al*. [53]
Nine ISSR primers	Three *J. curcas* accessions and eight *Jatropha* species	Clustering of *J. curcas* accessions	Senthil Kumar *et al*. [54]
Two hundred x, 100 ISSR and 50 organelle-specific primers	Eight *Jatropha* species along with a natural hybrid occurring in India	High genetic variation (98.5% polymorphism) among species used in the study	Basha and Sujatha [55]
Nineteen morphological and 21 ISSR markers	Five *J. curcas* accessions from Coimbatore and seven *Jatropha* species native to India	Importance of ISSR markers in genetic diversity assessment of *Jatropha* species	Vijayanand *et al*. [56]
Twenty-seven ISSR markers	Thirty accessions of *J. curcas* and two accessions each of three species	*J. curcas* accessions from Mexico were genetically diverse and the three species formed separate clusters	Tanya *et al*. [57]
Thirty-one SSR markers	Six species of *Jatropha* and *J. tanjorensis*	Assessed the cross-species transferability of *J. curcas* microsatellite markers	Pamidimarri *et al*. [58]
Nine SSR primers	Forty-one accessions from two provenances each from Brazil, Mexico, Columbia	High level of polymorphism with 2–8 alleles per locus	Bressan *et al*. [59]
Fifty-one EST-derived SSR markers	Twenty-five accessions of *J. curcas,* five *Jatropha* species and castor	Low to moderate level of informativeness within the EST-SSRs and 57.0–95.6% transferability among *Jatropha* species	Yadav *et al*. [60]

investigated the genetic diversity of 42 germplasm lines collected from different regions in India using RAPD and inter simple sequence repeat (ISSR) markers and revealed low inter-accessional variability. Kumar *et al.* [31] measured the level of genetic diversity in 26 *J. curcas* accessions collected from India. Results indicated that 26 decamer primers produced 6011 amplification products, of which 30.9% were found to be polymorphic and the size of bands ranged from 300 to 2500 bp. Out of 43 RAPD primers, ten polymorphic primers (percentage polymorphic bands – *PPB*=75.2%) were generated for genetic diversity evaluation of wild and cultivated varieties of 40 *J. curcas* accessions from different geographical regions in India [65]. Similarly, assessments of genetic diversity of *J. curcas* accessions collected from India were conducted by several researchers [28,34,37], and their results showed the level of genetic diversity is moderate in Indian germplasm. However, other researchers concluded that the level of genetic diversity is low in Indian *J. curcas* germplasm [30,66]. These conflicts may be related to sampling size or limited markers. Both limited sampling size and limited markers could result in the risk of overestimating or underestimating the diversity indices. Rafii *et al.* [39] reported the results of RAPD analysis of the genetic diversity of 48 *J. curcas* accessions from different locations in Malaysia. The results indicated the existence of a high level of genetic variation among the accessions. Chen *et al.* [40] reported that Chinese *J. curcas* accessions had rich genetic diversity using RAPD markers.

Rosado *et al.* [35] performed a genetic diversity survey of 192 *J. curcas* accessions collected from different geographical regions throughout Brazil using RAPD and simple sequence repeat (SSR) markers. Only 23 of the 381 RAPD markers were polymorphic (6.2%) and the six SSR primers generated only eight different alleles in all the 192 germplasm accessions analysed, indicating a narrow genetic diversity in Brazil *J. curcas* germplasm. In Africa, to exploit the *J. curcas* germplasm for production of commercial biofuel in Kenya, Machua *et al.* [67] determined the genetic diversity and genetic structure of 160 individuals collected from eight populations in Kenya using RAPD primers. Their results showed that the *J. curcas* germplasm of Kenya has a broad genetic base, which will be useful for breeding and genetic improvement programmes. In contrary, RAPD analysis of 40 accessions from Ghana with ten RAPD primers revealed an average polymorphism of 24.9%, indicating a narrow genetic base [68].

ISSR analysis

Technically simple, ISSR analysis has been successfully used in assessment of genetic variation in *J. curcas* and genetic relatedness between *Jatropha* species from India, China and Brazil. Basha and Sujatha [43] characterized 42 *J. curcas* accessions of native germplasm along with a non-toxic genotype from Mexico and reported moderate polymorphism (33.5%) with ISSR markers. Analysis of worldwide germplasm of *J. curcas* representing 13 countries revealed distinctness of non-toxic Mexican accessions from other accessions [46]. In this study, molecular data were corroborated with proximate composition data, which showed the association of molecular markers with the presence/absence of phorbol esters. In an accession from El Salvador, a unique allele specific to the accession was detected through SSR analysis, which reiterates the need for characterization of germplasm from other Central American regions as well. Genetic diversity in six wild populations of *J. curcas* collected from Northeast India, was assessed using ISSR and directed amplification of minisatellite DNA (DAMD) markers [69]. The study showed that variation at intra-population level was 68.9%.

The genetic diversity of eight populations from China was estimated using ISSR primers, revealing a high level of genetic variation at species level (*PPB*=91.0%, *He*=0.3070) [26]. The coefficient of genetic differentiation within populations was 70.6%. He *et al.* [25] investigated the genetic diversity and genetic structure of nine populations in China. Their results suggested a high level of genetic variation existed among the different populations. Ou *et al.* [32] examined 11 populations in China and also reported high polymorphism at species level and the distinctive differentiation among populations. Likewise, a set of 224 accessions including 219 from different geographical regions in South China and five from the neighbouring country Myanmar was analysed [48]. These studies reported a high level of genetic diversity in *J. curcas* accessions in China based on ISSR molecular profiles, but indicated that *J. curcas* germplasm in China might have been introduced from different places. The genetic variability and genetic relationships of 332 *J. curcas* cultivated accessions from 12 locations in Brazil were investigated using ISSR primers [36]. Results showed that the genetic diversity of *J. curcas* in Brazil was high at species level. In addition, Maghuly *et al.* [70] assessed the genetic diversity of *J. curcas* from 12 countries using ISSR markers and Ecotilling technique and showed clear variations not only between individuals but also between different regions.

SSR markers

Owing to their abundance and inherent potential for variation, SSRs (namely microsatellites) have become a valuable source of genetic markers in various aspects of molecular genetic studies. Sun *et al.* [44] developed 17 genomic SSRs, out of which only one was polymorphic among the 58 accessions of *J. curcas* collected across China. Similarly, Cai *et al.* [71] investigated the genetic diversity of 219 *J. curcas* accessions from China using SSR markers and revealed a low genetic diversity in the Chinese germplasm. Pamidimarri *et al.* [72, 73] isolated SSR

markers and investigated the genetic diversity of *J. curcas* accessions from India, and showed the narrow genetic diversity in *J. curcas* accessions. Ricci *et al*. [74] also reported low polymorphism in 64 genotypes from five geographic locations (Brazil, Cape Verde, Cuba, Mozambique and Senegal) using 32 SSR markers. Ambrosi *et al*. [75] analysed 26 accessions from different geographical regions (including Mexico, South America, Asia and Africa), using 10 RAPD, 6 ISSR and 10 SSR markers. Low genetic variability was documented not only among accession groups but also among accessions of different geographical origin, with the exception of Mexican landraces. Tanya *et al*. [76] characterized 26 Mexican, three Chinese, three Thai and four Vietnamese accessions using SSR markers. Five of these loci clearly displayed distinct banding patterns between 26 Mexican accessions (non-toxic) and the 10 Asian accessions (toxic). In the studies of Bressan *et al*. [59], nine polymorphic microsatellite loci with 2–8 alleles per locus were identified, of which six loci showed transferability to three congeners: *Jatropha podagrica, Jatropha pohliana* and *Jatropha gossypiifolia*. Based on the whole genome sequences, Sato *et al*. [77] identified about 41 000 SSR loci in the 289 Mb sequences of the *J. curcas* genome. From these, 100 SSR markers were developed and examined for polymorphism among 12 *J. curcas* varieties obtained from Indonesia, Thailand, China, Mexico, Guatemala, Tanzania, Madagascar, Cape Verde and Uganda. Their results showed that the polymorphism of those SSR markers in the limited accessions tested was low, but the accessions from Mesoamerican regions were genetically distinct from other regions. These SSR loci identified from the whole genome sequences largely widen the scope and utility of SSR markers in genetic diversity assessment and marker-assisted breeding programmes of *J. curcas*.

Expressed sequence tag (EST)-SSRs

EST-SSRs markers are those microsatellite loci derived from ESTs and have been applied to investigating genetic diversity of *J. curcas* over the last 3 years. Yadav *et al*. [60] mined SSRs from 13 201 ESTs of *J. curcas* and developed 21 EST-SSR markers for *J. curcas*. Using 21 EST-SSRs, a total of 51 alleles in 25 accessions from India were detected with an average of 2.42 per primer pair. The PIC value ranged from 0.04 to 0.61 with an average of 0.25, revealing low to moderate level of informativeness with EST-SSRs in the *J. curcas* accessions tested. Yang and Liu [78] developed 11 EST-SSRs and investigated the genetic diversity of 24 accessions from China. Their results exhibited that a low genetic diversity in Chinese germplasm. Wen *et al*. [33] studied the genetic relationships between 45 *J. curcas* accessions from different countries using 36 EST-SSRs and 20 genomic-SSRs designs based on cassava sequence information. A total of 183 polymorphic alleles were detected, indicating that the *J. curcas* germplasm tested in the study has a moderate level of genetic diversity.

Regardless of the source of the germplasm that was subjected to characterization, genetic variation detected using SSR markers was rather low in *J. curcas*. Compared with AFLP, RAPD, ISSR makers, SSR (including EST-SSRs) markers exhibited lower genetic diversity in *J. curcas* germplasm. This is probably because of detection of variations across the entire genome using AFLP, RAPD, ISSR makers, whereas identification of the variation confined to the repeat region using SSR markers.

Sequence-characterized amplified regions (SCARs)

SCARs are usually dominant markers; however, some of them can be converted into co-dominant markers by digesting them with restriction enzymes. In *J. curcas*, the development and utility of SCAR markers so far are limited. Basha and Sujatha [43] developed two diagnostic SCAR markers (ISPJ-1 and ISPJ-2) for differentiating the Indian and Mexican genotypes. Subsequently, 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 were developed [46]. Mastan *et al*. [79] converted one of the polymorphic RAPD primer (OPQ-15) to SCAR marker that discriminates the toxic and edible accessions.

Single-nucleotide polymorphisms (SNPs)

SNP markers have emerged as an increasingly valuable marker system for assessing population genetic structure in different species in recent years. However, studies based on SNPs are limited in *J. curcas*. Since the sequence information for diverse *J. curcas* accessions is unavailable, and as these markers represent the most abundant source of genetic polymorphism, there is an urgent need to develop SNP markers and exploit their potential in various applications of molecular biology in *J. curcas*. Maghuly *et al*. [70] attempted to identify SNPs through Ecotilling, which allows high-throughput analyses of natural genetic diversity particularly in plants with limited genetic diversity. Ecotilling was applied to 12 genes related to stress tolerance, toxin and oil metabolism, showing a clear variation among *J. curcas* germplasm. Yang [80] sequenced three gene fragments, namely, *ITS* (internal transcribed spacer), *PGIC* (cytosolic phosphoglucose isomerase) and *GAPDH* (glyceraldehyde-3-phosphate dehydrogenase) in 15 *J. curcas* accessions, including three of its allies (*J. gossypiifolia*, *J. podagrica* and *Jatropha integerrima*). The results showed that within *J. curcas* germplasm, nucleotide sequences were highly conserved, but the variation was high among inter-species. Popluechai *et al*. [47] identified alleles of *J. curcas* oleosin gene (*JcOle3*) and SNPs in its intron in *J. curcas* accessions, species and hybrids that

could serve as markers in phylogenetic or breeding studies. Silva-Junior *et al.* [81] reported on the discovery of a 768 high-quality SNPs for *J. curcas* derived from a pool of genetically diverse accessions using Illumina sequencing and an SNP selection pipeline. These SNPs would facilitate further breeding and genetic improvement of *J. curcas* in practice.

Cross-genera and Cross-species Transferability of Molecular Markers

With the advent of next generation sequencing (NGS) techniques, genome sequencing has become relatively easy. The whole genomes of *J. curcas* and castor bean (*Ricinus communis*) were sequenced and a high degree of synteny was observed between the genomes of these two genera [77, 82]. Conventional methods of developing SSR markers are prohibitively expensive, time-consuming and labour-intensive. Hence, development of molecular markers through comparative genomics assumes importance. Yadav *et al.* [60] evaluated the cross-taxa transferability of polymorphic EST-SSRs derived from *J. curcas.* They reported 57.0–95.6% transferability among five species of *Jatropha* and 47.0% transferability across genera (*R. communis*). In contrary, Sharma and Chauhan [83] employed 302 SSR markers from the whole genome sequence of castor bean to assess the genetic diversity of 49 *J. curcas* genotypes and 8 *Jatropha* species, which showed approximately 70% transferability. Similarly, Wen *et al.* [33] used SSR markers from EST and genome sequences of *Manihot esculenta* (Cassava) to analyse the genetic diversity among 45 *J. curcas* accessions collected from Indonesia, South America, Grenada and China. Fifty-six EST-SSRs and G-SSRs were successfully used for characterization of the accessions, which detected 183 polymorphic alleles with estimated mean genetic diversity index of 0.557. They reported that the germplasm investigated has a broad genetic background with a correlation between the genotype and geographic origin. Pamidimarri *et al.* [58] studied the cross-species amplification of 49 SSR markers derived from *Jatropha* in six *Jatropha* species: *J. gossypiifolia*, *J. podagrica, J. integerrima*, *Jatropha multifida*, *Jatropha glandulifera* and *Jatropha tanjorensis*, of which 31 markers showed cross-species amplification in all the six species tested. The study revealed the potential of these markers developed from *Jatropha*, in species differentiation, molecular identification and characterization of interspecific hybrids and genetic improvement of the species through marker-assisted breeding programmes for economically important traits.

Genetic Relationships among *Jatropha* Species and their Genetic Diversity

Interspecific hybridization played a vital role in genetic improvement of several crop plants. The genus *Jatropha* could also benefit from 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.* 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 [84, 85]. 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. Variation in fatty acid profile significantly influences the cetane number [86] and interspecific derivatives with altered desirable cetane value could be developed. *Jatropha* species are rich sources of hydrocarbons and *J. multifida* with big round seeds possesses higher oil content (50%) as compared with *J. curcas* (23–38%) [84]. Barriers between these two species are weak [55] and the cross-combination can aid in enhancement of oil content. Thin hull types are desirable for efficient recovery of oil and seeds of *Jatropha* species (*J. podagrica, J. integerrima* and *J. gossypiifolia*) have thin hull when compared with *J. curcas.* Determination of the energy values of the 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) [4, 85]. *Jatropha mahafalensis* is predicted to have equal energetic promise. The species, *J. multifida, J. podagrica, J. integerrima* and *J. gossypiifolia* are well known and cultivated throughout the tropics as ornamental plants. *Jatropha gossypiifolia*, a facultative annual, has heavy-fruit-bearing ability and is adapted to saline regions in Northeast Thailand and India. *J. gossypiifolia* is reported to have 18.5% ricinoleic acid in its seed oil [87] and physico-chemical properties of biodiesel derived from this species is in the acceptable range for use in diesel engines [88]. 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 *J. curcas* and *J. gossypiifolia* [55, 89]. *J. tanjorensis* is more vigorous and less attacked by insect pests and diseases. Large number of crosses with the putative parental species may result in development of backcross material for use in the breeding programmes. *Jatropha nana* and *Jatropha villosa* are found in dry stony places; *J. nana* and *Jatropha heterophylla* are dwarfs of African type. The crop should be of manageable height for mechanization. Availability of species with such diverse plant types and wide adaptability offers immense scope for improving the genetic architecture and agronomic attributes of *J. curcas. Jatropha platyphylla* from Mexico with 60% kernel oil is reported to be free of phorbol esters [90]. Phylogenetic advancement of the genus *Jatropha* has evolved with adaptations to arid conditions and *Jatropha* species adapted towards the Northern hemisphere could be a valuable source for development of drought-resistant cultivars. 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 [91].

Determination of genetic relationships among species is 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 [92], petiolar anatomy [93]; cross-ability relationships [1] and phenetic and cladistic analysis based on morphological characters [2, 94]. Molecular markers reveal more quickly and accurately, genetic differences far exceeding those obtainable using morphological or biochemical methods without confounding the influence of environment. Nuclear and plastid DNA analysis represent an important tool for phylogenetic and diversity analysis of plants. Molecular characterization and phylogenetic relationships among seven *Jatropha* species (*J. curcas, J. glandulifera, J. gossypiifolia, J. integerrima, J. multifida, J. podagrica* and *J. tanjorensis*) were determined using RAPD and AFLP primers, which showed maximum relatedness between *J. curcas* and *J. integerrima* [52]. Twelve *Jatropha* species including five *J. curcas* accessions from different states in India were investigated using RAPD markers [51]. According to their analysis, three distinct clusters were generated: one comprising all accessions of *J. curcas*, the second included six species: *J. gossypiifolia*, *Jatropha ramanadensis*, *J. podagrica*, *J. tanjorensis, J. villosa,* and *J. integerrima*. *J. glandulifera* belonged to the third cluster, indicating its higher genetic distinctness from other species.

Vijayanand *et al.* [56] studied the genetic diversity of Indian *Jatropha* species using a combination of morphological and ISSR markers. Among all the characters, the highest range was exhibited by plant height and the lowest value by the number of branches. Twenty-one ISSR primers generated 156 polymorphic alleles with an average of 7.47 alleles per primer. Maximum diversity was observed between *J. villosa* and *J. integerrima* and the least diversity between two accessions of *J. curcas*. ISSR markers differentiated the accessions into a wide genetic diversity as compared with the morphological data. Senthil Kumar *et al.* [54] analysed the genetic diversity among eight *Jatropha* species and three *J. curcas* accessions. The polymorphism was 98.1% with nine ISSR primers, indicative of a high level of genetic variation among the genotypes studied. The UPGMA cluster analysis indicated three distinct clusters, one comprising all accessions of *J. curcas*, the second cluster included four *Jatropha* species (*J. tanjorensis*, *J. gossypiifolia*, *J. podagrica* and *Jatropha maheshwarii*) and the third cluster included four species (*J. villosa, J. multifida*, *J. integerrima* and *J. glandulifera*).

The genetic relationships of *Jatropha* species including *J. curcas*, *J. gossypiifolia*, *J. glandulifera*, *J. integerrima*, *J. podagrica*, *J. multifida*, *J. villosa*, *J. villosa* var. *ramnadensis*, *J. maheshwarii* and a natural hybrid, *J. tanjorensis*, were also determined using ISSR, RAPD and SSR markers [55]. This study showed high interspecific genetic variation (*PPB*=98.5%) and maternal inheritance of chloroplast-specific markers based on characterization of the interspecific hybrids. The UPGMA dendrogram indicated that *J. curcas, J. integerrima* and *J. gossypiifolia* clustered together and were the closest relatives, which was also supported by the findings of Pamidimarri *et al.* [52]. Basha and Sujatha [55] used 50 organelle-specific SSR markers for characterization of eight *Jatropha* species along with the natural hybrid *J. tanjorensis*, which established *J. gossypiifolia* as the maternal parent of the hybrid. Tanya *et al.* [57] used ISSR markers to assess genetic variation among 30 accessions of *J. curcas*, two accessions each of *J. gossypiifolia*, *J. integerrima* and *J. podagrica*, along with three accessions of *R. communis*. *J. curcas* from Mexico gave the highest genetic diversity, whereas *R. communis* accessions showed the lowest genetic diversity. Pamidimarri *et al.* [53] studied the phylogenetic relationships among seven species of *Jatropha* using ITS sequences that showed medium genetic diversity among *Jatropha* species, and phylogenetic closeness of *J. curcas* with *J. integerrima.*

Thus, molecular characterization of *Jatropha* species has been confined to taxa naturalized in India. High level of interspecific differentiation in *Jatropha* was reported corroborating with the morphological differentiation of the species. However, the use of a few markers or variations at a single locus is inadequate to draw meaningful conclusions about the genetic relationships. Molecular studies should substantiate the classical taxonomic classifications based on morphological, cytological and cross-ability success and aid in resolving ambiguities in phylogenetic relationships. Molecular studies related to taxonomic classification should take into account the pioneering work of Dehgan [1] and consider the phylogenetic relationships, evolutionary trends, cross-ability 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 of *Jatropha* species native to India using chloroplast-specific microsatellite primers revealed maximum variability in the intergenic regions of ORF77–ORF82 and *rps12–rps19* regions which could be used for distinguishing *Jatropha* species and identification of haplotypes [55].

Potential Application of Genetic Improvement by Inter-Species Crosses

Wilbur [95] and Dehgan and Webster [94] regarded *J. curcas* as the most primitive member of the genus because of its ability to interbreed with species from the subgenera, its palmately lobed leaves, arborescent 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 were produced with *J. curcas* [1, 55]. Artificial hybrids between *J. curcas* and other species were attempted, but the most successful cross combination was with *J. integerrima* [1, 96–100]. Dehgan [1] attempted interspecific hybridization of 20 species in eight of the ten sections and the study was confined to identification of cross-ability 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. All F_1 hybrids except *J. curcas*×*J. multifida* were more vigorous than their parental species and flowered earlier. Pollen fertility in the F_1 hybrids varied between 42–69% [52] and 12–66.2% [1]. Although the F_1 hybrids were partially sterile, 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. [55, 99, 100]. Basha and Sujatha [55] demonstrated the possibility of obtaining hybrids with *J. curcas* as pollen parent crossed to *J. multifida, J. maheshwarii, J. gossypiifolia* and *J. villosa.*

In the genus *Jatropha*, existence of natural hybrid complexes is reported such as *J. curcas-canascens* complex in Mexico [101], *J. integerrima-hastata* complex in Cuba and West Indian islands [102] and *J. curcas-gossypiifolia* (*J. tanjorensis*) in India [89]. Hence, germplasm exhibiting gross morphological differences should be subjected for pollen studies and lines with 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 hybridity. RAPD markers confirmed the hybridity of *J. tanjorensis* as depicted, based on cytological and biochemical studies [89]. Use of organelle-specific markers further confirmed the direction of this natural cross and unravelled *J. gossypiifolia* as the maternal parent [55].

Marker-Assisted Selection (MAS)

MAS is a powerful tool for the indirect selection of difficult traits at an early stage, for accelerating the process of traditional plant breeding and for facilitating the improvement of traits that cannot be improved by conventional methods. The essential requisites for molecular mapping of a trait of interest are an appropriate mapping population, robust high-throughput molecular systems that can generate highly polymorphic informative markers, high-density linkage maps and suitable biometric tools for linkage analysis and map construction. In *Jatropha*, molecular markers have been used only to assess the genetic relatedness in *J. curcas* and between different species, while applications such as gene tagging, mapping, MAS or map-based cloning of genes coding for agronomically important traits are in their infancy. Recent advances in development of genomic and EST-SSRs and other genomic resources can make MAS a reality in *Jatropha* once marker trait associations are studied and linkage maps are constructed using appropriate segregating mapping populations.

Studies on development of framework linkage maps in *J. curcas* have been initiated. At Temasek Lifesciences Laboratory (TLL), a linkage map with 219 microsatellites, 200 SNPs and 160 AFLP markers has been constructed using backcross populations [103]. 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 constructed [104]. At The Centre for Novel Agricultural Products (CNAP), >400 SNPs were detected that could be sufficient for a dense map and in marker-assisted breeding [105]. Sun *et al.* [106] constructed a linkage map using a backcross population with 105 SSRs covering 643.8 cM of the genome, which resulted in identification of 28 quantitative trait loci (QTLs) for 11 growth and seed traits. These linkage maps would serve as framework maps for mapping economically important traits.

Following the studies on estimation of genetic diversity using molecular markers, the need for correlating the phenotypic characters with molecular variation was realized. All the studies that have used the non-toxic accessions from Mexico showed distinct molecular profiles with various markers [42, 43, 45, 46, 76, 107]. Likewise, correlation of phenotypic variation with molecular polymorphism indicated that time of flowering, inflorescence type and number, leaf colour and texture were the traits contributing to variation [108]. In the study of Pecina-Quintero *et al.* [38], a 100% pistillate accession showed 13 rare fragments in AFLP analysis. Recently, accessions with nil curcin have been reported from Thailand [109]. These accession-specific markers have great value in molecular fingerprinting of genotypes and in marker-assisted breeding programmes. In *J. curcas*, the genes involved in biosynthesis of triacylglycerols, phorbol esters, genes encoding curcin, disease-resistance genes, MADS box genes and flowering-related genes have been identified, which could accelerate the process of molecular breeding [76]. Wide genetic variation for seed oil content and fatty acid composition has been reported in the Mesoamerican populations [110]. Liu *et al.* [111] identified 18 QTLs underlying the oil traits and 3 expression QTLs (eQTLs) of the oleosin acid genes through QTL mapping with a backcrossing population consisting of 286 individuals. The QTLs and eQTLs, especially qC18:1–1, qOilC-4 and qOleIII-5 with contribution rates (R^2) higher than 10%, controlling oleic acid, total oil content and oleosin gene expression, respectively, are expected to provide indispensable data for initiating molecular breeding to improve seed oil traits in *Jatropha*, which is the key for a candidate crop for biodiesel production. Further, the EST databases being developed from developing seeds of *J. curcas* and

their functional annotation will aid in selective breeding of quantitative traits in the crop [112, 113]. The identification of genes related to *Jatropha* toxic components can accelerate the development of genetic strategies to produce dual-purpose varieties of *J. curcas* with low toxicity, increasing the possibility of using the seed meal as animal feed. Modification of the fatty acid composition of oil makes it more suitable for biodiesel production. Availability of plants bearing only pistillate flowers are of significance as these could be exploited in hybrid breeding programmes. Since *J. curcas* has great propensity for vegetative propagation, maintenance of the trait is relatively easy and several accessions could be tested for their combining ability and heterosis. These genetic and genomic resources may accelerate the identification of molecular markers and trait genes to develop elite cultivars with superior yields and profitability.

Conclusions and Future Perspectives

Assessment of *J. curcas* germplasm using morphological and molecular markers indicated low phenotypic and genotypic diversity in local populations in Asian regions and close clustering of accessions from Africa and Asia indicating a common ancestor. Narrow genetic base in African and Asian regions could be attributed to few introductions, the predominance of asexual mode of reproduction and/or because of the occurrence of apomixis and could probably have a common ancestor. Phenotypic diversity in most cases was not associated with genotypic diversity, indicating a strong influence for environment. Analysis of global diversity using different types of molecular markers confirms the observation of Heller [3], who showed the distribution and spread of *J. curcas* in the tropical belt via the Cape Verde islands. All the studies establish the availability of rich allelic diversity in the South American, Mexican and Meso-American regions that harbour accessions with useful and novel genes that provide a good basis for widening the genetic base of *J. curcas*. 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 disclose variation, molecular measures of genetic diversity have a very limited ability to predict quantitative genetic variability [44]. Hence, morphological characterization and estimates of molecular diversity need to be combined to identify divergent material for breeding and also for construction of linkage maps, diversity analysis, QTL/association mapping and molecular breeding of *J. curcas*. There is an immediate need for characterization of *Jatropha* species endemic/native to the Central and South American regions 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.

The EST-SSRs developed from the related genera such as *Hevea*, *Ricinus* and *Cassava* had greater transferability rates and thus offer a great potential for their use in marker-assisted breeding programmes in *Jatropha*. Efforts to identify linkage of molecular markers with traits of interest are lacking in *Jatropha*, in order to practice MAS. However, markers based on RAPD, ISSR, SSR and AFLP analysis capable of discriminating toxic and non-toxic accessions of *J. curcas* are available, which can be used for selection and marker-assisted breeding towards the production of *J. curcas* varieties with low or null phorbol esters. Once saturated genetic linkage maps are constructed, and marker trait linkages established, marker-assisted introgression of target traits and map-based cloning of the genes involved can be practiced with great success in *Jatropha*.

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