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Evaluation of genetic diversity among *Jatropha curcas* (L.) by RAPD analysis

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The genetic diversity and pedigree analysis of *Jatropha curcas* (L.) was evaluated through RAPD markers. A total number of 10 accessions, collected from different ecoclimatic zones of India, were screened with 43 random decamer primers to evaluate polymorphism. Selected 10 primers generated 125 bands, 76 of which were found to be polymorphic. The amplification products ranged from 9-14 bands for different primers. Each primer produced on an average 12.5 bands per primer, of which 7.6 were polymorphic. UPGMA method was used to construct dendrogram and genetic distance matrix was determined using squared Euclidean distances. Cluster analysis of data using UPGMA algorithm placed the ten accessions into two main clusters, the genetic dissimilarity matrix between genotypes ranged from 0-8 and the principal component analysis placed the 10 accessions into three groups.

Keywords: Cluster analysis, DNA fingerprinting, genetic diversity, *Jatropha curcas*, PCR, principal component analysis, RAPD

Introduction

Jatropha curcas (L.), a native of America and a member of family *Euphorbiaceae*, is a perennial, monoecious shrub or small tree and found throughout most of the tropics. It can grow well under any unfavourable agro-climatic conditions for its low moisture demand, low fertility requirement and tolerance to high temperature[1]. Economic importance of *J. curcas* has recently been increasing because of its seed oil that can be used as a fuel. Now-a-days, biofuel is being looked at an important alternative fuel in the overall energy security world over. *J. curcas* shows promise as an petro crop to be used for producing biodiesel[2]. The advantages in usage of biodiesel are its renewability, better quality exhaust gas emission and biodegradability. Moreover, it does not contribute to the rise in the level of carbon dioxide in the atmosphere[3]. *J. curcas* is an antifeedant agent[4] and herbal drug in dental complaints[5]. The milky sap of *J. curcas* is used in Mesoamerica for the treatment of different dermato-mucosal disease[6]. Extract from the plant is known for its medicinal properties and has shown effect on a wide array of organisms including insect, molluscs and nematodes[7]. It is a multipurpose tree to be fit for agro-forestry[8].

Conservation of biodiversity is one of the major concerns faced by mankind. Molecular markers are proved to be a valuable tool in the characterization and evaluation of genetic diversity within the species[9]. It has been shown that different markers reveal different classes of variation[10]. DNA-based marker fingerprinting can distinguish species rapidly using small amounts of DNA. Such markers are not typically influenced by environmental conditions and, therefore, can be used to describe patterns of genetic variation among plant populations, and to identify duplicate accessions within germplasm collections. Various approaches are available for DNA fingerprinting, such as, amplified fragment length polymorphism (AFLP)[11], restriction fragment length polymorphism (RFLP)[12], simple sequence repeats (SSRs)[13] and randomly amplified polymorphic DNA (RAPD)[14]. Among them, RAPD is an inexpensive and rapid method not requiring any information regarding the genome of the plant and has been widely used to ascertain the genetic diversity in several plants[15], RAPD analysis requires only a small amount of

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genomic DNA, but can produce high level of polymorphism and may facilitate more effective diversity analysis in plants. RAPD analysis provides information that can help define the distinctiveness of species and phylogenetic relationships at molecular level. Use of such techniques for germplasm characterization may facilitate the conservation and utilization of plant genetic resources, permitting the identification of unique or sources of genetically diverse genotypes. These molecular markers have been successfully used in *J. curcas* for detecting genetic diversity and relationship of inter and intra populations[16,17]. The present study aims at to assess the genetic relationship between different *J. curcas* accessions collected from various parts of India.

Materials and Methods

Plant Material and Reagents

Ten Accessions were collected from various ecoclimatic zones of India. Of which, IC-1, -2 and -3 were from Mahendragiri Hills, Orissa; IC-4, -5 and -6 from Araku Valley, Bisupuram, Visakhapatnam (Andhra Pradesh); and IC-7, -8, -9 and IC-10 from Biligiri Rangana Hills (B R Hills) of Karnataka (Table 1). The specimens were identified by authenticated taxonomic expert and voucher specimens were deposited in the Herbarium, Oil Technological Research Institute, Anantapur, and germplasms were also maintained in the experimental garden. Initially, a total of 43 random primers (OPERON Technologies, Inc., USA) were used to screen the template DNA. The chemicals used in the present work were *Taq* DNA polymerase (Fermentos, Pvt Ltd, India), dNTPs (Genetix, New Delhi, India); an extraction buffer consisting of 2% CTAB (w/v), 0.5 *M* Tris HCl (*p*H 8); 0.5 *M* EDTA (*p*H 8.0); 5.0 *M* NaCl, 1% PVP; 3 *M* sodium acetate solution (*p*H 5.2), ribonuclease-R (10 mg/mL), chloroform:isoamyl-alcohol (24:1), phenol:chloroform:isoamylalcohol (25:24:1 v/v/v), ethanol (70%, 100%), TE buffer (10 m*M* Tris HCl, 1 mM EDTA, *p*H 8.0).

Extraction and Purity of Genomic DNA

Genomic DNA was extracted from fresh leaves of each *J. curcas* accessions. The extraction was done with slight modifications of CTAB isolation protocol[18,19] for poly phenol, polysaccharide and tannin free DNA. The genomic DNA isolated was quantified spectrometrically by measuring absorbance at 260 nm and purity of DNA was checked. Stock DNA was diluted to make a working solution of 50 ng/µL for further PCR analysis. DNA concentration and purity was also determined by running the samples on 1.4% agarose gel and the nucleic acid concentration was calculated based on the intensities of band when compared with the λ DNA marker[20].

Primer Selection and RAPD Analysis

Ten *J. curcas* accessions were used for the evaluation. Of 43 primers used, only 10 best RAPD decamers, viz., OPB-07, OPB-10, OPC-02, OPE-20, OPG-14, OPK-03, OPN-07, OPR-14, OPR-16, OPC-18 were selected for the study (Table 2). As per the PCR protocol[28], the reactions were carried out in a DNA thermocycler (UVI Gene Technologies, Pvt Ltd, India). Each 20 µL reaction volume contained about 50 ng of template DNA, 1× PCR buffer (10 m*M* Tris HCl, *p*H 8.3; 50 m*M* KCl), 2.5 m*M* $MgCl_2$, 200µ*M* dNTP mix, 0.5 µ*M* of single primer, 0.2 U of *Taq* DNA polymerase. The reaction master mix for 20 µL PCR was prepared as 2.0 µL template DNA, 1.6 µL dNTPs, 2.0 µL primer, 2.0 µL assay buffer 10×, 0.4 µL *Taq* DNA polymerase, 12 µL sterile millipore water. The thermocycler was programmed for an initial denaturation of 5 min at 94°C, followed by 35 cycles of denaturation of 1 min at 94°C, 1 min of annealing at 36°C. Extension was carried out at 72°C

Table 1—List of *J. curcas* genotypes and details of collection sites

No.	Acc. no.	Collection site	State	Region	Latitude and longitude
1	IC-1	Mahendragiri Hills,	Orissa	India	18° 58′ N 84° 20′ 33 E
2	IC-2	Mahendragiri Hills,	Orissa	India	18° 58′ N 84° 21′ 20 E
3	IC-3	Mahendragiri Hills,	Orissa	India	18° 57′ N 84° 21′ 20 E
4	IC-4	ArakuValley,Visakhapatnam	Andra Pradesh	India	18° 20′ N 82° 51′ 00 E
5	IC-5	ArakuValley,Visakhapatnam	Andra Pradesh	India	18° 20′ N 82° 51′ 02 E
6	IC-6	ArakuValley,Visakhapatnam	Andra Pradesh	India	18° 20′ N 82° 50′ 58 E
7	IC-7	B R Hills	Karnataka	India	11° 54′ N 77° 08′ 59 E
8	IC-8	B R Hills	Karnataka	India	11° 54′ N 77° 09′ 16 E
9	IC-9	B R Hills	Karnataka	India	11° 54′ N 77° 08′ 17 E
10	IC-10	B R Hills	Karnataka	India	11° 54′ N 77° 08′ 21 E

Table 2—List of polymorphic RAPD primers and number of PCR amplified bands generated from *J. curcas* accessions

No.	Primer	Sequence (5′-3′)	Polymorphic bands	Monomorphic bands	Total bands	% of polymorphism	% of monomorphism
1	OPB-07	GGTGACGCAG	10	3	13	76.92	23.07
2	OPB-10	CTGCTGGGAC	6	8	14	42.85	57.14
3	OPC-02	GTGAGGCGTC	5	7	12	41.66	58.33
4	OPE-20	AACGGTGACC	10	4	14	71.42	28.57
5	OPG-14	GGATGAGACC	10	3	13	76.92	23.07
6	OPK-03	CCAGCTTAGG	12	1	13	92.30	7.69
7	OPN-07	CAGCCCAGAG	7	7	14	50.0	50.0
8	OPR-14	CAGGATTCCC	5	7	12	41.66	58.33
9	OPR-16	CTCTGCGCGT	5	6	11	45.45	54.54
10	OPC-18	TGAGTGGGTG	6	3	9	66.66	33.33
	Total		76	49	125	60.8	39.2

for 2 min and final extension at 72°C for 5 min, and a hold temperature of 4°C at the end. RAPD fragments were separated electrophoretically on 1.4% agarose gels in 1× TBE buffer, stained with ethidium bromide (0.5 µg/mL), and photographed on a UV transilluminator using gel documentation system.

Statistical Analysis of DNA Fingerprinting and Estimation of Genetic Diversity by using RAPD Marker

Each reproducible band was visually scored for the presence (1) or absence (0) for all the *J. curcas* accessions studied and the binary data for each primer were used for statistical analysis with the computer package "STATISTICA". A UPGMA dendrogram was constructed based on joining tree clustering. The dissimilarity matrix was computed using squared euclidian distance (SED) that estimated all pairwise differences in the amplification products. The genetic distance was computed as:

$\sum^{n}_{j=I} dj2$, where $dj = (X_{ik} - Xjk)$

Where X_{ik} refers to binary code of i^{th} tree for allele "k" and X_{jk} refers to the binary code of the j^{th} tree for allele "k". Only clear and unambiguous bands were taken into account and the bands were not scored if they were faint or diffused, as such fragments possess poor reproducibility. The band sizes were determined by comparing with the 100 bp DNA ladder, which was run along with the amplified products. Relationship among 10 *J. curcas* accessions was portrayed graphically in the form of dendrogram. Principal components analysis (PCA) technique helps in covering a set of variable into a few dimensions using which the genotypes/clones under study can be depicted in a two or three dimensional space[21].

Results and Discussion

Ten *J. curcas* accessions were analyzed using 43 random decamers, of which 10 primers were selected for having best stained, polymorphic reproducibility for further studies. The details of the nucleotide sequences of selected primers used to generate PCR products, summary of the total number of polymorphic and monomorphic DNA fragments generated, and percentage of polymorphism and monomorphism are presented in Table 2. A total of 125 marker levels were amplified across the genotypes, of which 76 (60.8%) were polymorphic and 49 (39.2%) monomorphic bands. A wide variation in the number of polymorphic bands ranging from 5-12 was observed. The highest 12 bands were observed for primers OPK-03 and 10 bands for primers OPB-07, OPE-20 and OPG-14. The average number of polymorphic bands per primer was 7.6 and the percentage of polymorphism ranges from 41.66 (OPR-14) to 92.30 (OPK-03) (Table 2). Thus, the present results indicate the variable potentiality of the primers in resolving the variation in genotypes studied (Fig. 1a & b).

A genetic distance matrix produced from the SED was used to quantify difference among genotypes. The genetic dissimilarity matrix between genotypes ranged from 0-8 (Table 3). The dendrogram of *J. curcas* accessions was constructed based on RAPD data (Fig. 2). All accessions initially fall under two major clusters (at 6 dissimilarity). Cluster I contains IC-5, IC-6, and IC-8C, while Cluster II is further divided in two major groups A and B (at 5.7 dissimilarity). The group A contains IC-3, IC-4, IC-7 and IC-10, while the group B contains IC-1, IC-2 and IC-9. The range of genetic distance among 10 accessions varied from 0 to 8 (Table 3). Such wide

range of dissimilarity values suggest that the germplasm collections are from genetically different eco-climatic geographical zones. The *J. curcas* germplasm collection represents a genetically diverse population and this might be attributed to a high level of cross-pollination in this species[22,23]. The high diversity revealed by RAPD is in agreement with the conclusion that outbreading plants species retain considerable variability[24]. This was further supported by molecular marker studies[25]. The maintenance of a high genetic variance within population was favoured by genetic systems of the species like gene flow, outbreeding, mutation, high genetic load, etc[26].

To visualize the genetic relatedness among the *J. curcas* genotypes in detail, PCA was made for 125 RAPD bands generated by 10 decamer primers. The results of analysis are presented three dimensionally in Fig. 3. It is evident from the data that *J. curcas*

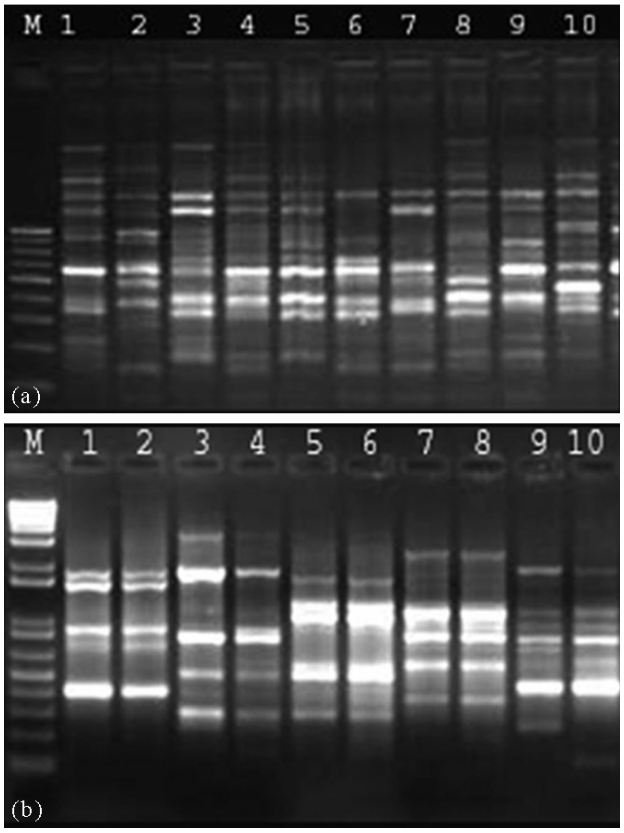

Fig. 1 (a & b)—RAPD polymorphism amongst 10 *Jatropha curcas* genotype detected with OPK-03 (a) & OPE-20 (b). (M: Mol marker, Lanes 1-10: Represents IC-1 to IC-10 *J. curcas* accessions)

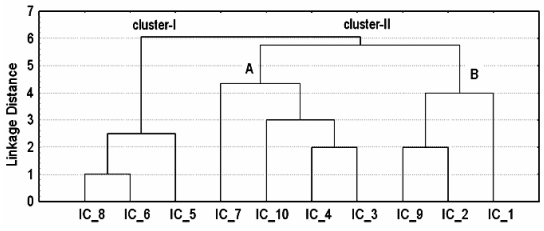

Fig. 2—The dendrogram of *J. curcas* accessions based on RAPD data

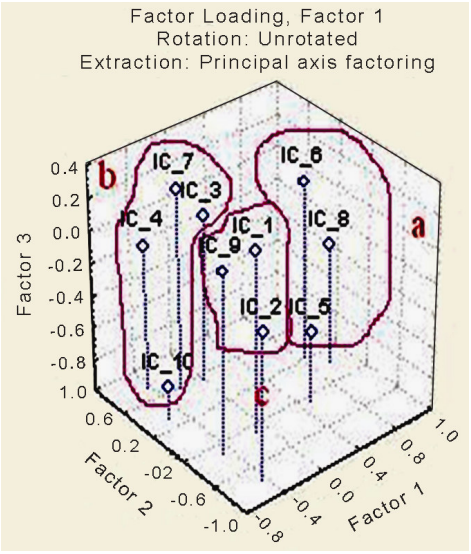

Fig. 3—Principal component analysis of *J. curcas* genotypes generated by RAPD markers

Table 3—Genetic distance matrix among *J. curcas* accessions developed by SED based on the RAPD data using UPGMA cluster analysis

	IC_1	IC_2	IC_3	IC_4	IC_5	IC_6	IC_7	IC_8	IC_9	IC_10
IC_1	0.00									
IC_2	3.00	0.00								
IC_3	6.00	7.00	0.00							
IC_4	6.00	7.00	2.00	0.00						
IC_5	5.00	4.00	7.00	7.00	0.00					
IC_6	6.00	7.00	4.00	6.00	3.00	0.00				
IC_7	5.00	6.00	5.00	3.00	6.00	5.00	0.00			
IC_8	5.00	6.00	5.00	7.00	2.00	1.00	6.00	0.00		
IC_9	5.00	2.00	5.00	5.00	6.00	7.00	6.00	8.00	0.00	
IC_10	6.00	5.00	4.00	2.00	5.00	8.00	5.00	7.00	5.00	0.00

were more dispersed on the PCA plot, which reflects a wider genetic base. It shows a clear-cut separation of ten genotypes into three groups, each group being distinct from other group. Group a contains IC-5, IC-6, and IC-8; while group b contains IC-3, IC-4, IC-7 and IC-10, and IC-1, IC-2 and IC-09 are the part of group c. In general, the results obtained from PCA are in agreement with the dendrogram generated by UPGMA method, which is a further confirmation of the genetic relationships delineated by cluster analysis[27]. Ecological and geographical differentiations are two important factors, which influences breeding and sampling strategies of tree crops[28]. Variation in genetic diversity within the species is usually related with geographic range, mode of reproduction, mating system, seed dispersal and fecundity[29]. The genetic diversity detected in the present study may be due to all these prevalent background factors as the genotypes of *J. curcas* studied are widely distributed in different eco-geographical regions. Similar conclusions were made by Gupta *et al*[29] while accessing genetic variation in 14 accessions of *J. curcas* from different agro climatic regions of India using RAPD markers. They reported wide genetic base in *Jatropha.* However, in other study[17], modest level of genetic variability was reported in *J. curcas* germplasm from India. The result of the present study shows that *J. curcas* germplasm within India constitutes a broad genetic base. From the clustering pattern and genetic relationship obtained using RAPD markers, breeders can identify the diverse genotype from different clusters and employ them in their further breeding programmes.

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References

1 Koushik N, Kumar K, Kumar S, Koushik N & Roy S, Genetic variability and divergence studies in seed traits and oil content of Jatropha (*Jatropha curcas* L.) accessions, *Biomass Bioenergy*, 31 (2007) 497-502.

2 Foidl N & Elder P, Agro-industrial exploitation of *Jatropha curcas*, in *Biofuel and industrial products from Jatropha curcas,* edited by G M Gubitz, M Mittelbach & M Trabi (Graz University of Technology, Austria) 1997, 139-148.

3 Korbitz W, Biodiesel production in Europe and America: An encouraging prospect, *Renew Energy*, 16 (1999) 1078-1083.

4 Adebowale K O & Adedire C O, Chemical composition and insecticidal properties of underutilized *Jatropha curcas* seed oil, *Afr J Bot*, 5 (2006) 901-906.

5 Girach R D, Ahmad A & Baghrendah M, *Jatropha curcas* L.: A potential source of herbal drug in dental complaints, *Hamdard Medicus*, 5 (1995) 94-100.

6 Marroquin E A, Blanco J A, Granados S, Caceres A & Morales C, Clinical trials of *Jatropha curcas* sapin treatment of common worts, *Fitoterapia*, 68 (1997) 160-162.

7 Jain C & Trivedi P C, Nimaticidal activity of certain plants against root-knot nematode, *Meloidogyne incognita* infecting chick pea, *Ann Plant Prot Sci*, 5 (1997) 171-174.

8 Wood P J & Burely J, *A tree for all reasons: The introduction and evaluation of multipurpose trees for agro forestry*, (ICRAF, Nairobi, Kenya) 1991, 58p.

9 Zoghlami N, Chrita I, Bouamama B, Gargouri M & Zemni H, Molecular based assessment of genetic diversity within Barbary fig (*Opuntia indica*) in Tunisia, *Sci Hortic*, 113 (2007) 134-141.

10 Powell W, Morgante M, Andre C, Hanafey M, Vogel J *et al.*, The comparison of RFLP, RAPD, AFLP and SSR (Microsatellite) markers for germplasm analysis, *Mol Breed*, 2 (1996) 225-238.

11 Zabeau S M & Vos P, Selective restriction fragment amplification: A general method for DNA fingerprinting. *Eur Pat No. 92402629* (1993) 0534858A1.

12 Botstein D, White R L, Skolnick M & Davis R W, Construction of a genetic linkage map in man using restriction fragment length polymorphism, *Am J Hum Genet*, 32 (1980) 314-331.

13 Tautz D, Hyper variability of simple sequences as a general source of polymorphic DNA markers, *Nucleic Acids Res*, 17 (1989) 6463-6471.

14 Williams J G K, Kubelik K J, Livak K J, Rafalski J A & Tingey S V, DNA polymorphisms amplified by arbitrary primers is useful genetic markers, *Nucleic Acids Res*, 18 (1990) 6531-6535.

15 Deshwall R P S, Singh R, Malik K & Randhawa G J, Assessment of genetic diversity and genetic relationships among 29 populations of *Azadirachta indica* using RAPD markers, *Genet Resour Crop Evol*, 52 (2005) 285-292.

16 Ganesh Ram S, Parthiban K T, Senthil Kumar R, Thiruvengadam V & Paramathma M, Genetic diversity among *Jatropha* species as revealed by RAPD markers, *Genet Resour Crop Evol*, 55 (2008) 803-809.

17 Basha S D & Sujatha E M, Inter- and intra-population variability of *Jatropha curcas* (L.) characterized by RAPD and ISSR markers and development of population specific SCAR markers, *Euphytica*, 156 (2007) 375-386.

18 Doyle J J & Doyle J L, A rapid DNA isolation procedure for small quantities of fresh leaf tissue, *Phytochemistry*, 19 (1987) 11-15.

19 Subramanyam K, Muralidhara Rao D & Devanna N, Novel molecular approach for optimization of DNA isolation and PCR protocol for RAPD analysis and genetic diversity assessment of *Jatropha curcas* (Euphorbiaceae), *Curr Biotica*, 3 (2009) 1-13.

20 Sambrook J, Fritsch E F & Maniatis T, *Molecular cloning—A laboratory manual,* 2nd edn (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, USA) 1989.

21 Ludwig A J & Reynolds F J, A primer on methods and computing, in *Statistical ecology* (John Wiley and Sons, Inc., New York, USA) 1988.

22 Solomon Raju A J & Ezradanam V, Pollination ecology and fruiting behaviour in a monoecious species, *Jatropha curcas* L. (*Euphorbiaceae), Curr Sci,* 83 (2002) 1395-1398.

23 Dhillon R S, Hooda M S, Handa A K, Ahlawat K S, Kumar Y *et al,* Clonal propagation and reproductive biology in *Jatropha curcas, Indian J Agrofor*, 8 (2006) 18-27.

24 Hamrick J L, Isozymes and analysis of genetic structure in plant populations, in *Isozymes in plant biology* , edited by E D Soltis & P S Soltis (Champan and Hall, London) 1990, 87-105

25 Hamrick J L & Godt M J W, Allozyme diversity in plant species, in *Plant population genetics, breeding and genetic resources* , edited by A H D Brown, M T Clegg, A L Kahler & B S Weir (Sinauer Associates, Sunderland, MA, USA) 1989, 43-63.

26 Matyas C, Climatic adaptation of trees: Rediscovering provenance tests, *Euphytica*, 92 (1996) 45-54.

27 Shashidhara G, *Genetic evaluation of elite sandle wood (Santalum album* L.*) clones using RAPD markers.* Ph D Thesis, University of Agricultural Sciences, Bangalore, India, 2002.

28 Namkoong G, Genetics and the forests of the future, *Unasylva*, 152 (1986) 2-18.

29 Gupta S, Srivastava M, Mishra G P, Naik P K & Chauhan R S, Analogy of ISSR and RAPD markers for comparative analysis of genetic diversity among different *Jatropha curcas* genotypes. *Afr J Biotechnol*, 7 (2008) 4230-4243.