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Genetic Diversity and Phylogenetic Relationships among *Jatropha curcas* L. Genotypes from Eastern India

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Genetic diversity and relationships among 31 genotypes collected from wide geographical range in eastern India was studied employing Random Amplified Polymorphic DNA (RAPD) markers. 19 markers (identified out of 25) produced 139 band in total. Off these 139 bands, 131 bands were polymorphic exhibiting a high polymorphism of 94.24%. Similarity indices estimated on the basis of RAPD primers ranged widely from 0.44 to 0.83 which suggests that these accessions represent genetically diverse population possibly due to predominance of cross pollination and seed source variability. Genotyping data obtained for RAPD primer across collected accessions were used to generate the UPGMA - based phylogenetic tree which shows two major clusters. The cluster I consisted of 16 genotypes from two states Jharkhand and Odisha (Chotanagpur and Eastern plateau region) while 15 accessions from West Bengal and Bihar (Indo Gangetic plains region) were grouped together in the Cluster II. This clear alignment of accessions into two different clusters as per geographical regions was probably due to different growing conditions in two clusters.

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1. INTRODUCTION

Jatropha curcas Linn. (Family- Euphorbiaceae) plant is remarkable for its drought hardiness, rapid growth, easy propagation, small gestation period, wide adaptability and optimum plant size for convenient seed collection [1-2]. Worldwide it is deemed a promising species for biodiesel production having a high non-edible seed oil content. The seeds contain 46-58% of oil on kernel weight and 30-40% on seed weight [3]. In fact the potentially high yield of oil per unit land area in *J. curcas* is second only to oil palm [4]. Furthermore, the seed oil quality is suitable for production of biodiesel as it contains more than 75% unsaturated fatty acids [5]. Apart from being a potential biofuel crop *J. curcas* has multiple utilities. Preparations of all plant parts, including seeds, leaves and bark are used in traditional medicine and for veterinary purposes. The latex of *J. curcas* has also been reported to be an abortifacient and is efficacious in dropsy, sciatica and paralysis [6]. It 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 [7]. Nath and Dutta [8] demonstrated the wound-healing properties of 'cur-cain', a proteolytic enzyme isolated from the latex of the plant. Kone-Bamba et al. [9] reported coagulating effects of the latex on blood plasma. 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 [10].

Limited success has been achieved in worldwide introduction of *J. curcas* for varied purposes due to unreliable and low seed set and oil yields resulting in poor economic returns [11]. The Species is characterized by variable and unpredictable yield due to unidentified reasons [12]. Cultivation of *J. curcas* involves risk because it is a wild species and no varieties with desirable traits for specific growing conditions are available [13]. The major constraint in achieving higher quality oil yield of *J. curcas* is lack of information about its genetic variability, oil composition and absence of suitable ideotypes for different cropping systems. Thus, the positive attributes of this plant are also not fully understood in terms of breeding and utilization [4] which limits its large-scale cultivation and warrants the need for genetic improvement and breeding in the species.

J. curcas is a highly cross-pollinated species which is anticipated to contain wide genetic variability offering significant scope for selecting superior genotypes for enhanced productivity. The promise of this valuable crop can only be realized completely through development and release of high yielding variety/clones which needs sufficient information regarding the extent and pattern of genetic variation in *J. curcas* populations. Modern techniques have accelerated characterization of *J. curcas* germplasm at molecular level [11,14] and even whole genome sequencing is available [15]. However, information regarding the extent and pattern of genetic variation in *J. curcas* population is limited [16-20]. Overall, scanty information on genetic base, genetic makeup and the patterns of genetic diversity of *J. curcas* is a bottleneck in development of varieties/ clones with superior traits of commerce. Thus, genetic diversity and relationships among 31 genotypes collected from wide geographical range in eastern India was studied employing Random Amplified Polymorphic DNA (RAPD) markers.

2. MATERIALS AND METHODS

2.1 Plant Material

Germplasm of 31 genotypes of *J. curcas* collected from different locations of Eastern India in the states of Bihar, Jharkhand, Odisha and West Bengal (Table 1; Fig. 1) maintained as a germplasm garden at Institute of Forest Productivity, Ranchi (India) was utilized.

2.2 DNA Isolation

Total genomic DNA was extracted from leaves using the CTAB method [21] with minor modifications. The yield and purity of DNA were measured using UV spectrophotometer (Spectroquant Pharo 300 Merck). The purity of DNA was determined by calculating the ratio of absorbance at 260 nm to that of 280 nm.

2.3 PCR Amplifications

A set of 25 random decamer oligonucleotides (Bangalore Genei, India) were used as single primers for the amplification of RAPD fragments (Table 2). The G=C content varied from 60-70%

while the melting temperature (Tm) was 32-34 ^{0}C.

The RAPD was carried out in themocyler (Gene Amp-PCR System, Applied Biosystems). The RAPD-PCR was programmed as I-94^{0}C for 5 minutes- Initial denaturation; II-94^{0}C for 1 minute- Denaturation; III-36^{0}C for 1 minute- Annealing; IV-72^{0}C for 2 minutes- Extension; V-72^{0}C for 5 minutes- final extension followed by 4^{0}C for infinity to hold the samples. The steps II, III and IV were programmed to run for 45 cycles.

PCR fragments were separated electrophoretically on 1.5% agarose gel containing ethidium bromide using 1 x TAE buffer. The PCR products were visualized under UV light in a Gel Doc Unit (ULTRACAM). The banding pattern were photographed and stored as digital pictures (Fig. 2). The reproducibility of the amplification was confirmed by repeating experiment as required.

To analyze RAPD data, the total numbers of unique bands were counted for each primer used. The presence or absence of each individual band was recorded for each lane on the gel representing a different plant sample. For this a matrix was created with each sample representing one column and each band in one row. The presence of a band was recorded as a one (1) and the absence as a zero (0). Only clear, unambiguous and reproducible bands were considered for scoring. The RAPD bands obtained from different primers in the clones of *J. curcas* were scored in the form of binary data of 1 and 0 which were put in the form of data matrix. Recorded data was put in a specified format into software for further assessment.

Table 1. Details of *J. curcas* genotypes from different areas of Eastern India

S. No.	Accessions	Location	District	State
1	JBT-44/5	Chandwa	Latehar	Jharkhand
2	JBT-44/6	Satwarwa	Palamu	Jharkhand
3	JBT-44/15	Sonpurwa	Garhwa	Jharkhand
4	JBT-44/19	Tildag	Garhwa	Jharkhand
5	JBT-44/20	Chaenpur	Palamu	Jharkhand
6	JCRCKG	Burmu	Ranchi	Jharkhand
7	JCGLBB	Basia	Gumla	Jharkhand
8	JCGLPD	Dhauntatoli	Gumla	Jharkhand
9	JCGLSH	Hundratoli	Gumla	Jharkhand
10	JCBKDT	Dantu	Bokaro	Jharkhand
11	JCBKBD	Bardih	Bokaro	Jharkhand
12	JCSDFP	Kolebira	Simdega	Jharkhand
13	JCSDBK	Karanjtoli	Simdega	Jharkhand
14	JBT-45/2	Nandangarg	W. Champaran	Bihar
15	JBT-45/4	Santpur	E.Champaran	Bihar
16	JBT-45/6	Siwan	E.Champaran	Bihar
17	JBT-45/9	Begusarai	Begusarai	Bihar
18	JBT-45/10	Balia	Begusarai	Bihar
19	JBT-45/11	Mokama	Patna	Bihar
20	JBT-45/12	Nalanda	Biharsharif	Bihar
21	JBT-45/13	Rajauli	Nawada	Bihar
22	AK-2	Midnapur	Midnapur	W. Bengal
23	AK-3	Kusto Colony	Durgapur	W. Bengal
24	AK-5	Saltora	Bamkura	W. Bengal
25	AK-6	Jhalgram	Midnapur	W. Bengal
26	BNDAK-7	Matha	Purulia	W. Bengal
27	BNDAK-8	Kulgoda	Purulia	W. Bengal
28	AK-9	Pathalmada	Purulia	W. Bengal
29	OR-1	Rourkela	Sundergarh	Odisha
30	OR-2	Talacher	Angul	Odisha
31	OR-3	Gania	Nayagarh	Odisha

Fig. 1. Area of collection of accessions in Eastern India

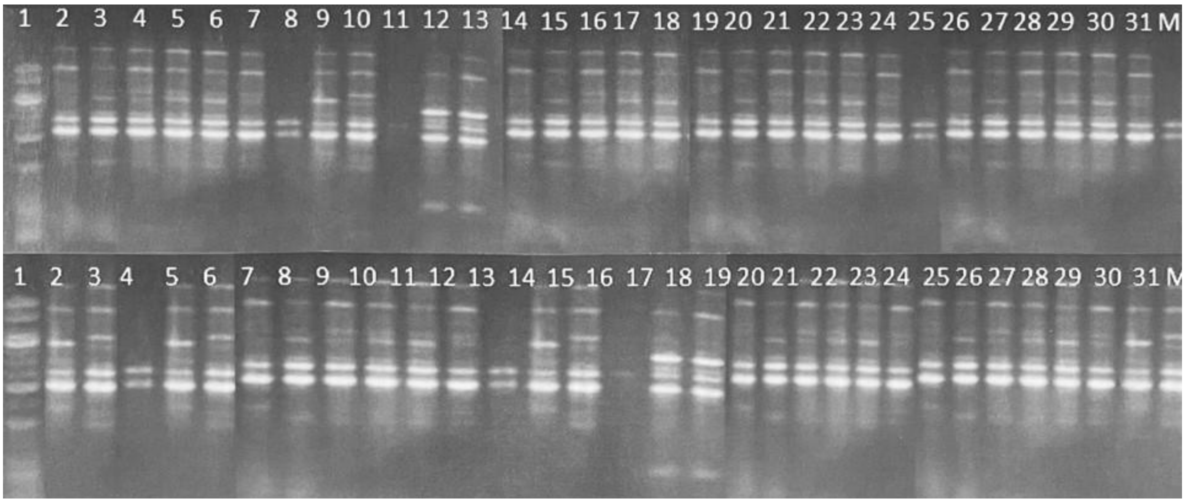

Fig. 2. RAPD patterns of 31 accession of *Moringa oleifera* generated by primer RPI 12 (above) and RPI 19 (below): M – 100 bp molecular weight ladder

Table 2. Details of the RAPD primers used in the study

Primer	Nucleotide sequence (5'-3')	G+C content (%)	Tm ^{0}C
RAPD-1	AAAGCTGCGG	60	32
RAPD-2	AACGCGTCGG	70	34
RAPD-3	AAGCGACCTG	60	32
RAPD-4	AATCGCGCTG	60	32
RAPD-5	AATCGGGCTG	60	32
RAPD-6	ACACACGCTG	60	32
RAPD-7	ACATCGCCCA	60	32
RAPD-8	ACCACCCACC	70	34
RAPD-9	ACCGCCTATG	60	32
RAPD-10	ACGATGAGCG	60	32
RAPD-11	ACGGAAGTGG	60	32
RAPD-12	ACGGCAACCT	60	32
RAPD-13	ACGGCAAGGA	60	32
RAPD-14	ACTTCGCCAC	60	32
RAPD-15	ACCCTGAGCC	70	34
RAPD-16	AGGCGGCAAG	70	34
RAPD-17	AGGCGGGAAC	70	34
RAPD-18	AGGCTGTGTC	60	34
RAPD-19	AGGTGACCGT	60	32
RAPD-20	AGTCCGCCTC	70	34
RAPD-21	CACGAACCTC	60	32
RAPD-22	CATAGAGCGG	60	32
RAPD-23	CCAGCAGCTA	60	32
RAPD-24	CCAGCCGAAC	70	34
RAPD-25	GAGCGCCTTC	70	34

The population genetic diversity parameters like percentage of polymorphic loci [22], genetic diversity [23], Shannon's Information Index [24] and genetic distance [25] were calculated using Popgene version 1.31 [26]. The total genetic diversity (HT), mean genetic diversity within population (HS) and the relative degree of genetic diversity between populations (GST) were measured using Nei's genetic diversity [27].

3. RESULTS AND DISCUSSION

Information of existing genetic diversity constitutes the basis of genetic improvement programmes and sustainable management of genetic resources. It depends upon multiple factors such as breeding systems, biological characteristics, evolutionary history, and natural selection impart genetic diversity and [28-29]. Since variability is a prerequisite for selection for improvement, it is indispensable to detect and document the amount of variation existing within and between the populations. Populations with low genetic variability have a reduced potential to adapt to environmental changes [30]. Therefore, genetic variation is important for the long term survival of a species [31]. *J. curcas* being a naturalized species, its ability to grow in varied eco-climatic zones and its wide range of distribution embraces a considerable scope of genetic variation. Characterization of the available germplasm is needed for breeding programs to be efficient [32-33]. However, the degree of genetic diversity in and within natural populations in and outside the Centre of origin in poorly studied for the species, hampering domestication of *J. curcas* for large-scale cultivation.

High efficiency and low expense RAPD markers having the capability to scan and detect multiple loci across all the regions of genome have been found highly suited for genetic variation and phylogeny studies in tree species. In the present study RAPD provided large number of reliable and reproducible fingerprint profiles for a collection of 31 accessions of *J. curcas.* However, the availability of unique or rare fragments present in different accessions were limited. The details of RAPD primers, number of amplified products and percentage polymorphism obtained by analyzing primers have been provided in Table 3. 139 band in total were observed. Off these 131 bands were polymorphic exhibiting a high polymorphism of 94.24%.

Table 3. Primer identity with total number of bands obtained and polymorphism percentage

Primer	Polymorphic bands	Monomorphic bands	Total bands	% Polymorphism
RAPD-3	7	0	7	100
RAPD-4	8	1	9	88.88
RAPD-6	6	0	6	100
RAPD-7	7	1	8	87.85
RAPD-8	6	0	6	100
RAPD-9	4	0	4	100
RAPD-10	12	1	13	92.3
RAPD-12	9	2	11	81.8
RAPD-13	7	0	7	100
RAPD-15	5	0	5	100
RAPD-16	6	0	6	100
RAPD-17	5	0	5	100
RAPD-18	4	1	5	80
RAPD-19	6	0	6	100
RAPD-21	11	2	13	84.6
RAPD-22	6	0	6	100
RAPD-23	8	0	8	100
RAPD-24	5	0	5	100
RAPD-25	9	0	9	100
Total	131	8	139	94.24

The genetic variation statistics for the loci have been summarized in the Table 4. The observed numbers of alleles (Na) ranged from 1.80 to 2.00 with a mean value of 1.94 while the range of the effective numbers of alleles (Ne) was found to be 1.44 to 1.85 with a mean value of 1.64. The Nei's gene diversity (H) was 0.36±0.15 and Shanon's information index (I) was recorded to be 0.53±0.20. Both values are comparatively higher than those reported for RAPD markers in *J. curcas* in earlier studies [34-35]. This reflects the heterozygous nature of the population under study. The high value of Shannon's information index in the present study (>0.5) is an indication of the presence of high genetic diversity. Overall the markers were very informative in recording highly divergent genotypes of *J. curcas* from eastern India.

Similarity indices estimated on the basis of 19 primers ranged widely from 0.44 to 0.83 which suggests that these accessions represent genetically diverse population possibly due to predominance of cross pollination and seed source variability (Table 5). The highest genetic distance (0.79) was found between AK6 and JCRCKG that have come from West Bengal and Jharkhand, respectively while the lowest genetic distance (0.19) was there between JCGLBB and JCGLPD, both from Jharkhand. The high diversity revealed by RAPD markers is in agreement with the conclusion that out-breeding plant species retain considerable variability [36]. Still in absence of the pedigree data, the higher probability of origin of all such accessions from the same source and eventual distribution to other locations in a region/state cannot be ruled out.

To understand genetic diversity in the germplasm analyzed, genotyping data obtained for all the nineteen RAPD primer across 31 collected accessions were used to generate the UPGMA (Unweighted Pair Group Method with Arithmetic Mean) - based phylogenetic tree which shows two major clusters (Fig. 3). The accessions JBT-45/5 and JBT-45/13 were seen to be at two ends of the phylogenetic tree exhibiting high diversity. The cluster I was consisted 16 genotypes from two states Jharkhand and Odisha (Chotanagpur and Eastern plateau region) while 15 accessions from West Bengal and Bihar (Indo Gangetic plains region) were grouped together in the Cluster II. This is clear alignment of accessions as per geographical regions possibly due to different dissimilar growing conditions. Grouping according to geographical differences has been observed in other studies also [34,37-38]. The Cluster I formed two sub-clusters with one of them with accessions only from Jharkhand while the other sub-cluster had accessions both from Jharkhand and Odisha. Cluster II formed three sub-clusters among which one formed of only two accessions JBT-45/12 and JBT-45/13 (Fig. 3). The clusters seen in the dendrogram were also analyzed for their genetic distinctiveness.

Coefficient of genetic differentiation [23] which is the measure of diversity within groups in population genetics was calculated which was 1 indicting that the population in study is highly diverse.

The prior assessments of genetic variations among Jatropha germplasms using molecular markers show the presence of high genetic diversity for the Central and South American regions and insignificant genetic variation from Asia and Africa [11,39]. The RAPD technique has been used for assessment of genetic diversity for *J. curcas* which revealed low to moderate level of genetic diversity of Indian germplasms [40-43]. Several other molecular studies later employing variety of DNA markers have also underlined widespread genetic monomorphism and low genetic variability in *J. curcas* [39,44-46]. The possible reason may be absence of the anthropogenic and environmental influences in generating genetic variability mainly in the introduced conditions. It seems that adaptive genomic characters imparting tolerance of growth in wide disturbed scenarios have most probably been acquired by the species before its global distribution. Richards et al. [47] 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. Furthermore, a limited stock has been vegetatively and apomictically propagated, since *J. curcas* is known to exhibit apomixis [48]. Another reason behind low variability seems to be the 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 [11].

Variation in genetic diversity within the species is usually related with geographical range, mode of reproduction, mating system, seed dispersal and fecundity. The genetic diversity in the present investigation might be due to geographical difference and seed source variability as well as high level of cross pollination in the species. Gupta et al [34] have also recorded wide genetic base in RAPD evaluation of 13 Indian accessions from different agroclimatic zones. Likewise Rafii et al. [35] found accessions from same and near states or regions to be grouped together according to their geographical origin. According to Pamidimarri and Reddy [49], Portuguese seafarers introduced accessions from Mexico and Central America to India through two dispersal routes: one brought *J. curcas* through Africa, Madagascar and finally to India, while the other passed through Spain on its way to India. This may also be a reason for geographical separation of the accessions in studies with Indian collections.

Table 4. Summary of genetic variation statistics for all loci

Locus	Sample Size	Na	Ne	H	I
RAPD-3	31	2.00	1.78	0.43	0.62
RAPD-4	31	2.00	1.56	0.34	0.52
RAPD-6	31	2.00	1.83	0.45	0.65
RAPD-7	31	2.00	1.73	0.40	0.57
RAPD-8	31	2.00	1.83	0.45	0.64
RAPD-9	31	2.00	1.85	0.46	0.65
RAPD-10	31	2.00	1.58	0.33	0.50
RAPD-12	31	2.00	1.63	0.35	0.51
RAPD-13	31	2.00	1.44	0.29	0.46
RAPD-15	31	2.00	1.63	0.38	0.57
RAPD-16	31	2.00	1.48	0.30	0.47
RAPD-17	31	1.80	1.60	0.31	0.44
RAPD-18	31	1.80	1.47	0.26	0.39
RAPD-19	31	1.83	1.54	0.33	0.48
RAPD-21	31	1.92	1.67	0.36	0.52
RAPD-22	31	2.00	1.63	0.36	0.53
RAPD-23	31	1.88	1.69	0.38	0.55
RAPD-24	31	1.80	1.62	0.34	0.49
RAPD-25	31	1.89	1.55	0.33	0.50
Mean	31	1.94	1.64	0.36	0.53
Standard Deviation		0.22	0.32	0.15	0.20

Table 5. Nei's analysis of genetic similarity (above diagonal) and genetic distance (below diagonal) in 31 accessions of *J. curcas*

Pop ID	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
1	****	0.76	0.71	0.65	0.63	0.68	0.73	0.70	0.70	0.61	0.65	0.69	0.71	0.64	0.64	0.62	0.61	0.64	0.64	0.59	0.54	0.65	0.62	0.58	0.63	0.59	0.61	0.65	0.68	0.60	0.58
2	0.27	****	0.80	0.67	0.58	0.58	0.63	0.56	0.62	0.60	0.60	0.65	0.63	0.62	0.58	0.54	0.55	0.58	0.60	0.61	0.56	0.60	0.60	0.56	0.63	0.58	0.66	0.57	0.63	0.57	0.63
3	0.35	0.22	****	0.67	0.60	0.60	0.66	0.66	0.56	0.65	0.69	0.65	0.61	0.62	0.58	0.58	0.55	0.58	0.60	0.60	0.55	0.59	0.60	0.58	0.63	0.61	0.62	0.58	0.60	0.63	0.60
4	0.43	0.40	0.40	****	0.71	0.55	0.60	0.58	0.59	0.59	0.56	0.65	0.65	0.59	0.55	0.54	0.59	0.56	0.50	0.61	0.55	0.59	0.58	0.50	0.59	0.55	0.56	0.54	0.55	0.55	0.58
5	0.47	0.55	0.50	0.35	****	0.61	0.61	0.64	0.68	0.60	0.58	0.62	0.72	0.60	0.65	0.59	0.60	0.55	0.53	0.59	0.60	0.58	0.63	0.53	0.63	0.59	0.55	0.56	0.60	0.63	0.66
6	0.38	0.55	0.50	0.60	0.49	****	0.67	0.70	0.68	0.60	0.65	0.63	0.71	0.68	0.55	0.56	0.54	0.57	0.67	0.53	0.50	0.51	0.55	0.58	0.45	0.56	0.53	0.55	0.63	0.63	0.59
7	0.32	0.46	0.41	0.50	0.49	0.40	****	0.83	0.73	0.61	0.67	0.65	0.63	0.65	0.65	0.60	0.58	0.63	0.64	0.55	0.61	0.67	0.68	0.64	0.64	0.62	0.60	0.60	0.68	0.59	0.62
8	0.36	0.58	0.41	0.55	0.45	0.36	0.19	****	0.71	0.65	0.73	0.68	0.62	0.67	0.61	0.59	0.63	0.58	0.64	0.56	0.57	0.68	0.63	0.64	0.55	0.66	0.60	0.58	0.65	0.65	0.59
9	0.36	0.48	0.58	0.53	0.38	0.38	0.32	0.34	****	0.71	0.65	0.68	0.69	0.64	0.65	0.59	0.64	0.63	0.61	0.62	0.61	0.57	0.56	0.50	0.54	0.53	0.60	0.56	0.68	0.60	0.56
10	0.49	0.50	0.43	0.53	0.52	0.52	0.49	0.42	0.34	****	0.76	0.72	0.65	0.54	0.63	0.53	0.60	0.61	0.57	0.66	0.55	0.65	0.62	0.55	0.60	0.52	0.63	0.69	0.68	0.66	0.66
11	0.42	0.50	0.37	0.58	0.54	0.42	0.40	0.32	0.42	0.28	****	0.75	0.60	0.61	0.63	0.62	0.60	0.58	0.67	0.59	0.50	0.67	0.56	0.61	0.55	0.56	0.61	0.66	0.65	0.68	0.59
12	0.37	0.42	0.42	0.42	0.48	0.46	0.43	0.39	0.39	0.33	0.29	****	0.64	0.60	0.68	0.61	0.65	0.62	0.62	0.64	0.55	0.63	0.70	0.58	0.59	0.58	0.68	0.68	0.71	0.68	0.70
13	0.35	0.47	0.49	0.42	0.33	0.35	0.46	0.48	0.37	0.43	0.50	0.45	****	0.60	0.65	0.57	0.62	0.58	0.60	0.68	0.58	0.56	0.61	0.53	0.59	0.55	0.58	0.57	0.59	0.61	0.68
14	0.45	0.48	0.48	0.53	0.52	0.38	0.42	0.40	0.45	0.62	0.49	0.50	0.50	****	0.73	0.78	0.64	0.65	0.73	0.59	0.64	0.65	0.65	0.70	0.63	0.65	0.63	0.60	0.60	0.58	0.56
15	0.45	0.55	0.55	0.60	0.42	0.59	0.42	0.49	0.42	0.47	0.47	0.39	0.43	0.32	****	0.81	0.71	0.67	0.71	0.65	0.67	0.70	0.73	0.71	0.76	0.65	0.68	0.69	0.67	0.65	0.66
16	0.48	0.62	0.54	0.62	0.53	0.58	0.50	0.53	0.53	0.63	0.48	0.49	0.57	0.25	0.22	****	0.71	0.63	0.75	0.60	0.65	0.71	0.67	0.69	0.66	0.65	0.60	0.65	0.56	0.63	0.57
17	0.49	0.60	0.60	0.53	0.52	0.62	0.54	0.47	0.45	0.52	0.52	0.43	0.48	0.45	0.34	0.35	****	0.74	0.73	0.63	0.64	0.67	0.72	0.64	0.65	0.60	0.68	0.65	0.58	0.63	0.58
18	0.45	0.55	0.55	0.58	0.59	0.57	0.47	0.54	0.47	0.49	0.54	0.48	0.55	0.42	0.40	0.46	0.30	****	0.80	0.68	0.71	0.64	0.71	0.67	0.65	0.58	0.63	0.69	0.63	0.60	0.56
19	0.45	0.50	0.50	0.69	0.64	0.40	0.45	0.45	0.49	0.57	0.40	0.48	0.50	0.32	0.34	0.29	0.32	0.22	****	0.66	0.67	0.67	0.72	0.70	0.67	0.66	0.67	0.71	0.61	0.60	0.63
20	0.53	0.49	0.52	0.49	0.53	0.63	0.60	0.58	0.48	0.41	0.53	0.45	0.38	0.53	0.43	0.52	0.46	0.39	0.41	****	0.78	0.60	0.61	0.58	0.68	0.60	0.62	0.64	0.58	0.54	0.63
21	0.62	0.58	0.60	0.60	0.52	0.70	0.49	0.57	0.49	0.59	0.70	0.60	0.55	0.45	0.40	0.43	0.45	0.34	0.40	0.25	****	0.67	0.65	0.60	0.70	0.62	0.61	0.63	0.55	0.56	0.59
22	0.42	0.50	0.53	0.53	0.54	0.67	0.40	0.38	0.57	0.42	0.40	0.46	0.58	0.42	0.36	0.35	0.40	0.45	0.40	0.50	0.40	****	0.73	0.67	0.71	0.60	0.67	0.72	0.63	0.65	0.63
23	0.48	0.52	0.52	0.54	0.46	0.60	0.39	0.46	0.58	0.48	0.58	0.36	0.49	0.43	0.31	0.40	0.33	0.35	0.33	0.49	0.43	0.31	****	0.72	0.76	0.67	0.73	0.74	0.62	0.63	0.68
24	0.54	0.58	0.55	0.69	0.64	0.54	0.45	0.45	0.70	0.59	0.49	0.55	0.63	0.36	0.34	0.37	0.45	0.40	0.36	0.55	0.52	0.40	0.33	****	0.73	0.72	0.64	0.68	0.58	0.60	0.59
25	0.47	0.46	0.46	0.53	0.47	0.79	0.45	0.59	0.62	0.52	0.59	0.53	0.53	0.47	0.28	0.41	0.42	0.42	0.40	0.39	0.36	0.34	0.27	0.32	****	0.71	0.71	0.75	0.58	0.62	0.68
26	0.53	0.54	0.49	0.59	0.53	0.58	0.48	0.41	0.63	0.66	0.58	0.54	0.59	0.43	0.43	0.42	0.50	0.55	0.41	0.52	0.48	0.50	0.40	0.33	0.35	****	0.69	0.65	0.60	0.64	0.70
27	0.49	0.41	0.48	0.58	0.59	0.64	0.52	0.52	0.52	0.47	0.49	0.39	0.55	0.47	0.38	0.50	0.38	0.47	0.40	0.48	0.49	0.40	0.31	0.45	0.34	0.37	****	0.78	0.73	0.66	0.73
28	0.43	0.57	0.54	0.62	0.58	0.60	0.50	0.55	0.58	0.37	0.41	0.38	0.57	0.50	0.37	0.42	0.43	0.37	0.35	0.45	0.46	0.33	0.30	0.39	0.29	0.42	0.25	****	0.69	0.65	0.71
29	0.38	0.46	0.50	0.60	0.52	0.47	0.38	0.42	0.38	0.38	0.42	0.35	0.53	0.52	0.40	0.58	0.54	0.47	0.49	0.55	0.59	0.47	0.48	0.54	0.54	0.50	0.32	0.37	****	0.75	0.69
30	0.50	0.57	0.47	0.59	0.46	0.46	0.53	0.43	0.50	0.41	0.39	0.38	0.49	0.55	0.43	0.47	0.46	0.50	0.50	0.62	0.58	0.43	0.47	0.50	0.48	0.45	0.41	0.42	0.29	****	0.73
31	0.55	0.47	0.52	0.54	0.41	0.53	0.48	0.53	0.58	0.41	0.53	0.36	0.38	0.58	0.41	0.57	0.55	0.58	0.46	0.47	0.53	0.46	0.38	0.53	0.39	0.36	0.31	0.34	0.37	0.32	****

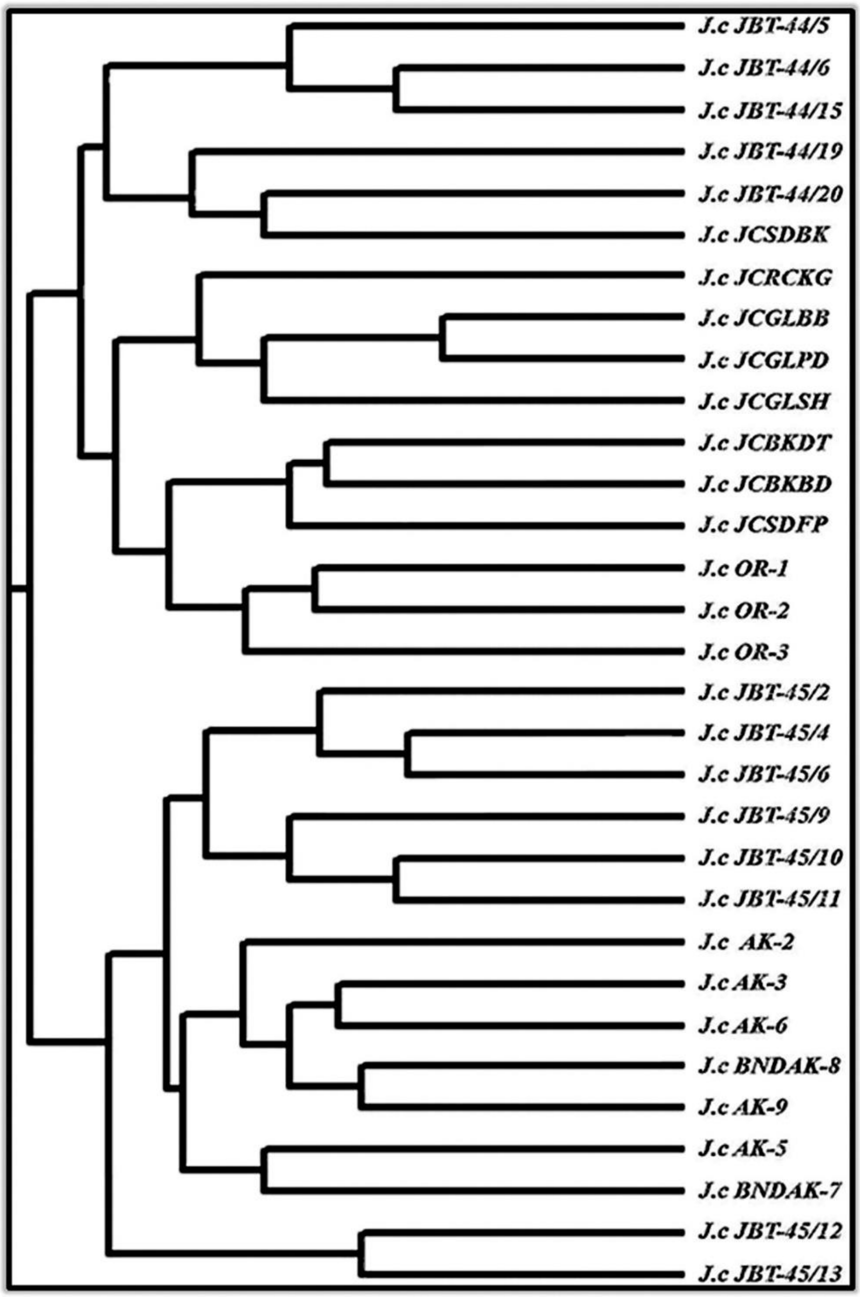

Fig. 3. Cluster generated from the Jaccard similarity coefficient and UPGMA clustering using RAPD markers between 31 *J. curcas* accessions

4. CONCLUSION

Estimating genetic diversity is essential to ensure success in the management of genetic resources, planning and adoption of strategies for genetic breeding in promising species like *J. curcas*. RAPD markers with their high level of polymorphism and ability to decipher genetic variability and relationships appeared to be viable system for undertaking such studies in *J. curcas.* In contrast to most studies we found ample genetic diversity among the analyzed accessions from four states of eastern India indicating that these accessions represent genetically diverse population due to predominance of cross pollination and seed source variability. A clear cut clustering pattern of the accessions as per geographic regions was recorded which may possibly be due to different genetic makeup of the accessions as a result of evolution in markedly different growing conditions.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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