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Full Length Research Paper

Genetic diversity assessment of wild and cultivated varieties of *Jatropha curcas* (L.) in India by RAPD analysis

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The present study deals with evaluation of genetic diversity and pedigree analysis through RAPD analysis. A total number of 40 *Jatropha curcas* accessions collected from different geographical regions and 43 random decamer primers were screened to assess polymorphism. 10 primers were amplified and 94 polymorphic bands were found out of 125 scored. Accounting for 75.2 % polymorphism across the genotypes 12.5 bands per primer, out of 9.4 were polymorphic. Jaccard's coefficient of similarity varied from 0.00 to 1.00 indicative of high levels of genetic variation among the genotypes studied. Cluster analysis of data using UPGMA algorithm placed the 40 accessions into 2 main clusters, with cluster II divided into six sub-clusters. The result provides valid guidelines for the collection, conservation and characterization of *Jatropha curcas* genetic resources.

Key words: *Jatropha curcas*, DNA fingerprinting PCR, RAPD, genetic diversity, cluster analysis, PCA.

INTRODUCTION

Jatropha curcas (L.) species is a perennial, monoecious shrub, native of America and widely distributed in the tropics. *J. curcas*, a member of the Euphorbiaceae family can grow well under any unfavorable agro climatic conditions, because of its low moisture demands, fertility requirements and tolerance to high temperatures (Diwaker et al., 1993; Tiwari et al., 1994). It is found throughout most of the tropics and known by nearly 200 names, which indicate its significance (Koushik et al., 2007). In the recent past, the economic importance of *J. curcas* has been increasing because of the use of its oil as a fuel; biofuel is being looked at as an important alternative fuel in overall energy security worldwide. It shows promise for use as an oil crop for bio-diesel (Henning, 1998). The main advantages of using bio-diesel are its renewability, better quality exhaust gas emission and biodegradability. It does not contribute to a rise in the level of carbon dioxide in the atmosphere (Beet et al., 2002). *J. curcas* is an antifeedant agent (Meshram et al., 1994; Adebowale and Adedire, 2006), a little known herbal drug in dental complaints (Girach et al., 1995) and its milky sap is used in Mesoamerica for the treatment of different dermato-mucosal diseases (Maroquin et al., 1997). Extracts from the plant are also known for their medicinal properties and their effects on a wide array of organisms including insect pests, molluscuns and nematodes (Jain et al., 1997). It is a multipurpose tree species fit for agro-forestry and other afforestation programmes (Wood et al., 1991).

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Due to the multiple uses and the ability of this tree to thrive in arid and semi arid environments, it has become increasingly important to describe and characterize these valuable resources. Little work has been done so far on the germplasm collection and evaluation of genetic diversity in order to preserve this species. In fact the conservation of biodiversity is one major issue facing man-kind. This is a challenging goal since up to now knowledge regarding the amount of genetic variation and genetic relationship by using molecular markers was very little in *J. curcas* (Sudheer pamidiamarri et al., 2006). At present, molecular markers have been proved to be valuable tools in the characterization and evaluation of genetic diversity within the species (Nejia Zoglami et al., 2006). It has been shown that different markers reveal different classes of variation (Powell et al., 1996; Russel et al., 1997). These molecular markers had been successfully used in *J. curcas* for detecting genetic diversity and relationship (Ganesh ram et al., 2008; Basha et al., 2007). Of these techniques, RAPD has several advantages, such as simplicity of use, low cost and the use of small amount of plant material (Nejia Zoglami et al., 2006). The aim of this study was to produce a suitable marker for the investigation of DNA polymorphism in *J. curcas*, useful in the analysis of genetic diversity and ecotype identification and establish a genetic relationship between accessions obtained from various parts of India.

DNA marker based fingerprinting can distinguish species rapidly using small amounts of DNA and therefore can assist to deduce reliable information on their phylogenetic relationships. DNA markers are not typically influenced by environmental conditions and therefore can be used to help describe patterns of genetic variation among plant populations and to identify duplicated accessions within germplasm collections. Various approaches are available for DNA fingerprinting such as amplified fragment length polymorphism (AFLP) (Zabeau and Vos, 1993), restriction fragment length polymorphism (RFLP) (Botstein et al., 1980), simple sequence repeats (SSRs) (Tautz, 1989) and randomly amplified polymorphic DNAs (RAPD) (Williams et al., 1990). Among these, RAPD is an inexpensive and rapid method not requiring any information regarding the genome of the plant and has been widely used to ascertain genetic diversity in several plants (Belaj et al., 2001; Deshwall et al., 2005). RAPD analysis requires only a small amount of genomic DNA and can produce high levels of polymorphism and may facilitate more effective diversity analysis in plants (Williams et al., 1990). 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 genotypes or sources of genetically diverse genotypes. RAPD analysis has been used for genetic diversity assessment and for identifying germplasm in a number of plant species (Kapteyn and Simon, 2002; Welsh and McClelland, 1990). The present study was undertaken to assess the genetic relationships between different *Jatropha* species using RAPD markers.

MATERIALS AND METHODS

Plant material and reagents

Extensive field trips were carried out to collect *J. curcas* seeds from different geographical locations in india. The pooled accessions were germinated and germplasm maintained in JNTU-OTRI.Anantapur. Out of 90 germplasms collection of *J. curcas,* 40 random accessions were taken for analysis (Table 1). Random primers (10-mers) were obtained from OPERON Technologies, Inc., USA (Table 1), *Taq* DNA polymerase, (Fermentos, Pvt Ltd, and INDIA), dNTPs (Genetix, New Delhi, India) and PCR (UVI Gene technologies Pvt Ltd INDIA). An extraction buffer consisting of 2% CTAB (w/v), Tris HCl pH 8.0 (0.5 M); EDTA pH 8.0 (0.5 M); NaCl (5.0 M), PVP. 3 M sodium acetate solution (pH5.2), ribonuclease R (10 mg/ml), chloroform :Isoamylalcohol (24:1), phenol:chloroform:isoamylalcohol (25:24:1v/v/v), ethanol (70%, 100%) and TE buffer (Tris HCl, 10mM, EDTA, 1 mM, pH 8.0).

Extraction and purity of genomic DNA

Genomic DNA was extracted from freshly harvested leaves of each *J. curcas* accessions. DNA extraction was improved with slight modifications of CTAB DNA isolation protocol (Doyle and Doyle, 1987; Sul et al., 1996; Aljanabi et al., 1999*)* for polyphenol, polysaccharide, tannin free DNA. The method involves CTAB extraction employing PVP (0.01 g per 300 mg of leaves) while grinding, successive long term chloroform: isoamylalcohol (24:1), phenol, chloroform, Isoamylalcohol (25:24:1, v/v/v) extractions and an overnight 5 µl of RNase treatment. The pellet was air dried and resuspended in TE buffer. All the centrifugation steps were carried out at room temperature to avoid precipitation with CTAB and DNA degradation and to obtain good quality DNA. The genomic DNA isolated was quantified spectrometrically by measuring absorbance at 260 nm (BL198 ELICO, India). The purity of DNA was determined by calculating the ratio of absorbance at 260 nm to that of 280 nm. Stock DNA was diluted to make a working solution of 50 ng/µl for PCR analysis. DNA concentration was also determined by running the samples on a 1.4% agarose gel based on the intensities of bands by comparison with the Lambda DNA marker (Sambrook et al., 1989).

Primer selection and RAPD analysis

The 10 RAPD decamers; OPB-07, OPB-10, OPC-02, OPE-20, OPG-14, OPK-03, OPN-07, OPR-14, OPR-16 and OPC-18 (Table 2) were selected from among 43 RAPD primers based on their ability to detect distinct polymorphic amplified products across the

Table 1. *J. curcas* collection from different parts of the country.

Accession number	Collection site	State	Region
OTRI\JC\01	Nalla malai	Andrapradesh	India
OTRI\JC\08	Thirumala hills	Andrapradesh	India
OTRI\JC\11	Mahendra giri hills	Orissa	India
OTRI\JC\12	Araku valley	Andrapradesh	India
OTRI\JC\13	Band	Orissa	India
OTRI\JC\15	Sambal pur	Orissa	India
OTRI\JC\18	Dhekamal	Orissa	India
OTRI\JC\20	Madhurai	Tamilnadu	India
OTRI\JC\25	Coimbatthoor	Tamilnadu	India
OTRI\JC\29	Pandicheri	Tamilnadu	India
OTRI\JC\32	Pudikuttai	Tamilnadu	India
OTRI\JC\38	GKVK, Bangalore	Karnataka	India
OTRI\JC\39	UAS, Dharward	Karnataka	India
OTRI\JC\41	Ranchi	Jarkhand	India
OTRI\JC\44	Patna	Bhihar	India
OTRI\JC\48	Odakalli	Kerala	India
OTRI\JC\49	Trichur	Kerala	India
OTRI\JC\51	Bhupal	Madhya Pradesh	India
OTRI\JC\55	Jabalpur	Madhya Pradesh	India
OTRI\JC\60	Indore	Madhya Pradesh	India
OTRI\JC\62	Dehradun	Uttaranchal	India
OTRI\JC\64	NBRI, Lucknow	Uttar Pradesh	India
OTRI\JC\66	Agra	Uttar Pradesh	India
OTRI\JC\67	Bithur	Uttar Pradesh	India
OTRI\JC\69	Karnal	Haryana	India
OTRI\JC\71	Firojpur, gurgaon	Haryana	India
OTRI\JC\72	Panipat	Panjab	India
OTRI\JC\78	Luthiana	Panjab	India
OTRI\JC\79	Mohali	Panjab	India
OTRI\JC\84	Palampur	Panjab	India
OTRI\JC\87	Jorhat	Assam	India
OTRI\JC\89	Raghunath pur	West Bengal	India
OTRI\JC\92	Mohan pur	West Bengal	India
OTRI\JC\96	IARI-New Delhi	New delhi	India
OTRI\JC\101	Ahmedabad	Gujarath	India
OTRI\JC\108	Anand	Gujarath	India
OTRI\JC\120	Shilling	Meghalaya	India
OTRI\JC\123	Sephajala wild life santuri	Tripura	India
OTRI\JC\127	Raipur	Chatthis garh	India
OTRI\JC\129	Akola	Maharastra	India

accessions. 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, 1X PCR buffer (10 mM Tris Hcl pH 8.3; 50 mM KCl), 2.5 mM $MgCl_2$ (Bangalore Genei Pvt. Ltd., Bangalore) 200µM dNTP Mix (Genetix, New Delhi, India), 0.5 µM of single primer (OPERON Technologies, Inc., USA) and 0.2 U of *Taq* DNA polymerase (Fermentos, Pvt Ltd, India). 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 ml assay buffer 10 X, 0.4 µl taq DNA polymerase, and12 µl sterile millipore water. The thermocycler was

programmed for an initial denaturation step 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 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 1X TBE buffer, stained with ethidium bromide (0.5 µg/ml) and photographed on a UV transilluminator using a digital camera. DNA from each plant was amplified with the same primer more than once and the banding patterns were compared to Lambda. DNA *EcoR*1- *Hin*d111 double digest was used as molecular marker (Bangalore Genei, Bangalore, India) to know the size of the fragments.

Statistical analysis

RAPD markers across 40 accessions were visually scored for the presence (1) or absence (0) for each primer. By comparing the banding patterns of genotypes for a specific primer, genotype-specific bands were identified and faint or unclear bands were not considered. The binary data so generated were used to estimate levels of polymorphism by dividing the polymorphic bands by the total number of scored bands. Pair-wise similarity matrices were generated by Jaccard's coefficient of similarity (Jaccard, 1908) and a dendrogram was constructed by using the unweighted pair group method with arithmetic average (UPGMA) with the MVSP 3.0 (multi variate statistical package (Kovach Computing Services, 1987-1998) to show a phenetic representation of genetic relationships as revealed by the similarity coefficient. The binary data was also subjected to principal component analysis (PCA) using the "statistica" package. Principal components analysis is described as combining two correlated variables into one factor. However, basically, the extraction of principal components amount to variance maximizing (varimax) rotation of the original variable space. Principal components analysis is a data reduction method used for reducing the number of variables. The variances extracted by the factors are called the risen values. This name derives from the computational issues involved.

RESULTS

RAPD Polymorphism

J. curcas species were analyzed using 42 random decamers used to amplify DNA from 40 accessions. 10 primers were found to produce reproducible bands and thus selected for further analysis of the 40 accessions. The details of the names and nucleotide sequences of primers used to generate 125 PCR products and summary of the total number of polymorphic and monomorphic DNA fragments and percentage of polymorphism and monomorphism are shown in Table 3.

A total of 125 bands were scored of which 94 (75.2%) were polymorphic and 31 (25.347%) were monomorphic across the genotypes. On an average, total number of bands per primer was 12.5 bands of which 9.4 were polymorphic. A wide variation in the number of bands ranging from 6 -12; 12 bands for primers OPE-20, OPK-03, 11 bands for primer OPB-07 and 10 bands for primers OPG-14, OPN-07 and OPR-14 were observed which indicate the potentiality of various primers in resolving variations in genotypes studied (see Figures 1 and 2).

Table 2. RAPD primers (decamer) used for analysis.

Primer code	Nucleotide sequence (5^1-3^1)
OPB-07	GGTGACGCAG
OPB-10	CTGCTGGGAC
OPC-02	GTGAGGCGTC
OPE-20	AACGGTGACC
OPG-14	GGATGAGACC
OPK-03	CCAGCTTAGG
OPN-07	CAGCCCAGAG
OPR-14	CAGGATTCCC
OPR-16	CTCTGCGCGT
OPC-18	TGAGTGGGTG

Genetic diversity

Jaccard's genetic similarity co-efficient varied from 0.00 to 1.00 (Table 4). The highest genetic similarity of 1.00 was between JC 78, JC 32 and JC 0.80 and between JC 120 and JC 44 while the lowest of 0.10 was between JC 79, JC 55, JC 79 and JC 62. UPGMA cluster analysis of the Jaccard's similarity coefficient generated a dendrogram (Figure 3) which illustrated the overall genetic relationship among the accessions studied. UPGMA dendrogram showed two main clusters split at Jaccard's similarity co-efficient of 0.10. Cluster I include JC 18. Cluster two indicated the six sub clusters (Table 5). Association among the 40 accessions was also resolved by PCA (Figure 4); 36 out of 40 separated into two groups while the remaining 4 are fall-outs of the graph. The overall grouping pattern of PCA corresponded well with the clustering pattern of the dendrogram (Figure 5). In agreement with the dendrogram, JC 18, JC 01 and JC 62 did not group with any other accessions in the PCA; also confirming their genetic distinctness from all other *J. curcas* accessions.

DISCUSSION

Based on RAPD polymorphism (Table 3), the primers namely; OPE- 20 and OPK-03 showed maximum poly-

Table 3. List of RAPD primers and number of PCR products amplified bands generated from 40 *J. curcas* accessions.

Primer	Polymorphic bands	Monomorphic bands	Total bands	% polymorphism	% monomorphism
OPB-07	11	*2*	13	84.61	15.38
OPB-10	9	5	14	64.28	35.71
OPC-02	8	4	12	66.66	33.33
OPE-20	12	2	14	85.57	14.28
OPG-14	10	3	13	76.92	23.07
OPK-03	12	1	13	92.30	7.69
OPN-07	10	4	14	71.42	28.57
OPR-14	10	2	12	83.33	16.66
OPR-16	6	5	11	54.54	45.45
OPC-18	6	3	9	66.66	33.33
Total	94	31	125	74.629	25.347

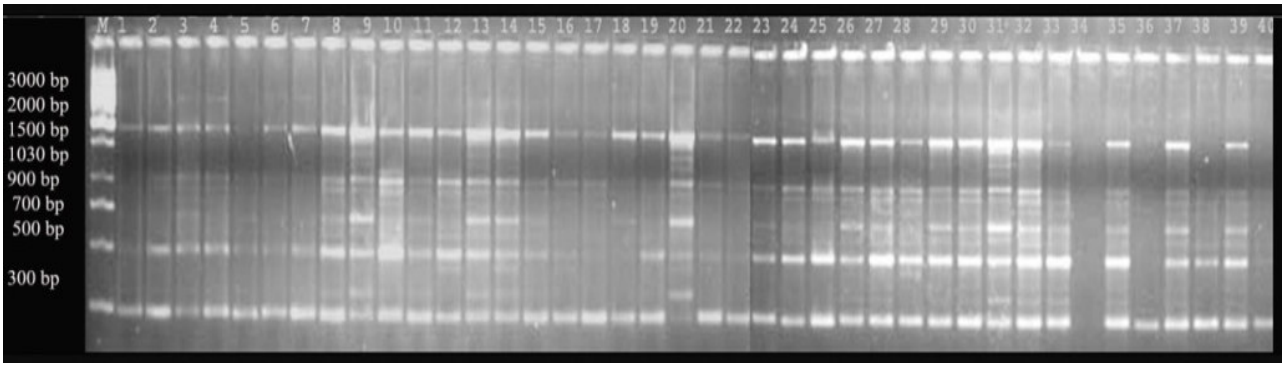

Figure 1. RAPD polymorphism for *J. curcas* genotypes detected with OPB-07.

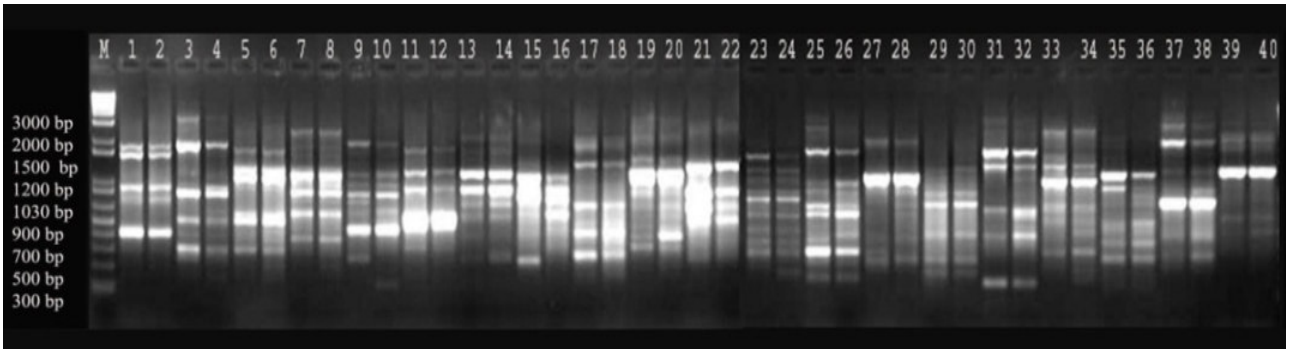

Figure 2. RAPD polymorphism for *J. curcas* genotypes detected with OPE-20.

morphic bands of 12, OPB-07 showed 11 while OPG- 14, OPN- 07 and OPR-14 showed 10 bands. The average number of polymorphic bands per primer is 9.4 and the percentage of polymorphism ranges from 54.54 (OPR-16) to 92.30% (OPK-03) (Table 3). Recently Basha and Sujatha (2007) had reported low levels of molecular diversity among Indian accessions of *J. curcas* germplasm indicating a narrow genetic base. Ganesh ram et

Table 4. Jaccards similarity coefficient of 40 *Jatropha Curcas accessions.*

	JC 01	JC 08	JC 11	JC 12	JC 13	JC 15	JC 18	JC 20	JC 25	JC 29	JC 32	JC 38	JC 39	JC 41	JC 44	JC 48	JC 49	JC 51	JC 55	JC 60	JC 62	JC 64	JC 66	JC 67	JC 69	JC 71	JC 72	JC 78	JC 79	JC 84	JC 87	JC 89	JC 92	JC 96	JC 101	JC 108	JC 120	JC 123	JC 127	JC 129
JC 01	1.00																																							
JC 08	0.40	1.00																																						
JC 11	0.17	0.33	1.00																																					
JC 12	0.17	0.33	1.00	1.00																																				
JC 13	0.17	0.14	0.14	0.14	1.00																																			
JC 15	0.20	0.00	0.17	0.17	0.75	1.00																																		
JC 18	0.00	0.00	0.00	0.00	0.17	0.20	1.00																																	
JC 20	0.40	0.33	0.60	0.60	0.33	0.40	0.00	1.00																																
JC 25	0.00	0.40	0.40	0.40	0.17	0.00	0.00	0.17	1.00																															
JC 29	0.00	0.25	0.43	0.43	0.25	0.13	0.29	0.25	0.50	1.00																														
JC 32	0.00	0.14	0.60	0.60	0.14	0.17	0.17	0.33	0.40	0.67	1.00																													
JC 38	0.00	0.33	0.60	0.60	0.33	0.17	0.00	0.33	0.75	0.67	0.60	1.00																												
JC 39	0.00	0.20	0.50	0.50	0.20	0.25	0.00	0.50	0.25	0.33	0.50	0.50	1.00																											
JC 41	0.25	0.38	0.38	0.38	0.38	0.25	0.25	0.57	0.25	0.63	0.38	0.38	0.29	1.00																										
JC 44	0.14	0.29	0.29	0.29	0.50	0.33	0.14	0.50	0.33	0.57	0.29	0.50	0.40	0.71	1.00																									
JC 48	0.14	0.50	0.50	0.50	0.29	0.14	0.14	0.50	0.33	0.57	0.29	0.50	0.40	0.71	0.67	1.00																								
JC 49	0.22	0.50	0.50	0.50	0.33	0.22	0.22	0.33	0.38	0.56	0.50	0.50	0.25	0.50	0.30	0.44	1.00																							
JC 51	0.29	0.43	0.43	0.43	0.43	0.29	0.13	0.25	0.29	0.33	0.25	0.43	0.14	0.30	0.22	0.38	0.75	1.00																						
JC 55	0.33	0.29	0.13	0.50	0.33	0.14	0.29	0.14	0.38	0.29	0.29	0.17	0.50	0.43	0.25	0.44	0.38	1.00																						
JC 60	0.40	0.60	0.60	0.60	0.14	0.17	0.00	0.60	0.17	0.25	0.33	0.33	0.50	0.38	0.29	0.50	0.50	0.43	0.29	1.00																				
JC 62	0.60	0.29	0.29	0.29	0.29	0.33	0.14	0.50	0.00	0.22	0.29	0.13	0.17	0.50	0.25	0.25	0.44	0.38	0.67	0.50	1.00																			
JC 64	0.25	0.20	0.50	0.50	0.20	0.25	0.00	0.50	0.00	0.14	0.20	0.20	0.33	0.29	0.17	0.40	0.25	0.33	0.17	0.50	0.40	1.00																		
JC 66	0.29	0.43	0.43	0.43	0.25	0.13	0.13	0.25	0.29	0.50	0.43	0.43	0.14	0.44	0.22	0.38	0.75	0.71	0.57	0.43	0.57	0.33	1.00																	

Table 4. Contd.

JC 67	0. 14	0. 50	0. 29	0. 29	0. 13	0. 00	0. 14	0. 13	0. 60	0. 57	0. 50	0. 50	0. 17	0. 33	0. 25	0. 25	0. 63	0. 38	0. 43	0. 29	0. 25	0. 00	0. 57	1. 00														
JC 69	0. 00	0. 50	0. 20	0. 20	0. 20	0. 00	0. 00	0. 20	0. 67	0. 33	0. 20	0. 50	0. 33	0. 29	0. 40	0. 40	0. 25	0. 14	0. 17	0. 20	0. 00	0. 00	0. 14	0. 40	1. 00														
JC 71	0. 40	0. 33	0. 33	0. 33	0. 14	0. 17	0. 17	0. 60	0. 17	0. 25	0. 33	0. 14	0. 20	0. 57	0. 29	0. 29	0. 33	0. 11	0. 29	0. 33	0. 50	0. 20	0. 25	0. 29	0. 20	1. 00													
JC 72	0. 17	0. 60	0. 60	0. 60	0. 14	0. 00	0. 00	0. 33	0. 75	0. 43	0. 33	0. 60	0. 20	0. 38	0. 29	0. 50	0. 50	0. 43	0. 13	0. 33	0. 13	0. 20	0. 43	0. 50	0. 50	0. 33	1. 00												
JC 78	0. 00	0. 14	0. 60	0. 60	0. 14	0. 17	0. 17	0. 33	0. 40	0. 67	1. 00	0. 60	0. 50	0. 38	0. 29	0. 29	0. 50	0. 25	0. 29	0. 33	0. 29	0. 20	0. 43	0. 50	0. 20	0. 33	0. 33	1. 00										
JC 79	0. 13	0. 43	0. 43	0. 43	0. 25	0. 13	0. 29	0. 25	0. 50	0. 50	0. 25	0. 43	0. 14	0. 44	0. 38	0. 57	0. 56	0. 50	0. 10	0. 25	0. 10	0. 14	0. 33	0. 38	0. 33	0. 25	0. 67	0. 25	1. 00									
JC 84	0. 00	0. 14	0. 33	0. 33	0. 14	0. 17	0. 40	0. 14	0. 40	0. 43	0. 60	0. 33	0. 20	0. 22	0. 13	0. 13	0. 50	0. 25	0. 13	0. 14	0. 13	0. 00	0. 25	0. 50	0. 20	0. 33	0. 33	0. 60	0. 43	1. 00								
JC 87	0. 17	0. 60	0. 33	0. 33	0. 33	0. 17	0. 00	0. 33	0. 40	0. 43	0. 33	0. 60	0. 50	0. 38	0. 50	0. 50	0. 50	0. 43	0. 50	0. 60	0. 29	0. 20	0. 43	0. 50	0. 50	0. 14	0. 33	0. 33	0. 25	0. 14	1. 00							
JC 89	0. 50	0. 43	0. 43	0. 43	0. 25	0. 29	0. 13	0. 67	0. 13	0. 33	0. 25	0. 25	0. 33	0. 63	0. 57	0. 57	0. 40	0. 33	0. 38	0. 67	0. 57	0. 33	0. 33	0. 22	0. 14	0. 43	0. 25	0. 25	0. 33	0. 11	0. 43	1. 00						
JC 92	0. 50	0. 40	0. 17	0. 17	0. 17	0. 20	0. 00	0. 40	0. 20	0. 13	0. 17	0. 17	0. 25	0. 25	0. 33	0. 14	0. 22	0. 13	0. 33	0. 40	0. 33	0. 00	0. 13	0. 33	0. 25	0. 40	0. 17	0. 17	0. 13	0. 17	0. 40	0. 50	1. 00					
JC 96	0. 40	0. 60	0. 14	0. 14	0. 14	0. 00	0. 17	0. 14	0. 17	0. 25	0. 14	0. 14	0. 00	0. 38	0. 13	0. 29	0. 50	0. 43	0. 50	0. 33	0. 50	0. 20	0. 67	0. 50	0. 20	0. 33	0. 33	0. 14	0. 25	0. 14	0. 33	0. 25	0. 17	1. 00				
JC1 01	0. 14	0. 50	0. 50	0. 50	0. 29	0. 14	0. 14	0. 50	0. 33	0. 57	0. 50	0. 50	0. 40	0. 71	0. 43	0. 67	0. 63	0. 38	0. 43	0. 50	0. 43	0. 40	0. 57	0. 43	0. 40	0. 50	0. 50	0. 50	0. 38	0. 29	0. 50	0. 38	0. 14	0. 50	1. 00			
JC1 08	0. 33	0. 29	0. 29	0. 29	0. 50	0. 33	0. 14	0. 50	0. 14	0. 38	0. 29	0. 29	0. 17	0. 71	0. 43	0. 43	0. 44	0. 38	0. 67	0. 29	0. 67	0. 40	0. 57	0. 25	0. 17	0. 50	0. 29	0. 29	0. 22	0. 13	0. 29	0. 38	0. 14	0. 50	0. 67	1. 00		
JC1 20	0. 00	0. 33	0. 33	0. 33	0. 33	0. 17	0. 17	0. 33	0. 40	0. 67	0. 33	0. 60	0. 50	0. 57	0. 80	0. 80	0. 33	0. 25	0. 29	0. 33	0. 13	0. 20	0. 25	0. 29	0. 50	0. 14	0. 33	0. 33	0. 43	0. 14	0. 60	0. 43	0. 17	0. 14	0. 50	0. 29	1. 00	
JC1 23	0. 20	0. 40	0. 17	0. 17	0. 40	0. 20	0. 00	0. 40	0. 50	0. 29	0. 17	0. 40	0. 25	0. 43	0. 60	0. 33	0. 22	0. 13	0. 33	0. 17	0. 14	0. 00	0. 13	0. 33	0. 67	0. 40	0. 40	0. 17	0. 29	0. 17	0. 40	0. 29	0. 50	0. 17	0. 33	0. 33	0. 40	1. 00
JC1 27	0. 33	0. 50	0. 29	0. 29	0. 13	0. 14	0. 33	0. 29	0. 14	0. 22	0. 29	0. 13	0. 17	0. 33	0. 11	0. 25	0. 63	0. 38	0. 25	0. 50	0. 43	0. 17	0. 38	0. 43	0. 17	0. 50	0. 29	0. 29	0. 38	0. 50	0. 29	0. 38	0. 33	0. 50	0. 43	0. 25	0. 13	0. 14	1. 00	
JC1 29	0. 33	0. 29	0. 29	0. 29	0. 29	0. 33	0. 33	0. 29	0. 00	0. 22	0. 13	0. 13	0. 17	0. 33	0. 25	0. 43	0. 44	0. 57	0. 25	0. 50	0. 43	0. 40	0. 38	0. 11	0. 00	0. 13	0. 13	0. 13	0. 38	0. 13	0. 29	0. 57	0. 14	0. 29	0. 25	0. 25	0. 29	0. 00	0. 43	1. 00

et al. (2008) detected polymorphism in *Jatropha* species with 26 RAPDprimers (80.2%) across 8 species which were considerably higher. In the present study, 40 *J. curcas* accessions from India showed high percentage of 75.2%. Jaccard's genetic similarity co-efficient varied from 0.00 to 1.00. Similarly wide range in similarity values had been observed in many other perennial species level studies (Nair et al., 1999; Sarmah et al., 2007). Association between the accessions OTRI\JC\78 (Luthiana, panjab), OTRI\JC\32 (pudikuttai, Tamilnadu) in the sub cluster D shared 100% similarity; accessions OTRI\JC\120 (shillang, Meghalaya), OTRI\JC\44 (Patna, bhihar) in the sub cluster E shared 80% similarity (Table 5) even though they are collected from different geographical regions. It is possible that genotypes from different geographical regions can be genetically similar (Neeraj jain et al., 2003).

All genotypes from different geographical regions showed close resemblance and fall under single sub-populations. This association between genotypes from contiguous regions may be the result of similar agro-climatic conditions or due to

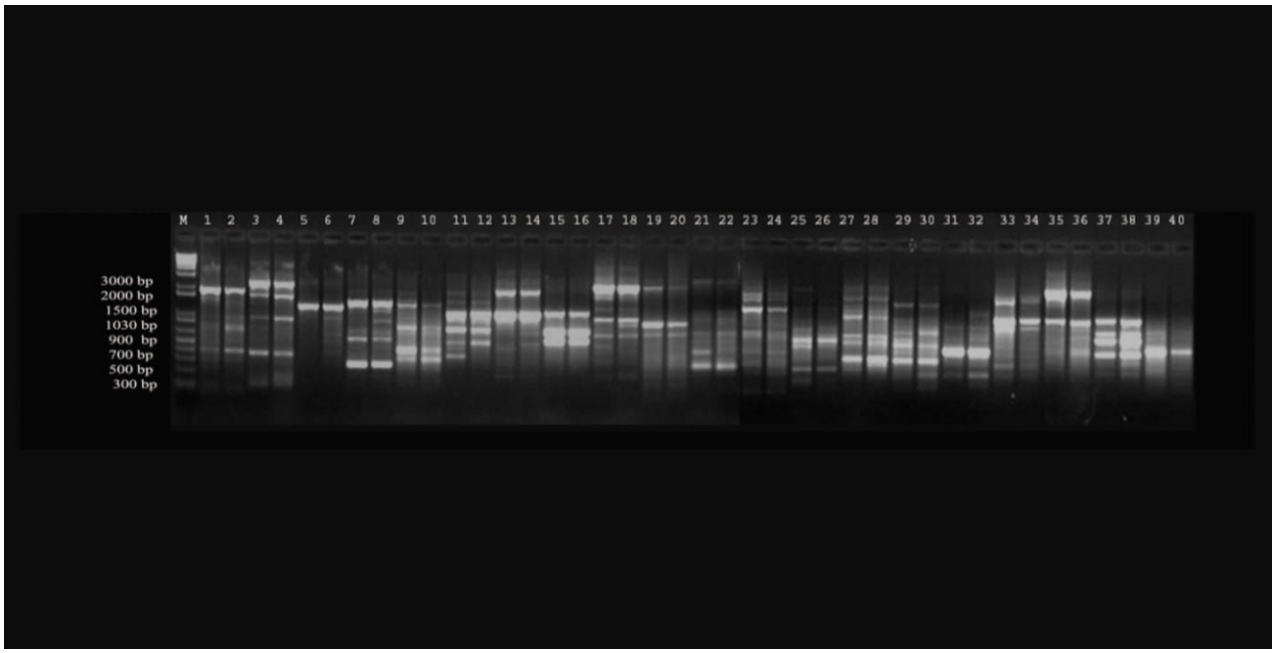

Figure 3. RAPD polymorphism for *Jatropha Curcas* genotypes detected with OPk-03.

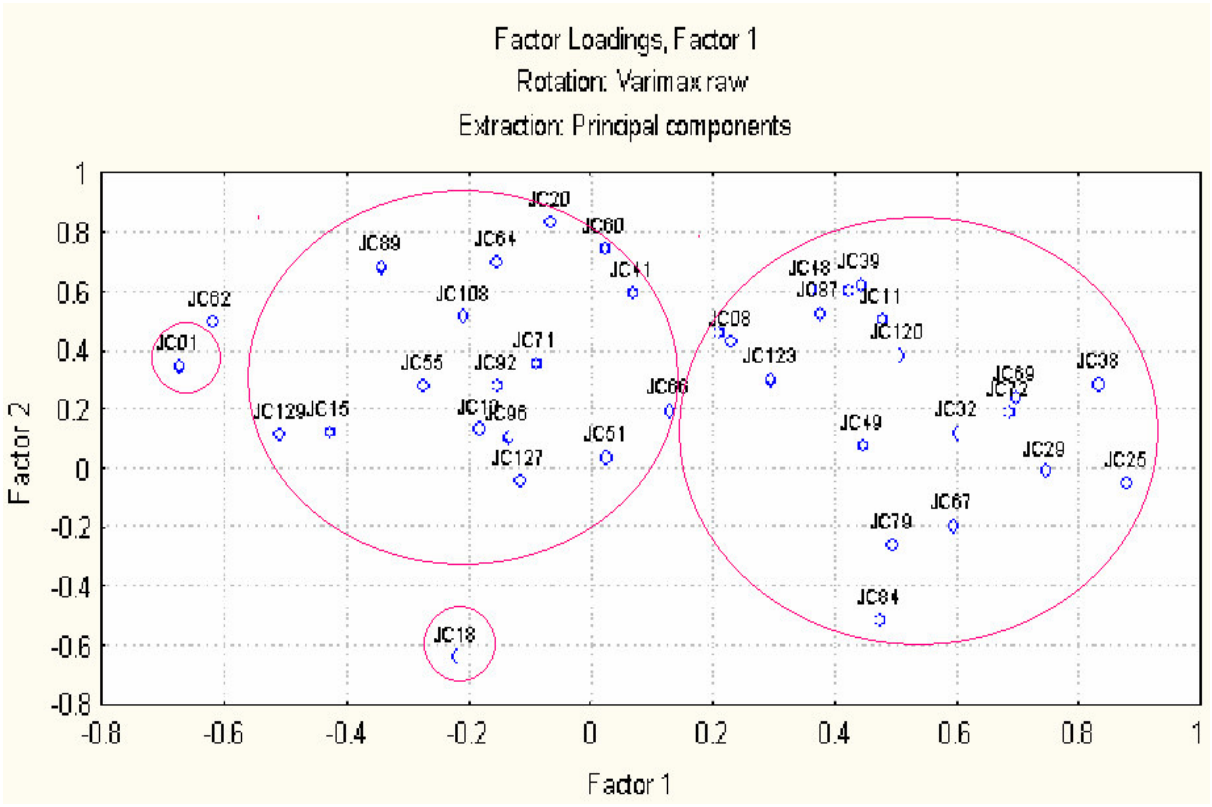

Figure 4. Genetic relationship of 40 *J. curcas* accessions based on principal component analysis.

Table 5. Representation of accessions in different clusters.

Cluster	Accession	Location	State
Cluster-I	OTRI\JC\18	Dhekamal	Orissa
Cluster-II			
A	OTRI\JC\13	Band	Orissa
	OTRI\JC\15	Sambalpur	Orissa
B	OTRI\JC\127	Raipur	Chatthis garh
	OTRI\JC\71	Firojpur, gurgaon	Haryana
	OTRI\JC\96	IARI-New Delhi	New delhi
	OTRI\JC\66	Agra	Uttar Pradesh
	OTRI\JC\51	Bhupal	Madhya Pradesh
	OTRI\JC\49	Trichur	Kerala
	OTRI\JC\62	Dehradun	Uttaranchal
	OTRI\JC\55	Jabalpur	Madhya pradesh
	OTRI\JC\108	Anand	Gujarath
	OTRI\JC\101	Ahmedabad	Gujarath
	OTRI\JC\41	Ranchi	Jarkhand
C	OTRI\JC\129	Akola	Maharastra
	OTRI\JC\64	NBRI, Lucknow	Uttar Pradesh
	OTRI\JC\39	UAS, Dharward	Karnataka
	OTRI\JC\60	Indore	Madhya Pradesh
	OTRI\JC\89	Raghunath pur	West Bengal
	OTRI\JC\20	Madhurai	Tamilnadu
	OTRI\JC\12	Araku valley	Andrapradesh
	OTRI\JC\11	Mahendra giri hills	Orissa
D	OTRI\JC\84	Palampur	Panjab
	OTRI\JC\78	Luthiana	Panjab
	OTRI\JC\32	Pudikuttai	Tamilnadu
	OTRI\JC\29	Pandicheri	Tamilnadu
	OTRI\JC\79	Mohali	Panjab
	OTRI\JC\67	Bithur	Uttar Pradesh
	OTRI\JC\72	Panipat	Panjab
	OTRI\JC\38	GKVK, Bangalore	Karnataka
	OTRI\JC\25	Coimbatthoor	Tamilnadu
E	OTRI\JC\123	Sephajala wild life santuri	Tripura
	OTRI\JC\69	Karnal	Haryana
	OTRI\JC\48	Odakalli	Kerala
	OTRI\JC\120	Shilling	Meghalaya
	OTRI\JC\44	Patna	Bhihar
	OTRI\JC\87	Jorhat	Assam
	OTRI\JC\08	Thirumala hills	Andrapradesh
F	OTRI\JC\92	Mohan pur	West Bengal
	OTRI\JC\01	Nalla malai	Andrapradesh

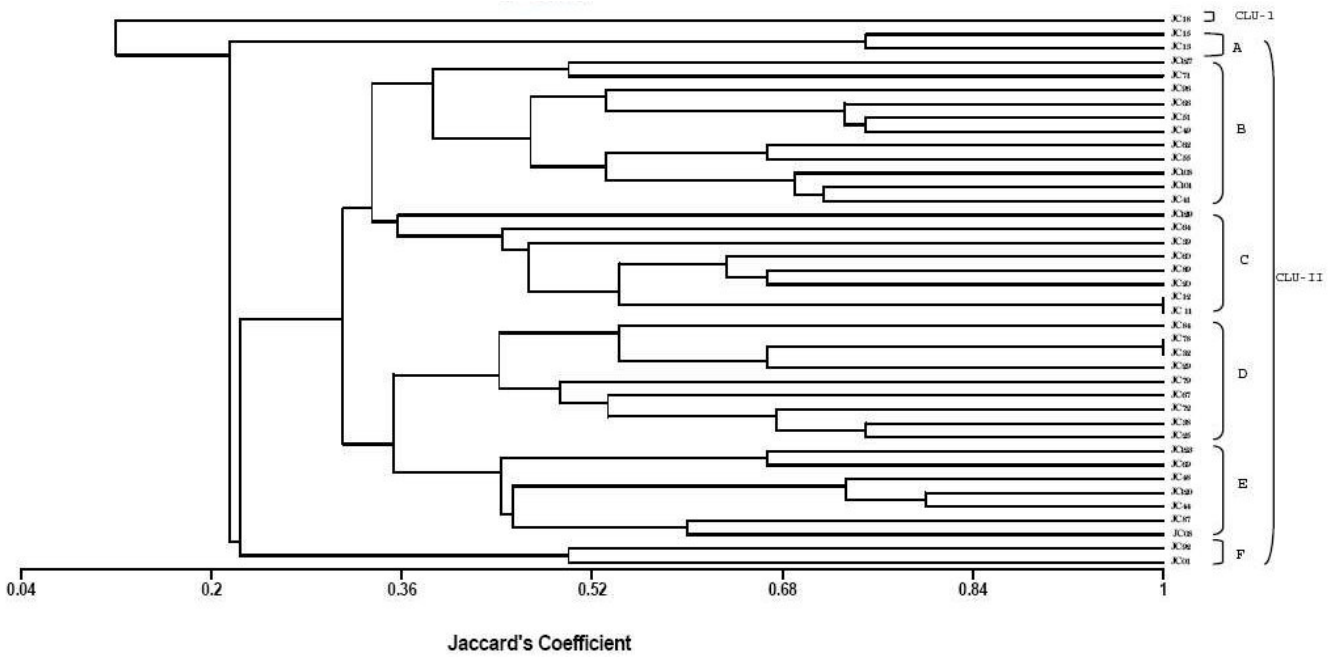

Figure 5. UPGMA-Dendrogram.

seed movement and gene flow (Padmesh et al., 1999). In practice, better under-standing of the distribution of genetic variation at the intra specific level would help to identify superior genotype(s) for cultivar up-grades and as well as to evolve strategies for the establishment of effective *in situ* and *ex situ* conservation programmes (Bhutta et al., 2006; Basha et al., 2007). Although such empirical determination of diversity can be obtained by evaluating morphological, physiological and biochemical traits, the study also reveals the limitations of conventional taxonomic tools in resolving the taxonomic confusion prevailing in plant classification. The technical simplicity of the RAPD technique has facilitated its use in the analysis of genetic relationships in several genera (Wilikie et al., 1993; Demeke, 1992; Nair et al., 1999). The major concern regarding RAPD generated phylogenies includes homology of bands showing the same rate of migration and cause and origin of sequence in the genome (Stammers et al., 1995). In spite of this limitation, RAPD markers have the greatest advantage of its capability to scan across all regions of the genome hence its suitability for phylogenetic studies at species levels (Wilikie et al., 1993; Demeke, 1992).

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