Tropical Agricultural Research & Extension 15(1): 2012

ASSESSMENT OF MACROGEOGRAPHICAL GENETIC VARIATIONS *IN JATROPHA CURCAS* L IN INDIA USING ALLOZYME AND RAPD MARKERS

Warrier RR*, Devika Nagalakshmi B, Savitha C, Anandalakshmi R, Nicodemus A, Singh GB
Institute of Forest Genetics and Tree Breeding, PB No. 1061, Forest Campus, Coimbatore 641002 India

Accepted: 20 September 2011

ABSTRACT

***Jatropha curcas* L., an exotic introduced in to India by Portuguese about 500 years ago, was assessed for its genetic base in the country as a step to initiate breeding programmes. Genetic diversity of 56 accessions of Jatropha estimated from allozymes and from Random Amplified Polymorphic DNA (RAPDs) revealed eighteen allozyme loci and 120 RAPD markers. According to allozyme data, species genetic diversity (p = 26.67%, A = 1.533 and He =0.205), was low. Inbreeding coefficients suggest that populations are structured in genetic neighborhoods. The RAPDs also showed similar levels of genetic diversity (p = 35.73% and He = 0.181) at the species level. Nei's genetic distances estimated both from allozymes and RAPDs indicated low differentiation among populations. No significant associations among genetic and geographical variables were observed, suggesting that such low genetic variation in this allogamous species could be due to the few introductions that have spread across the country primarily, through vegetative propagation. The species has not been improved for productivity and thus naturally occurring unadapted populations are a result of the few initial introductions.**

***Key words*:** Allozyme; Genetic diversity; *Jatropha curcas;* Population structure; RAPD

INTRODUCTION

India, an emerging major player in the global economy, consumes over 127 million t of crude oil a year and imports about 70% of it thus paying a huge import bill. To tide over this and to overcome the oil crisis in future, the Planning Commission of India initiated a National Mission on Biodiesel to identify suitable tree borne oil seeds, amongst which *Jatropha curcas* was recognised as a potential petrocrop.

Success of improvement programmes lies in the identification of genetically divergent material and development of genetically superior stocks. Breeding of inter and intra specific Jatrophas for exploitation of hybrid vigour is one of the most economical means to tree improvement. Elsewhere in the world, systematic germplasm collection and evaluation programme to identify superior material from the existing natural variations, establishment of clonal seed orchards, development of mass multiplication techniques to ensure easy supply of elite planting material, a series of multi-locational trials under different agro-climatic conditions to establish authentic data on yield estimates and economic of production have been taken up on a massive scale (Muys *et al.* 2007).

In India, currently, the Jatropha promotion programmes use the material available ''wild'' locally (Basha *et al.* 2009). Perusal of the mode of introduction of the species into India reveals that the possibility for a spectrum of genetic variation, other than the ones forced by edaphic and climatological factors, is not very wide. Though high yielding varieties and cultivars have been identified, there exists skepticism over subjecting them to breeding for exploitation of hybrid vigour due to the species alleged narrow genetic base. In this scenario, spending valuable financial resources, time and human endeavor on plant improvement, i.e. collection and evaluation of germplasm, including establishment of clonal and seedling seed orchards, multi-locational trials, vegetative multiplication gardens under different agro-climatic conditions for supply of elite planting material on a massive scale may not pay rich dividends. Further, it is of paramount importance to evaluate and maintain genetic variability within stands because plantations consisting of genetically uniform materials are highly vulnerable to major climatic fluctuations and pests. The present paper details the study taken up to evaluate Jatropha germplasm and identify genetic

*Corresponding author: *rekha@icfre.org*

variability to further breeding programmes. In our study on *Jatropha curcas*, the molecular marker analyses attempts to explain the extent of variation existing in *Jatropha curcas* in India, how it is distributed and information to assess if the current levels of diversity is sufficient for improvement and / or domestication. Our hypothesis was based on the fact that the species would have high intrapopulation polymorphism and high polymorphism among populations due to its cross pollinating nature.

MATERIALS AND METHODS

Materials: Seed material of 56 accessions from various States of India from where Jatropha has been selected for various national programmes (Warrier *et al*. 2010) were collected and used. Thirty seedlings per accession were studied for allozyme variations and ten seedlings for DNA. The seeds were germinated on sand beds and maintained. Young leaves were used for the analyses.

Preparation of extract: Thirty seedlings per accession were studied. Leaf samples, free of any visible infestations, were collected, brought to the laboratory in ice and stored at 4ºC for further analysis. About 100 mg of tissue was washed once in deionised water and mashed individually in a pre-chilled mortar in 500µl of 0.1 M Tris-HCl buffer (pH 6.8) containing 10% glycerol and 0.056 M β-mercaptoethanol. The resultant slurry was centrifuged at 12,000 rpm for 20 min. in a refrigerated centrifuge and the supernatant was stored at -20ºC before use.

Electrophoresis: Poly Acrylamide Gel Electrophoresis (PAGE) was carried out at low temperature as per standard procedure (Laemmli, 1970) with appropriate modifications. Allozyme zones were designated to define the general area on the zymogram within which the bands migrated. The relative position of each band was drawn schematically and the zymogram was scored. Scoring was made for those bands which were clearly visible. Genetic interpretation of enzyme phenotypes was based on observed differences in zymogram profile after activity staining of the gel. Variation in banding patterns was determined by the migration from the origin towards the anode.

Enzymes studied: Fifteen enzyme systems were selected for detailed analysis after a preliminary survey namely Aspartate Aminotra -nsferase (AAT), Alcohol Dehydrogenase (ADH), Peroxidase (POD), Iso Citrate Dehydro -genase (ICD), Formate Dehydrogenase (FDH), Shikimate Dehydrogenase (SKD), Malate Dehydrogenase (MDH), Lactate Dehydrogenase (LDH), Glucose 6 Phosphate Dehydrogenase (G6PD), Glyceraldehyde 3 Phosphate Dehydrogenase (G3PD), Aldolase (ALD), Superoxide Dis Mutase (SOD), Alpha Esterase (αEST), Glutamate Phosphate Dehydrogenase (GDH) and Beta Esterase (βEST).

Data analysis: The allozyme gels were genetically interpreted according to Karpa *et al*. (1997). The data was subjected to population genetic analysis using POPGENE software (Yeh *et al.* 1997). Genetic parameters within populations, including the percentage of polymorphic loci (*P*), expected heterozygosity (*He*), and fixation index (*F*), were calculated. To estimate outcrossing rates for these populations, we used the fixation index *F*, with outcrossing rate $t = (1 - F)/ (1 + F)$. Nei's (1973) total gene diversity (H_T), coefficient of gene differentiation (G_{ST}), and Nei's (1972) genetic identity (*I*) between populations were also computed. The coefficient of genetic differentiation among populations, G_{ST}, was used to estimate the level of gene flow, *Nm* (the number of migrants exchanged between local populations per generation), based on the relationship $G_{ST} = 1/(4Nm + 1)$, where G_{ST} is Nei's (1973) estimator of F_{ST} (Wright, 1951).

DNA extraction: 300mg of fresh leaf tissue was homogenized using mortar and pestle in liquid nitrogen. The powdered sample was taken in a centrifuge tube containing preheated CTAB isolation buffer(2% CTAB, 1.0M NaCl, 0.2% β Mercaptoethanol, 20*mM* EDTA, 100*mM* Tris HCl , 0.5 % PVP pH 8.0) and incubated at 60° C for 30min. The solution was extracted twice with equal volume of chloroform: isoamylalcohol (24:1) and centrifuged at 12,000g for 10 minutes. The supernatant was transferred to a clean tube, to which 2/3 volumes of ice cold propanol was added and allowed to precipitate at RT for 1hr. The supernatant was poured off following

spinning and the nucleic acids were resuspended in wash buffer (76% ethanol, 7.5*M* sodium acetate). The supernatant was poured off, the pellet was air dried and resuspended in 1ml TE (10*mM* Tris HCl, 1m*M* EDTA pH7.4). The samples were incubated at 37°C for 30 minutes following treatment with 10g/ml RNAse. Following this, the samples were diluted with 2 volumes of double distilled water or TE. Ammonium acetate (7.5*M* stock, pH7.7) was added to reach a final concentration of 2.5*M*. An equal volume of ice cold ethanol was added and gently mixed. The tubes were centrifuged, air dried and the DNA was re-suspended in 200µl of TE.

PCR protocol: Polymerase Chain Reactions (PCRs) for amplification of DNA extractions were carried out in a 25µl volume (Willams *et al.,* 1990). Twenty five custom decanucleotide primers synthesised from M/S 1st Base, Singapore were used for amplification. The reaction conditions were optimized. Mixtures (25µl total volume) were composed of dNTPs (200µM), $MgCl_2$ (1.5mM), 1X buffer, primer (0.2µM), DNA (100ng), Taq DNA polymerase (2 units). Negative control was included in which all the ingredients were present except template DNA. The amplifications were carried out using the DNA Engine thermal cycler (MJ Research, USA) programmed for 94°C for 5 min (one cycle); followed by 94°C for 1min, 35°C for 1min and 72°C for 2min (35 cycles); 72°C for 8min (one cycle), then 4°C (infinitive). Reactions were repeated twice to confirm amplification of samples. Amplification products (7µl) were mixed with 3µl loading buffer and separated on 1.2% agarose gels. The bands were visualized using ethidium bromide staining.

DNA analysis: For RAPD analysis, bands were identified by image analysis software for gel documentation (AlphaEaseFC Imaging Software Ver. 6.0, Alpha Innotech, JH Bio, USA). Smeared and weak bands were excluded. Assuming that each RAPD band represents a single diallelic locus in Hardy–Weinberg (H–W) equilibrium, the p/a data set was converted into allele frequencies, scored manually and transferred to the present-absent scale (1 or 0 for each allele and genotype) and adjusted for the fixation index (*F*), which was calculated based on allozyme data. An additional measure for partitioning genetic variation was obtained by the Shannon index (*S*) because it is relatively insensitive to the inability of RAPD in detecting heterozygous loci (Dawson *et al.* 1996). Data were subjected to analyses using POPGENE 3.2 software.

RESULTS AND DISCUSSION

Allozyme analysis-Fifteen enzyme systems provided a total of 18 loci for the 56 accessions of *Jatropha curcas*. Twenty eight percent (5/18) of the resolved allozyme loci were polymorphic (0.99 criterion). Out of these eighteen loci, 13 were invariably present in all the accessions while 5 were present in 9, 10, 11, 44, 46 accessions. The 13 loci, ADH1, AAT1, POD1, ICD1, SKD1, LDH1, G6PD1, G3PD1, ALD1, SOD1, αEST1, GDH1 and βEST1 were invariant in all the accessions analysed and were monomorphic. Unique alleles were however, not detected in any of the accessions. Rare alleles were present at the dimorphic MDH1 and FDH1 loci. It was

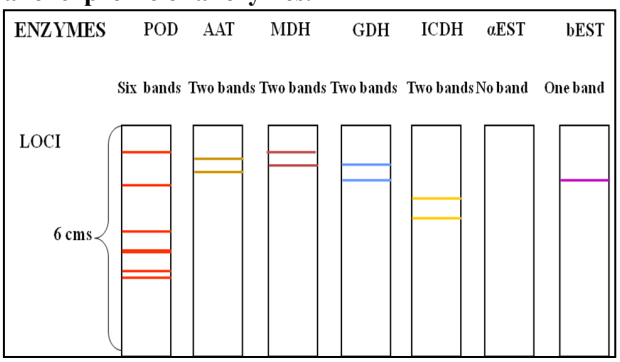
Table 1 A. Overall allele frequencies at eighteen loci in 56 accessions of *Jatropha curcas*

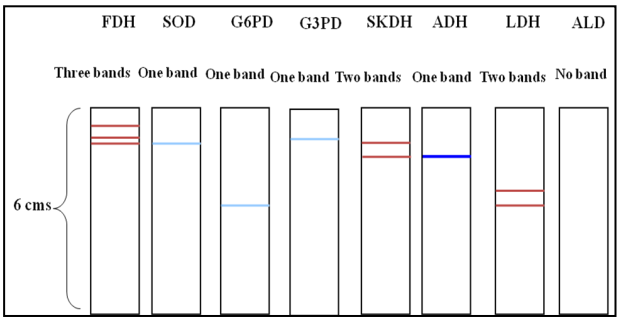
Allele	Locus										
	ADH1	AAT1	POD1	POD2	POD3	POD4	ICD1	FDH1	SKD1	MDH1	LDH1
A	1.00	1.00	0.99	0.67	0.66	0.69	1.00	0.69	1.00	0.67	1.0
B	-	-	0.01	0.20	0.20	0.19	-	0.19	-	0.20	-
C	-	-	-	0.12	0.14	0.12	-	0.12	-	0.12	-

Table 1 B. Overall allele frequencies at eighteen loci in 56 accessions of *Jatropha curcas*

Allele	Locus						
	G6PD1	G3PD1	ALD1	SOD1	EST1	GDH1	EST1
A	1.00	1.00	1.00	0.993	1.00	1.00	1.00
B	-	-	-	0.003	-	-	-
C	-	-	-	0.003	-	-	-

Fig. 1. Zymogram depicting relative mobility and designation of electrophoretic variants at different loci found among 56 accessions of *J. curcas* based on allelic profile of allozymes.





monomorphic to all other accessions. All the alleles were not equally distributed (Tables 1A and B). There were maximum variations in POD2, POD3, POD4, FDH1, and MDH1, and minimum in ADH1, AAT1, ICD1, SKD1, LDH1, G6PD1, G3PD1, ALD1, αEST1, GDH1 and βEST1 loci (Fig. 1).

The percentage of polymorphic loci (*P*) ranged from 11 to 28%, with a mean value of 26.67 ± 4.57%. The average number of alleles per locus (na) ranged from 1.22 to 1.56, with a mean value of 1.53 ± 0.09. The observed heterozygosity (*Ho*, ranging from 0.08 to 0.26 with an average of 0.15 ± 0.022) was slightly higher than the expected heterozygosity (*He*, ranging from 0.08 to 0.19 with an average of 0.14 ± 0.022). Negative fixation indices (*F*) were obtained in most populations studied, ranging from 0.006 to 0.194, indicating low or poor levels of outbreeding in these populations. Tests for Hardy-Weinberg equilibrium showed that polymorphic loci in most populations had significant heterozygote deficiency whereas a few other populations exhibited heterozygote excess. Nei's genetic identities (*I*) between populations varied from 0.05 to 0.29 with an average of 0.24 ± 0.04. Based on the genetic identity matrix, a UPGMA dendrogram was

Fig. 2 RAPD Profiles using primers OPE 13 (left) and OPB 05 (right) showing banding patterns

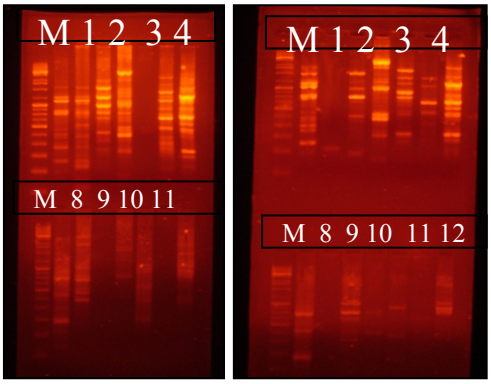
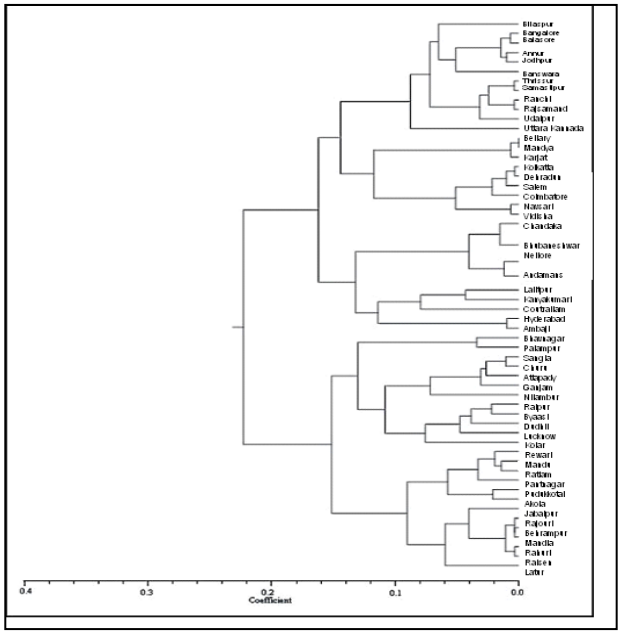


Fig. 3. Dendrogram (UPGMA) representing genetic relationships among 56 accessions of *J. curcas* based on genetic similarity matrix obtained using the allelic profile of allozymes.



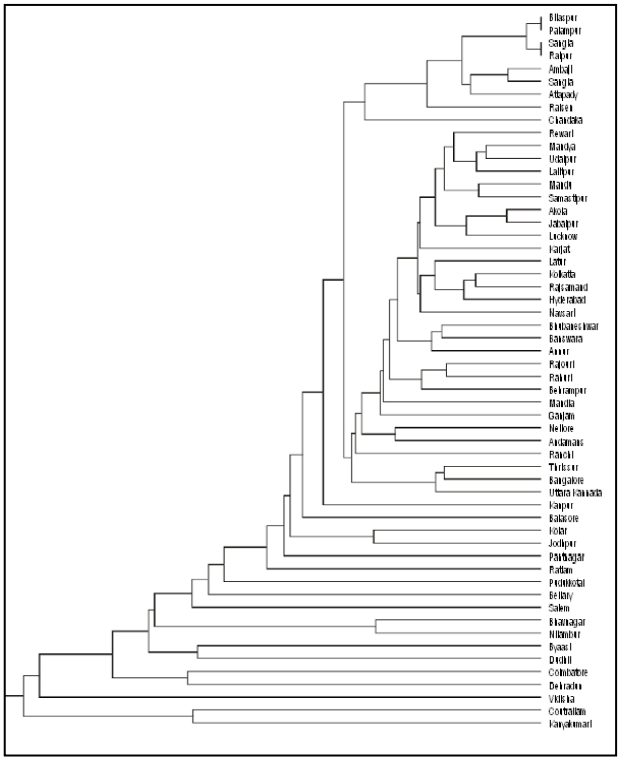
constructed showing the relationships among these populations (Fig. 3). The level of gene flow (*Nm*) was estimated to be 0.24 individual per generation between populations. Genetic identities varied from 0.93 to 1.00.

RAPD diversity-A total of 120 bands whose size ranged between 300 and 2000bp were produced using 25 primers (Fig. 2). Bands produced per primer ranged from 1.2 to 10.2 at the population level with an average of 5.2. Among the 254 loci, 47.2% were polymorphic at the species level and the remaining were consistently monomorphic. The percentages of polymorphic loci (*P*) for a single population ranged widely from 24 to 53% with an average of 35.73 ± 5.4%. Assuming Hardy-Weinberg equilibrium, the average gene diversity within populations was estimated to be 0.09, comparable to the adjusted estimate (*H*S =

Table 2. Comparison of genetic diversity detected by allozyme and RAPD markers

	Allozyme markers	RAPD markers
Polymorphic loci (*P*), %	26.67	35.73
Effective number of alleles per locus (ne)	1.270	1.102
Total gene diversity	0.392	0.393
Within population	0.107	0.074
Between populations	0.205	0.181
Genetic identity (*I*)	0.238	0.187
Gene flow (*Nm*)	0.971	2.257

Fig. 4. Dendrogram (UPGMA) representing genetic relationships among 56 accessions of *J. curcas* based on genetic similarity matrix obtained using the RAPD data



0.10), which takes into account the fixation index within each population. Genetic identities at the RAPD level varied from 0.951 to 0.998, not significantly different from the values obtained at the allozyme level. Based on the genetic identity matrix, a UPGMA dendrogram was constructed showing the relationships among these populations (Fig. 4). Heterozygosity is directly applied as a measure of genetic variation (Crow 1986). High levels of heterozygosity are important for survival over long life span of trees (Gregorius and Ziehe 1986), which is related with the low mortality of seedlings and plants (Mejnartowicz and Lewandowski 1994). Tree species in general are highly outcrossing and highly heterozygous as compared to herbaceous perennials and annuals, the average level of heterozygosity being twice that of the herbaceous plants (Hamrick *et al.* 1979).

It was observed that all the accessions had polymorphic loci ranging between 11.11 and 27.78 suggesting that there has been very poor chance for incorporation of new genes into the next generation. It may be noted that Jatropha is an entomophillous species and hence a high level of outcrossing is expected. However, only few populations showed maximum (27.78 %) polymorphic loci which suggest that large amounts of inbreeding exists within the accessions. The low heterozygosity could be ascribed to a common descent of the population. As the introduction of Jatrophas to the country is ambiguous, the results point to confirmation of a narrow genetic base as indicated by lower proportion of polymorphic loci. With increase in polymorphic loci, the expected heterozygosity and the average heterozygosity would simultaneously increase which is reflected in variation in the heterozygote levels increasing from 0.08 to 0.26. Studies have been reported on allozyme variation in *Santalum album* (Manojkumar *et al.* 1998), *Acacia* (Wickneswari and Norwati 1989), *Tectona grandis* (Kumaravelu 1979), Eucalyptus (Martin-Corder and Lopes 1997) and *Azadirachta indica* (Philomina 2000).

Reduced number of fertile unrelated trees and a low population size would also result in decreased diversity (Kang and Lindgren 1998). In the present study, allozyme analyses have provided information about the relative amounts of genetic variation present within Jatropha accessions distributed throughout the country. The genetic variability observed was lower than other plant species summarized by Hamrick *et al.* (1992). Hence, for improvement of this species, seeds from a wide genetic base need to be obtained to enable its subjection to various ecological conditions. The mean number of alleles per locus per population was 1.53, which was higher than the average of 1.44 in dicots. The mean expected heterozygosity per population (0.10) in this study was lower than the averages of both angiosperm (0.143), outcrossing wind-pollinated woody plants (0.154) and 17 tropical tree species (0.217) reported by various authors (Moran 1992; House and Bell, 1994; Ibrahim, 1996; Martins-Corder and Lopes 1997).

Gene diversity- An enormous amount of protein diversity is characteristic of natural populations (Mallet 1996). Their genetic structure is mainly influenced by the combined effects of random genetic drift, restricted gene flow and differential selection pressures. These effects lead to low 'within' population genetic variation and comparatively high 'among'

population genetic variation in species consisting of small and isolated populations (Holderegger and Schneller 1994; Nevo 1983). Generally, when populations are close together, having possibility of enough gene exchange, there should be few differences in gene frequency but if they are far apart there should be strong differences (Mallet 1996). This was in contrast to the results obtained in Jatropha where the plant populations have been spread all over the country as hedges. In our study, the genetic identity and distance values calculated between 56 accessions represented as an UPGMA dendrograms (Figures 2and3) was a fair representation of the Nei's genetic distances between the accessions, which showed many clusters. Analysis of genetic relationships revealed heterogeneous values for Nei's genetic identity (*i*). It ranged from 0.01 (minimum value) to 0.20 (maximum value). All the 56 accessions were clustered at various levels irrespective of the geographical similarity or distance. Most of the accessions showed similarity. Comparison of genetic variation using different marker systems showed that RAPDs consistently revealed higher levels of variability than allozymes in terms of percentage of polymorphic loci, gene flow and gene diversity. At the allozyme level, relatively low genetic variation exists in *J. curcas*, when compared with the average values for animal-pollinated outcrossing plant species (P = 50%, A = 1.99, H = 0.167). Low genetic differentiation among populations was detected (G_{ST} = 0.2048), which is similar to the reported average in outcrossing plant species (G_{ST} = 0.20; Hamrick and Godt, 1989). This level and pattern of genetic variation is more comparable to predominantly selfing plant species than those predominantly outcrossing species. In comparison with allozyme diversity, higher levels of genetic variation were detected at the RAPD level. However, the pattern of genetic diversity within and among populations was comparable between the two data sets. Estimates of G_{ST} based on RAPDs confirmed that natural gene flow between populations of *J. curcas* was apparently limited, as indicated by allozyme data. Nei's genetic identity estimate based on RAPDs also corroborates the allozyme estimate, showing that a low level of population genetic divergence has occurred in the species. Our results are consistent with other comparative studies that reported that more variation was detected at RAPD loci than at allozyme loci, but the same pattern of population genetic structure was revealed by the two sets of data.

The RAPD heterozygosity values were slightly higher than those observed using allozyme loci. However, testing the significance of difference will require more data. Likewise, the RAPD F_{ST} values were of comparable magnitude to the allozyme-based F_{ST}-values and indicated only very limited differentiation between populations. *Nm* estimates were high (Nm> 1). Both the RAPD H- and F_{ST}-values in *J. curcas* were comparable to allozyme-based figures in other species showing poor to low heterozygosity.

None of the accessions studied was collected from the same localities. Despite this, the genetic variability estimates were very low which might be due to the reduced gene flow between these populations. The 56 accessions have been brought from different places where they have grown in varied climatic and edaphic conditions. The dendrograms clearly depicted narrow genetic diversity among various accessions. Though the different accessions brought from different parts of the country were clustered, the genetic distances were very low. Accessions 12-17 from Kerala and Karnataka were clustered together while most accessions from Maharashtra and Madhya Pradesh were grouped together; Accessions 3-11 from the states of Karnataka, Madhya Pradesh, Gujarat, Himachal Pradesh also grouped together. This helped us to arrive at the conclusion of a lack of alliance between genetic data and geographical characteristics of the populations. Further, the differences between RAPD- and allozyme-based estimates of genetic diversity suggest that allozymes may not be a representative sampling of the entire genome. Yet, the numerical congruence between allozyme and RAPD data suggests an indication that in the case of *J. curcas* the allozyme data provide a good picture of the population genetic structure of this species.

Although many woody species maintain relatively high levels of genetic variability (Hamrick and Godt 1996), some examples exist of widespread conifers with limited genetic variation, such as *Pinus resinosa*

(Fowler and Morris 1977) and *Tsuga canadensis* (Zabinski 1992), with 0 and 10% polymorphism, respectively. The most common explanation for the reduction or absence of variation is that each species has gone through one or more population bottlenecks. The reduced polymorphism and partial inbreeding measured in accessions of Jatrophas, may be due to reduced gene flow. Though geographical range has been considered a good predictor of the levels of genetic variation in plant populations (Hamrick and Godt 1989), the present study revealed otherwise. Scher (1996) has reported similar observations in the rare Pacific yew (*Taxus brevifolia*) which occurs over a wide range but shows low polymorphism (<50%).

CONCLUSION

In conclusion, the present RAPD data follows a similar pattern as the co-dominant allozyme data on *J. curcas.* Trends suggested by allozyme data remain the same with RAPDs also (i.e. the within and between population differences). This congruence between allozyme and RAPD data strongly suggests the strength of allozyme studies which could provide valuable information on genetic diversity as co-dominant markers as do RAPDs. The 56 Indian accessions did not possess genetic distinctness and failed to be discriminated indicating a narrow genetic base of the material investigated. Further, the intra-population variation as determined by allozyme and RAPD studies was on par with the genetic variation detected between populations or land races. Such low genetic variation in an allogamous species like *J. curcas* could be due to the few introductions that have spread across the country primarily, through vegetative propagation. The species has not been improved for productivity and most of the projects that relied on naturally occurring unadapted populations are a result of the few initial introductions. Hence, there is an immediate need to widening or broadening the genetic base of *J. curcas* if any genetic improvement programmes are to be attempted.

ACKNOWLEDGEMENTS

The authors acknowledge Directors of AFRI, Jodhpur; IWST, Bangalore; FRC, Hyderabad; TFRI, Jabalpur, and Deans of College of Forestry, TNAU, Mettupalayam, and KAU, Trichur for providing the Jatropha accessions to carry out the present study. The authors are also grateful to Dr. Ajay Parida, Executive Director, MS Swaminathan Research Foundation (MSSRF), Chennai for evaluating the work.

REFERENCES

Basha SD Francis HPS Makkar KB and Sujatha M 2009 A comparative study of biochemical traits and molecular markers for assessment of genetic relationships between *Jatropha curcas* L germplasm from different countries. Plant Science 176: 812-823

Crow JF 1986 Basic concepts in population quantitative and evolutionary genetics WH Freeman and Comp., New York.

Dawson IK Simons AJ Waugh R and Powell W 1996 Diversity and genetic differentiation among subpopulations of *Gliricidia sepium* revealed by PCR-based assays. Heredity 75: 10-18

Fowler DP and Morris RW 1977 Genetic diversity in red pine: evidence for low genetic heterozygosity. *Canadian Journal of Forest Research.* **7**: 343-347

Gregorius HR and Ziehe M 1986 The significance of over- and underdominance for maintenance of genetic polymorphism II Overdominance and instability with random mating. *J Theor Biol* 118: 115-125

Hamrick JK and Godt MJW 1989 Allozyme diversity in plant species In: A H D Brown MT Clegg AL Kahler and BS Weir (Eds.) Plant population genetics breeding and genetic resources. Sinauer Associates Sunderland, Massachusetts. pp 43–63

Hamrick JL Godt MJW and Sherman-Broyles SL 1992 Factors influencing levels of genetic diversity in woody plant species. *New Forests* 6: 95-124

Hamrick JL Linhart YB and Mitton JB 1979 Relationships between life history characteristics and electrophoretically detected genetic variation in plants. *Ann. Rev. Ecol. Syst.* 10: 173 -200

Hamrick JL and Godt MJW 1996 Effects of life history traits on genetic diversity in plant species *Philos. Trans. R. Soc. Lond. B* 351:1291-1298

Holderegger R and Schneller JJ 1994 Are small isolated populations of *Asplenium septentrionale* variable? *Biol J Linnean Soc* 51:377-385

House APN and Bell JC 1994 Allozyme variation and mating system in *Eucalyptus urophylla* S T

Blake. *Silvae Genetica* 43(2-3): 167-176

Ibrahim AM 1996 Genetic variation in *Faidherbia albida*- implications for conservation of genetic resources and tree improvement. *Univ. Helsinki Tropic. Forest Rep.* 11: 86

Kang KS and Lindgren D 1998 Fertility variation and its effect on the relatedness of seeds in *Pinus densiflora Pinus thunbergii* and *Pinus koraiensis* clonal seed orchards. *Silvae Genetica* 47(4): 196-201

Karpa L Kresorich S Bhat KV Ayad WG and Hodgkin WG 1997 Molecular tools in plant genetic resource conservation: a guide to the technologies. IPGRI Tech. Bull. 2

Kumaravelu G 1979 Allozyme characterization of teak clones. *Indian Forester.* 95

Laemmli UK 1970 Electrophoresis. *Nature* 227: 680

Mallet J 1996 The genetics of biological diversity from variety to species. In: K J Gaston (Ed) *Biodiversity- A biology of numbers and differences* Blackwell Science, UK. pp 13-53

Manojkumar M Subramanian RS and Kumaravelu G 1998 Allozyme studies in clonal bank of sandal (*Santalum album* L) In: Advances in Forestry Research in India. Volume XVIII. 267p

Martins-Corder MP and Lopes CR 1997 Allozyme characterization of *Eucalyptus urophylla* (S T Blake) and *E grandis* (Hill ex Maiden) populations in Brazil. *Silvae Genetica* 46(4): 192-197.

Mejnartowicz L and Lewandowski A 1994 Allozyme polymorphism in seeds collected from IUFRO-68 Douglas-fir test-plantation. *Silvae Genetica* 43: 181-186

Moran GF 1992 Patterns of genetic diversity in Australian tree species. *New Forests* **6**: 49– 66

Muys B Achten W Mathijs E Singh V P and Verchot L 2007 Bio-diesel production from *Jatropha curcas* L : energy balance global warming potential and land use impact. Book of abstracts of the Third International Conference on Renewable Resources and Biorefineries, 4-6 June 2007, Ghent, Belgium. 55p

Nei M 1972 Genetic distance between populations. American Naturalist 106: 283-292

Nei M 1973 Analysis of gene diversity in subdivided populations. Proceedings of the National Academy of Sciences, USA. 70: 3321-3323

Nevo E 1983 In: Evolution from Molecules to Man D S Bendell (Ed) Cambridge University Press. 287-321

Philomina D 2000 Genetic analysis of one-parent families for variability diversity stability and propagation techniques in neem (*Azadirachta indica* A Juss). Ph.D thesis, TNAU, Coimbatore. 246 p

Scher S 1996 Genetic structure of natural *Taxus* populations in western North America. In: T B Smith and R K Wayne (Eds) Molecular Genetic Approaches in Conservation. Oxford University Press. New York. pp. 424-441

Singh MJ 2006 Biodiesel an Environmentally Friendly Alternate Fuel Experience of Indian Railways In: Brahma Singh R Swaminathan and V Ponraj (Eds) Biodiesel Conference Towards Energy Independence – Focus on Jatropha Paper presented at the conference held at Rashtrapati Nilayam Bolaram, Hyderabad on 9-10 June, 2006. pp 289-290

Warrier RR Priyadharshini P Senthil Vadivu S Devika Nagalakshmi D Savitha C Anandalakshmi R Nicodemus A and Gurudev Singh B 2010 Isozyme polymorphism to detect genetic diversity of *Jatropha curcas* (L) in India. *Annals of Tropical Research* 32 (1): 92-111

Wickneswari R and Norwati M 1993 Genetic diversity of natural populations of *Acacia auriculiformis. Aust. J. Bot.* 41: 65-77

Williams JGK Kubelik AR Livak KJ Rafalski JA and Tingey SV 1990 DNA polymorphisms amplified by arbitrary primers are useful as genetic markers *Nucleic Acids Res.* 18: 6531-6535

Wright J 1951 The genetic structure of populations *Ann Eugen* 15: 323-354

Yeh FC Yang RC Boyle TBJ Ye ZH and Mao JX 1997 POPGENE the user-friendly shareware for population genetic analysis. Molecular Biology and Biotechnology Centre, University of Alberta, Edmonton, Alberta, Canada.

Zabinski C 1992 Allozyme variation in eastern hemlock. *Can. J. For. Res.* 22:1838-1842