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Molecular Characterization of Genetic Diversity in *Jatropha curcas* L.

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ABSTRACT

Jatropha curcas L. (Euphorbiaceae) is a small tree with valuable attributes, including that as a commercially important non-edible oilseed that grows naturally in the equatorial Americas and has spread to other tropical countries. *J. curcas* has been found to be the most suitable tree species for production of biodiesel as it can be grown as a quick yielding plant even in problem soils and adverse climatic conditions. *J. curcas* has been neglected in the past and little systematic work has been done on productivity aspects. It is highly cross-pollinated and is known for continued seedling propagation, it is anticipated for the existence of wide genetic variability offering significant scope for selecting superior genotypes, which will help to improve productivity and provide sound scientific base to this crop. Few attempts have been directed to improve it as a crop plant and characterize it at molecular level. However, understanding the genetic relationship and variation is important for efficient parental selection and different techniques are being used in the study of variations in *J. curcas*. Application of molecular markers in *J. curcas* studies would be the right tool to differentiate plant varieties, for choosing right parents for cross pollination and for marker assisted breeding. In the present paper, we have reviewed the studies conducted at the molecular level to characterize the genetic diversity in *J. curcas*.

Keywords: AFLP, biofuel, EST, molecular marker, microsatellite, ISSR, RAPD

Abbreviations: **AFLP**, amplified fragment length polymorphism; **AM**, arbuscular mycorrhizal; **CPT**, candidate plus tree; **CTBP**, combinational tubulin-based polymorphism; **EST**, expressed sequence tag; **FIASCO**, fast isolation by AFLP of sequence-containing repeat; **GD**, genetic distance; **ISSR**, inter simple sequence repeat; **MI**, marker index; **PCA**, principal component analysis; **PCR**, polymerase chain reaction; **PIC**, polymorphism information content; **RAPD**, random amplified polymorphic DNA; **RFLP**, restriction fragment length polymorphism; **RIP**, ribosome inactivating protein; **SCAR**, sequence characterized amplified region; **SPAR**, single-primer amplification reaction; **SSR**, simple sequence repeat; **UPGMA**, unweighted pair group method with arithmetic mean

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INTRODUCTION

The genus *Jatropha* (Euphorbiaceae) has 172 species of which *Jatropha curcas* L., variously referred as physic nut, ratanjot or Barbados nut, is postulated to be the most primitive form, from which other *Jatropha* species have evolved with changes in growth habit and flower structures (McVaugh 1945; Dehgan and Webster 1979). It is an easy-to-establish perennial shrub normally up to 5 m in height and shows articulated growth with morphological discontinuity. The latex-containing branches bear 5 to 7 lobed alternately arranged leaves and a terminal inflorescence. The plant is monoecious and the flowers are generally unisexual with occasional hermaphrodite flowers. It is a diploid species with 2n = 22 chromosomes (Heller 1996).

A number of scientists have attempted to define the origin of *J. curcas*, but the source remains controversial. Martin and Mayeux (1984) identified the Ceara state in Brazil as a centre of origin but without giving any arguments. Dehgan and Webster (1979) cite Wilbur (1954) as arguing *J. curcas* being undoubtedly part of the flora of Mexico and probably of northern Central America and most likely originated there. According to other sources, the physic nut seems to be native to Central America as well as to Mexico where it occurs naturally in the forests of coastal regions (Aponte 1978). It is highly probable that the centre of origin of the physic nut is in Mexico (and Central America) since it is found in Africa and Asia only in cultivated forms. The "true" centre of origin, however, still has to be found. From the Caribbean, it was probably distributed by Portuguese seafarers via the Cape Verde Islands and former Portuguese Guinea (now Guinea Bissau) to other countries in Africa and Asia (Burkill 1966). Today it is found in forest and non-forest areas of almost all the tropical and subtropical countries and since it is not browsed by cattle, it is also cultivated as protection hedges around gardens and fields.

J. curcas is a multipurpose plant with numerable valuable attributes and considerable economic potential. Jatropha

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Invited Review

seeds contain 46-58% of oil on kernel weight and 30-40% on seed weight (Subramanian *et al.* 2005). In recent years, it has drawn attention as a source of seed oil that can provide an economically viable substitute for motor fuel (Takeda 1982; Ishii and Takeuchi 1987; Openshaw 2000; Adebowale and Adedire 2006; Chen *et al.* 2006). Among the oil-bearing tree species, *J. curcas* is desired due to its drought hardiness, rapid growth, easy propagation, small gestation period, wide adaptation, production on good and degraded soils and optimum plant size that makes seed collection more convenient (Jones and Miller 1991; Francis *et al.* 2005).

Apart from being a potential biofuel crop *J. curcas* has multiple utilities. Jatropha oil has high saponification value (in the range of 169.9 to 208.3 mg KOH/g oil, Adebowale and Adedire 2006; Akbar *et al.* 2009; Kywe and Oo 2009) and used in India and other countries for making soap. Preparations of all parts of the plant, including seeds, leaves and bark, fresh or as a decoction are used in traditional medicine and for veterinary purposes. The latex of *J. curcas* contains an alkaloid similar to quinine in properties called "Jatprophine" which is believed to have anti-cancerous properties and used for external application on skin diseases and rheumatism (Thomas *et al*. 2008). Nath and Dutta (1992) demonstrated the wound-healing properties of 'curcain', a proteolytic enzyme isolated from latex. Kone-Bamba *et al.* (1987) reported coagulating effects of the latex on blood plasma. Its latex has also been reported to be an abortifacient and is efficacious in dropsy, sciatica and paralysis (Frienvis 2008). The oil has a strong purgative action and is widely used for skin diseases and to soothe pain. A decoction of leaves is used against cough and as an antiseptic after birth. Extract from all plant parts have insecticidal properties (Grainage and Ahmed 1988) while seed extract/oil have been found to be particularly effective against cowpea beetle *Callosobruchus maculates* (Coleoptera: Bruchidae) and cotton ballworm *Helicoverpa armigera* (Lepidoptera: Noctuidae) (Adebowale and Adedire 2006; Arvinda *et al.* 2009).

J. curcas has remarkable capacity to survive in varied climatic conditions as in areas of low rainfall to almost on any type of soil whether gravelly, sandy or saline and thrives even on the poorest stony soils and rock crevices. To combat phosphate deficiency it establishes a symbiotic association with arbuscular mycorrhizal (AM) fungi present in soil; allowing penetration and elaboration of a complex network of arbuscular (tree shaped) hyphae in root cells that facilitates exchange of minerals and nutrient between plant and fungal symbiont. The AM fungus derives carbon and energy-rich photosynthetic products (Hamel 1991), in turn the plant is provided with all essential nutrients particularly P, Cu and Zn which are often present in low concentrations in soil (Liu *et al.* 2007). *J. curcas* sheds its leaves in winter which forms mulch around the plant base increasing organic matter and enhancing earthworm population around root zone, thus, increasing soil fertility. Also, it is a suitable species for soil conservation and stabilization of shifting sand dunes (Frienvis 2008).

Worldwide introduction of *J. curcas* for varied purposes had met with limited success due to unreliable and low seed set as well as oil yields resulting in low economic returns. *J. curcas* is a wild species and no varieties with desirable traits for specific growing conditions are available, which makes growing Jatropha a risky business (Jongschaap *et al.* 2007). The crop is also characterized by variable and unpredictable yield for reasons that have not been identified (Ginwal *et al.* 2004). This limits the large-scale cultivation and warrants the need for genetic improvement and breeding of superior genotypes of the species for which establishing genetic distances through DNA fingerprinting methods is required.

The major constraints in achieving higher quality oil yield of this crop are lack of information about its genetic variability, oil composition, and absence of suitable ideotypes for different cropping systems. Knowledge of genetic relationship and variation in the species is a pre-requisite in any breeding program because it permits the organization of germplasm, including elite lines, and provides for more efficient parental selection (Karp and Edwards 1998). Despite its ecological and economic importance, the taxonomy and genetic structure of the *Jatropha* genus is not entirely clarified due to the occurrence of natural hybridization among species (Airy Shaw 1972). Furthermore, the available germplasm lacks information on the genetic base (Sujatha *et al.* 2008). Hence, assessment of genetic diversity and its characterization becomes imperative for which molecular tools have rendered their assistance in the recent past. In the present paper, we have reviewed the studies conducted at the molecular level to characterize genetic diversity in *J. curcas*.

TRADITIONAL APPROACHES FOR GENETIC DIVERSITY ASSESSMENT

A wide variety of techniques have been used in the studies of forest tree relationship and variation. Studies have been conducted to identify genetic variation in populations, provenances and clones of *J. curcas* through traditional morphometric and biochemical marker techniques (Prabakaran and Sujatha 1999; Ginwal *et al.* 2004; Pant *et al.* 2006; Sunil *et al.* 2008). Although phenotypic traits cannot be reliable measures of genetic differences because of the influence of the environment on gene expression we feel it is appropriate to mention salient traditional approaches employed for assessment of variability in *J. curcas*. These studies have enriched the scientific understanding of nature of existing variation in the species (**Table 1**).

Sukarin *et al.* (1987) did not observe any differences in vegetative development and first seed yields among 42 clones originating from different locations in Thailand and planted in a provenance trial at the Khon Kaen Field Crops Research Center. Heller (1992; cited in Heller 1996) tested a collection of 13 provenances in multi-location field trials in two countries of the Sahel region: Senegal and Cape Verde. Significant differences in the vegetative development were detected among the various provenances at all locations. However, plants of various provenances appeared very uniform in morphological characters such as leaf shape. In a small sub-set of 10 *J. curcas* accessions from central India, seed oil content was significantly correlated with seed weight, stem diameter and total leaf area (Ginwal *et al.* 2004). In a range from 400 m to 100 m elevation, Pant *et al.* (2006) found a significant positive effect of altitude on various oil yield components, including number of branches per tree, number of fruits per branch and number of seeds per tree but a significant reduction was observed in kernel oil content (43.1% at low vs. 30.7% at higher elevations).

The predominance of environmental factors over genetic factors has been reported by Kaushik *et al.* (2006, 2007) within the small genetic resource base of *J. curcas* accessions from Haryana state in India, although seed size and oil content and seed weight could be genetically clustered and significantly differentiated. Rao *et al.* (2008) evaluated genetic association, and variability in seed and growth characters in 32 high-yielding candidate plus trees (CPTs; phenotypes judged, but not proven by test, to be unusually superior in some quality/s such as exceptional growth rate, desirable growth habit, high wood density, etc.) of *J. curcas* from different locations spread over 150,000 km^2 in India. Significant trait differences were observed in all the seed characters viz., seed morphology and oil content as well as in growth characters viz., plant height, female to male flower ratio and seed yield in the progeny trial. Broad sense heritability was high in general and exceeded 80% for all the seed traits studied. Sunil *et al.* (2008) recorded the phenotypic traits of *J. curcas* plants *in situ* at 4 different eco-geographical regions of India. They noticed pronounced differences in the 9 characters they assessed for a total of 162 accessions in the 4 zones. For example, the plant height of 80% accessions in one zone was less than 1.5 m while in

Table 1 Variability in morphological and biochemical characteristics in *Jatropha curcas* L.

Material	Characteristics	Differences observed	References
Clones (Thailand)	Vegetative development, first seed yield	Not significant	Sukarin *et al*. 1987
Provenances (Senegal and Cape Verde)	Vegetative development, leaf shape	Significant variation in leaf shape	Heller 1992
Accessions (central India)	Seed oil content, seed weight, stem diameter, total leaf area	Significant correlation of morphological features with oil content	Ginwal *et al*. 2009
Accessions from different elevations (Himachal Pradesh, India)	Branches/ tree, number of fruits/ branch, number of seeds/ tree	Reduction in oil content with increase in elevation	Pant *et al*. 2006
Accessions (Haryana, India)	Seed size, seed weight, oil content	Significant variation in seed weight and oil content	Kaushik *et al*. 2006, 2007
Candidate Plus Trees (India)	Seed and growth characteristics	Significant difference in seed morphology, seed weight, plant height, female to male flower ratio, seed yield and oil content	Rao *et al*. 2008
Accessions from different ecogeographical regions, India	Plant height, number of fruits, seed oil content	Significant difference in plant height	Sunil *et al*. 2008
J. curcas and related species (Tamil Nadu, India)	Isozymes	Differences in peroxidise activity and super-oxide dismutase activity	Prabakaran and Sujatha 1999
J. curcas and related species (Central Java and Indonesia)	Enzyme systems	Difference in sorbitol dehydrogenase, shikimate dehydrogenase, alcohol dehydrogenase and isocitrate dehydrogenase activity	Yunus 2007
Accessions from eastern India	Seed weight, seed morphology, seed aspect ratio, 2D surface area, oil content	Significant difference in seed weight and percentage oil content	Bhatia *et al*. 2009

another zone 60% of the accessions were larger than 1.5 m. Similar differences were noticed in number of fruits and seed oil content and composition. Like most earlier studies, Sunil *et al.* (2008) did not undertake genetic characterization of the accessions, thus the reason for the variability was not clear.

Only a limited number of studies involving biochemical markers for assessment of genetic diversity in *J. curcas* have been reported which mainly involved isozyme markers. For example, Prabakaran and Sujatha (1999) utilized the isozymes peroxidase and superoxide dismutase to ascertain the phylogeny of *J. curcas* with other species while Yunus (2007) was able to differentiate among *Jatropha* species from different regions in Central Java and Indonesia on the basis of enzyme-like sorbitol dehydrogenase, shikimate dehydrogenase, alcohol dehydrogenase and isocitrate dehydrogenase. Most of the studies to evaluate germplasm have been done utilizing materials collected from CPTs of different regions, different aged plants (3-20 years) and propagated through seeds or vegetative cuttings (Anonymous 2006). Comparison of yield-contributing traits based on such accessions results in erroneous conclusions about the superiority of the identified clone as it is strongly influenced by the mode of propagation, soil type, climatic conditions, age of the plant and plant density (Heller 1996).

MOLECULAR MARKERS

DNA markers provide an opportunity to characterize genotypes and to measure genetic relationships more precisely than other markers (Soller and Beckmann 1983). The advent of polymerase chain reaction (PCR) technology has offered new marker systems for diagnosis of genetic diversity in large scale studies (Saiki *et al.* 1988). Over the last 15 years, PCR technology has led to the development of a number of simple and quick techniques such as randomly amplified polymorphic DNA (RAPD), inter simple sequence repeat (ISSR) and amplified fragment length polymorphism (AFLP) markers which have been used by various workers for characterization of genetic diversity in forest tree species. Most of these marker systems along with some other novel approaches have been extensively utilized in genetic diversity assessment in *J. curcas* (Montes *et al.* 2009; Popluechai *et al.* 2009).

In recent years, a number of molecular studies on genetic diversity in *J. curcas* have been attempted (**Table 2**). Prior to this, the studies only involved the assessment of genetic diversity through morphometric and biochemical markers (oil contents), as discussed in the previous section. Many of the studies involving molecular characterization of *J. curcas* have originated from India, China and other countries where the species is not native (although naturalized in diverse agroclimatic conditions). Relatively less works have appeared from its centre of origin i.e., Central America as well as Africa and other regions of the tropics where it occurs extensively. Mainly the authors have either attempted evaluation of within species genetic variability or establishment of phylogenetic relationship of *J. curcas* with other species of the genera while some others have tried to characterize toxic and non-toxic varieties at molecular level.

Intra-specific genetic diversity

Assessment of within species genetic variation involving accessions/populations from different agro-climatic zones and/or geographical areas in India and China (with exception of some studies where accessions from Asia, Latin America and Africa were collectively evaluated) have been carried out employing a wide varieties of molecular marker systems to reveal low to moderate levels of genetic diversity. The extent of genetic diversity was assessed in a representative set of 42 accessions of *J. curcas* encompassing different agroclimatic zones of India along with a non-toxic genotype from Mexico. Molecular polymorphism was 42.0% with 400 RAPD primers and 33.5% with 100 ISSR primers between accessions indicating modest levels of genetic variation in the Indian germplasm. The within-population variation based on RAPD polymorphism was 64% and was at par with inter-population variation. Population-specific bands have been identified for accessions from Kerala (2 RAPD markers), Neemuch-1 from Rajasthan (1 each of RAPD and ISSR markers) and Mexican genotype (17 RAPD and 4 ISSR markers), which serve as diagnostic markers in genotyping (Basha and Sujatha 2007). However, among the 23 selected provenances from 300 collected provenances from all over India, Reddy *et al.* (2007) reported relatively lower polymorphism using RAPD (14-16%) and AFLP (8-10%) techniques.

Ranade *et al.* (2008) employed two single-primer amplification reaction (SPAR) methods to assess the diversity amongst the accessions of *J. curcas*, both amongst already held collections as well as from a few locations in the wild. They concluded that this relatively recently introduced plant species shows adequate genetic diversity in India and the accessions from the North East India were most distant from all other accessions in UPGMA analysis. The phylogenetic relationships of 13 *J. curcas* genotypes from different parts of India (Rajasthan, Uttaranchal, Uttar Pradesh and Orissa) were analyzed by Gupta *et al.* (2008) using 34 PCR markers (20 RAPDs and 14 ISSRs). Amplification of

Table 2 Molecular markers used for assessment of genetic diversity in *Jatropha curcas* L.

Species/Accessions	Marker used	% Polymorphism	Genetic diversity	Reference
42 accessions	RAPD	42.0	Moderate	Basha and Sujatha 2007
	ISSR	33.5		
23 provenances	RAPD	15	Low	Reddy *et al*. 2007
	AFLP	9		
12 *Jatropha* species	RAPD	80.2	High	Ganesh Ram *et al*. 2008
13 accessions	RAPD	84.26	High	Gupta *et al*. 2008
	ISSR	76.54		
58 accessions	AFLP	14.3	Low	Sun *et al*. 2008
7 *Jatropha* species	RAPD	68.48	Moderate	Pamidiamarri *et al*. 2009a
	AFLP	71.33		
8 *Jatropha* species and 3 *J. curcas* accessions	ISSR	98.14	High	Senthil Kumar *et al*. 2009
72 accessions from 13 countries	RAPD	61.8	Low to high	Basha *et al*. 2009
	ISSR	35.5		
28 accessions	RAPD	38	Moderate	Singh *et al*. 2009
48 accessions	AFLP	88	High	Tatikonda *et al*. 2009

genomic DNA of the 13 genotypes, using RAPD analysis, yielded 107 fragments that could be scored, of which 91 were polymorphic, with an average of 4.55 polymorphic fragments per primer. Number of amplified fragments ranged from one (OPA20, OPB19, OPD13) to nine (OPA18) and which varied in size from 200 to 2,500 bp. Percentage of polymorphism ranged from 40% (OPB18) to a maximum of 100% (14 primers). Resolution power ranged from a minimum of 0.153 (OPA20, OPB19) to a maximum of 11.23 (OPB15). The genotypes from Orissa (Orissa 6 and Orissa 7) appeared to be distinct from other genotypes.

Out of 25 ISSR primers used in the same study, only 14 primers were able to give rise to reproducible amplification products. These primers produced 81 bands across 13 genotypes, of which 62 were polymorphic with an average of 4.42 polymorphic fragments per primer. The number of amplified fragments ranged from two (ISSR 7, ISSR 8, ISSR 16) to nine (ISSR 12) and varied in size from 200 to 2,500 bp. Percentage of polymorphism ranged from 37.5% (ISSR 2, ISSR10) to a maximum of 100% (seven primers). The primers based on poly (GA) produced maximum number of bands (nine) while, poly (AT) and many other motifs gave no amplification at all with any of these thirteen genotypes (Gupta *et al.* 2008). RAPD markers were more efficient than the ISSR assay with regards to polymorphism detection, as they detected 84.26% polymorphism as compared to 76.54% for ISSR markers. However, resolving power (Rp), average bands per primer, Nei's genetic diversity (h), Shannon's Information Index (I), total genotype diversity among population (Ht), within population diversity (Hs) and gene flow (Nm) estimates were more for ISSR (7.098, 5.79, 0.245, 0.374, 0.244, 0.137 and 0.635, respectively) as compared to RAPD markers (5.669, 5.35, 0.225, 0.359, 0.225, 0.115 and 0.518, respectively). The regression test between the two Nei's genetic diversity indexes gave r2 = 0.3318, showing low regression between RAPD and ISSR based similarities. Regression value for ISSR and ISSR + RAPD combined data is moderate (0.6027), while it is maximum for RAPD and ISSR+RAPD based similarities (0.9125). Clustering of genotypes within groups was not similar when RAPD and ISSR derived dendrograms were compared, whereas the pattern of clustering of the genotypes remained more or less the same in RAPD and combined data of RAPD + ISSR (Gupta *et al.* 2008).

In China, Sun *et al.* (2008) assessed genetic relationships of 58 *J. curcas* accessions from different geographic locations based on simple sequence repeat (SSR) and AFLP analyses. Seventeen SSR markers were developed using the FIASCO (Fast Isolation by AFLP of Sequences Containing repeats) protocol; only one SSR primer was polymorphic with two alleles. The seven AFLP primer combinations amplified 70 polymorphic loci in total, 14.3% of which were polymorphic. The clustering of genotypes based on the AFLP markers shows that the genetic diversity of *J. curcas* in Guizhou region of China was notably different from the other samples. AFLP was also employed to assess the diversity in the elite germplasm collection of *J. curcas* from six different states of India. Forty-eight accessions were used with seven AFLP primer combinations that generated a total of 770 fragments with an average of 110 fragments per primer combination. A total of 680 (88%) fragments showed polymorphism in the germplasm analyzed, of which 59 (8.7%) fragments were unique (accession specific) and 108 (15.9%) fragments were rare (present in less than 10% accessions). In order to assess the discriminatory power of seven primer combinations used, a variety of marker attributes like polymorphism information content (PIC), marker index (MI) and resolving power (RP) values were calculated. Although the PIC values ranged from 0.20 (E-ACA/M-CAA) to 0.34 (E-ACT/M-CTT) with an average of 0.26 per primer combination and the MI values were observed in the range of 17.60 (E-ACA/M-CAA) to 32.30 (E-ACT/M-CTT) with an average of 25.13 per primer combination. Genotyping data obtained for all 680 polymorphic fragments were used to group the accessions analyzed using the UPGMA-phenogram and principal component analysis (PCA). Accessions coming from Andhra Pradesh were found to be diverse as these were scattered in different groups, whereas accessions coming from Chhattisgarh showed occurrence of higher number of unique/rare fragments (Tatikonda *et al.* 2009).

Recently, Montes *et al.* (2009) studied 225 accessions of *J. curcas* collected from over 30 countries in Latin America, Africa and Asia. Samples were analyzed (AFLP) at San Carlos University in Guatemala by nucleotide binding site (NBS)-profiling (conserved sequence based on NBS-gene family) in the Netherlands at Wageningen University and Research Centre - Plant Research International (Van der Linden *et al.* 2004). Genetic variability was low in African and Indian *J. Curcas* accessions, but high genetic variability was found in Guatemalan and other Latin American accessions. These studies have proved that molecular markers provide an efficient and quick tool in characterization of genetic diversity among the clones, accessions and populations of *J. curcas.* Several authors (e.g. Reddy *et al.* 2007; Basha and Sujatha 2007; Gupta *et al.* 2008; Sun *et al.* 2008) have also recorded relative effectiveness of different marker systems as well as low to moderate level of within species genetic diversity in *J. curcas*. However, considerable variability in morphometric features and oil yield in promising genotypes (CPTs) of *J. curcas* identified after rigorous selection in whole of eastern India comprising of four states namely Bihar, Jharkhand, Orissa and West Bengal (62 CPTs identified in more than 4 m km^2 area) during first phase of our research under the aegis of Indian Council of Forestry Research and Education has been confirmed through 20

RAPD markers in 28 CPTs/their clones (Bhatia *et al.* 2009; Singh *et al.* 2009).

Phylogenetic relationship of *J. curcas*

Efforts have been directed towards establishment of phylogenetic relationship of *J. curcas* with other related *Jatropha* species and natural hybrids (e.g. *J. tanjorensis* J. L. Ellis et Saroja.) through RAPD, AFLP, ISSR and other microsatellite markers in addition to some novel molecular techniques. Fortunately, the material used in these studies, sometimes, incorporated accessions from many countries (Costa Rica, India, Mexico, Nigeria, Thailand, etc.) which assisted in clear comparative analyses. Ganesh Ram *et al.* (2008) investigated genetic diversity of 12 *Jatropha* species (including 5 accessions of *J. curcas*) using RAPD markers. From the 26 RAPD primers used, 18 primers gave reproducible amplification banding patterns of 112 polymorphic bands out of 134 bands scored accounting for 80.2% polymorphism across the genotypes. Three primers viz., OPA 4, OPF 11 and OPD 14 generated 100% polymorphic patterns. However, the study with few data points (18 primers) resulted in several ambiguities in establishment of genetic relationships among *Jatropha* species.

Pamidiamarri *et al.* (2009a) studied the extent of genetic variability to establish phylogenetic relationship among seven species of *Jatropha viz*., *J. curcas*, *J. glandulifera* Roxb., *J. gossypifolia* L., *J. integerrima* Jacq., *J. multifida* L., *J. podagrica* Hook. and *J. tanjorensis* J. L. Ellis et Saroja using RAPD and AFLP markers. The percentage of loci that were polymorphic among the species was found to be 97.74% by RAPD and 97.25% by AFLP. The mean percentage of polymorphism was found to be 68.48% by RAPD and 71.33% by AFLP. They recorded maximum relatedness between *J. curcas* and *J. integerrima* which may be the reason for the success of inter hybrid crosses between these two species. However, neither RAPD nor AFLP data generated in this study supported the view of *J. tanjorensis*, a natural interspecific hybrid between *J. curcas* and *J. gossypifolia* as suggested by Prabakaran and Sujatha (1999) and emphasized that both RAPD and AFLP techniques are comparable in divergence studies of *Jatropha s*pecies.

Pamidimarri *et al.* (2009b) further studied phylogenetic relationships among these seven species using nuclear ribosomal DNA internal transcribed spacer (ITS) sequence (nrDNA ITS) and compared the results with multilocus marker analysis systems reported earlier (Pamidiamarri *et al.* 2009a) for the same genus. The size variation obtained among sequenced nrDNA ITS regions was narrow and ranged from 647 to 654 bp. The overall mean genetic distance (GD) of genus *Jatropha* was found to be 0.385. The present study also strongly supports high phylogenetic closeness of *J. curcas* and *J. integerrima* while *J. podagrica* was also found clustered with *J. curcas*.

Senthil Kumar *et al.* (2009) studied genetic diversity among eight *Jatropha* species and three *J. curcas* accessions were analyzed using ISSR markers. Nine ISSR primers generated reproducible amplification banding pattern of 61 polymorphic bands out of 64 scored accounting for 98.14% polymorphism across the species. The ISSR primers *viz*., I1, I2, I3, I4, I5, I6, I7 and I10 generated 100% polymorphic patterns. Jaccard's coefficient of similarity (Jaccard 1901) varied from 0.346 to 0.807, indicative of high level of genetic variation among the genotypes studied. The UPGMA cluster analysis indicated three distinct clusters, one comprising of all accessions of *J. curcas* L. (TNMJ1, TNMJ 22 and TNMJ 23), while second included four species *viz*., *J. tanjorensis* J. L. Ellis et Saroja, *J. gossypifolia* L., *J. podagrica* Hook and *J. maheshwarii* Subrum and M.P. Nayer and the third cluster included another four species *viz*., *J. villosa* Wight, *J. multifida* L., *J. integerrima* Jacq and *J. glandulifera* Roxb. The overall grouping pattern of clustering corresponds well with PCA confirming patterns of genetic diversity observed among the species.

Using nuclear and organelle specific primers for supporting interspecific gene transfer, Basha and Sujatha (2009) attempted characterization of *Jatropha* species occurring in India. DNA from 34 accessions comprising eight agronomically important species (*J. curcas*, *J. gossypifolia*, *J. glandulifera*, *J. integerrima*, *J. podagrica*, *J. multifida*, *J. villosa*, *J. villosa* var. ramnadensis and *J. maheshwarii*) and a natural hybrid, *J. tanjorensis* were subjected to molecular analysis using 200 RAPD, 100 ISSR and 50 organelle specific microsatellite primers from other angiosperms. The nuclear marker systems revealed high interspecific genetic variation (98.5% polymorphism) corroborating with the morphological differentiation of the species. Ten organelle specific microsatellite primers resulted in single, discrete bands of which three were functional disclosing polymorphism among *Jatropha* species. PCR products from two consensus chloroplast microsatellite primer pairs (ccmp6 and 10) revealed variable number of T and A residues in the intergenic regions of ORF 77–ORF 82 and rp12–rps19 regions, respectively in *Jatropha*. Artificial hybrids were produced between *J. curcas* and all *Jatropha* species used in this study with the exception of *J. podagrica*. Characterization of F1 hybrids using polymorphic primers specific to the respective parental species confirmed the hybridity of the interspecific hybrids. Characterization of both natural and artificially produced hybrids using chloroplast specific markers revealed maternal inheritance of the markers. While the RAPD and ISSR markers confirmed *J. tanjorensis* as a natural hybrid between *J. gossypifolia* and *J. curcas*, the ccmp primers (ccmp6 and 10) unequivocally established *J. gossypifolia* as the maternal parent.

As compared to multilocus markers like RAPD and AFLP, microsatellites have advantages like locus specificity, codominant nature, high reproducibility and substantial size polymorphism (Powell *et al.* 1996). Generation of novel molecular markers like microsatellites provides better tools to assess the amount and distribution of molecular diversity and for population genetic studies. Pamidimarri *et al.* (2009c) isolated 12 microsatellites from *J. curcas* and characterized them in 32 accessions collected from a natural population in Junagadh Gir forest region, Gujarat, India and the library was constructed following the FIASCO procedure (Zane *et al.* 2002) with minor modifications. Their cross-amplification was also checked in six common species of *Jatropha* (*J. glandulifera*, *J. gossypifolia*, *J. integerrima*, *J. multifida*, *J. podagrica* and *J. tanjorensis*).

A total of 12 polymorphic loci were identified, with highest number of alleles (11) given by marker jcds24 and lowest (two) by jcps1 and jcms30. The observed and expected heterozygosities ranged from 0.94 to 0.54 and from 0.95 to 0.56, respectively. Tests for Hardy–Weinberg equilibrium showed that loci jcds58, jcds66, jcps1, jcps6 and jcms30 were not in Hardy–Weinberg equilibrium. These deviations may be due to presence of null alleles or disturbances in natural dispersal of the race in the population by anthropogenic activity (Basha and Sujatha 2007; Pamidimarri *et al.* 2009a). No significant linkage disequilibrium was detected between any pair of loci after correcting for multiple comparisons.

Assessing genetic variation by RAPD, AFLP and combinatorial tubulin based polymorphism (cTBP) in 38 *J. curcas* accessions from 13 countries on 3 continents revealed narrow genetic diversity while the 6 *Jatropha* species from India exhibited pronounced genetic diversity indicating higher possibilities of improving *J. curcas* by interspecific breeding (Popluechai *et al.* 2009). The samples were initially examined using 10 RAPD primers. One cluster contained all of the 17 *J. curcas* accessions and the second contained the out-group *J. podagrica*, which showed an overall similarity of 52% with *J. curcas*. Among the *J. curcas* accessions the similarity coefficient was high (0.78) indicating a narrow genetic base. The two Indian accessions clustered separately while the Nigerian accession clustered with the remaining 14 Thai accessions. The 6 provenances of Thai accessions could not be clearly differentiated, reinforcing the narrow genetic base between provenances.

Popluechai *et al.* (2009) also used a novel, relatively unexploited technique of combinatorial tubulin based polymorphism (cTBP; Breviario *et al.* 2007) which uses variation in the length of the first and second intron of members of the plant β-tubulin gene family. The approach was successfully used earlier to detect intra and inter-species polymorphism in diverse plants including oilseed plants – rapeseed and peanut (Breviario *et al.* 2007) and palm (Breviario *et al.* 2009). Results showed that the four accessions from Costa Rica were clearly different to those from other parts of the world and they also exhibited intra-specific polymorphism in both intron I and II. In all these studies *J. curcas* accessions from different ecogeographic regions of India were 60 to 80% similar. Results from the studies above suggested the importance of testing accessions from wider eco-geographic regions of the world. However, the analysis of 38 accessions from 13 countries around the world, along with 6 different species of *Jatropha* from India, again indicated 75% similarity among the global *J. curcas* accessions (Popluechai *et al.* 2009).

Distinguishing toxic and non-toxic varieties

Molecular characterization of toxic and non-toxic varieties to develop PCR based molecular markers for distinguishing non-toxic from toxic or vice versa has also been attempted. The polymorphic markers were successfully identified specific to non-toxic and toxic varieties using RAPD and AFLP techniques. Totally 371 RAPD, 1441 AFLP were analyzed and 56 (15.09%) RAPD and 238 (16.49%) AFLP markers were found specific to either of the varieties. Genetic similarity between non-toxic and toxic varieties was found to be 0.92 by RAPD and 0.90 by AFLP fingerprinting and demonstrated that both techniques were equally competitive in identifying polymorphic markers and differentiating varieties (Pamidiamarri *et al.* 2008).

Basha *et al.* (2009) elucidated genetic background of 72 *J. curcas* accessions representing 13 countries has been using molecular analysis and biochemical traits. Seed kernel protein, oil content, ash content and phorbol esters revealed variation with accessions from Mexico containing low levels of phorbol esters. Molecular characterization disclosed polymorphism of 61.8 and 35.5% with RAPD and ISSR primers, respectively and Mantel test (Mantel 1967) revealed positive correlation between the two marker systems. A dendrogram based on pairwise genetic similarities and three-dimensional principal coordinate analysis (PCA) using data from RAPD and ISSR marker systems showed close clustering of accessions from all countries and grouped the Mexican accessions separately in clusters III, IV, V and VI.

Presence of the toxic phorbol esters is a major concern and analysis of 28 Mexican accessions resulted in identification of molecular markers associated with high and low phorbol ester content. The identified RAPD and ISSR markers were converted to SCARs for increasing their reliability and use in marker-assisted programs aimed at development of accessions with reduced toxicity. Twelve microsatellite primers differentiated the non-toxic Mexican accessions and disclosed novel alleles in Mexican germplasm. Amplification with primers specific to the curcin coding sequence and promoter region of ribosome-inactivating protein (RIP) revealed polymorphism with one primer specific to RIP promoter region specifically in accessions with low phorbol ester levels. Narrow genetic variation among accessions from different regions of the world and rich diversity among Mexican genotypes in terms of phorbol ester content and distinct molecular profiles indicates the need for exploitation of germplasm from Mexico in *J. curcas* breeding programs (Basha *et al.* 2009).

The findings of Popluechai *et al.* (2009) showed that the non-toxic Mexican accession (NTMA) clustered separately from other *J. curcas* accessions and that the genetic similarity coefficient between the Thai and the NTMA was high (0.76) as similarly noted by Basha and Sujatha (2007). Their assessment of nearly 52% similarity between the Thai *J. curcas* and *J. podagrica*, was also the same as reported by Ganesh Ram *et al*. (2008) in case of Indian *J. curcas* and *J. podagrica.* Comparison of global accessions of *J. curcas* to other *Jatropha* species resulted in *J. integerrima* and *J. gossypifolia* being closer to *J. curcas* than other species as noted by Pamidiamarri *et al.* (2009a) in comparing the Indian *J. curcas* to other *Jatropha* species from India. Such an overlap between local and global results indicates a narrow genetic diversity in *Jatropha*.

CONCLUSIONS AND FUTURE AVENUES

With advancement of scientific research in the arena of plant molecular biology a number of molecular techniques have been developed in recent years. Most of these have been efficiently employed for characterization of genetic diversity of *J. curcas* L. to produce similar notion of low level of genetic diversity in the species despite wide phenotypic variability and significant differences in oil content in accessions from different geographical regions. *J. curcas*, an undomesticated plant species uniquely exhibits naturally widespread genetic monomorphism as revealed in most of the molecular studies. The reasons for the global low genetic variability seen in *J. curcas* remain unclear. Most likely, the anthropogenic and environmental influences in generating genetic variability are missing because a) it is not a crop, b) is well-surviving, undomesticated plant, it is highly stress tolerant due to adaptive genomic characters probably acquired before its global distribution and c) a limited stock has been vegetatively and apomictically propagated, since *J. curcas* is known to exhibit apomixis (Bhattacharya *et al.* 2005). Furthermore, Richards *et al.* (2006) observed that 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.

Another reason behind this seems to be introduced nature of *J. curcas* in countries (like India) where these molecular studies have been conducted with limited numbers of accessions in most of the cases. Further, variable seed yield and oil content in different accessions not commensurate with genetic differences indicates towards overriding influence of prevailing environmental conditions for seed oil production. However, incorporation of genotypes from wide geographical area including Central America especially Mexico and Guatemala, Carrabin in addition to Africa and Asia for evaluation at molecular level will present a clear picture. Therefore, a collaborative global *Jatropha* genetic diversity evaluation effort is immediately needed for better use of this valuable species in breeding programs considering its potential of biodiesel and motor fuel production.

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