

## Comparison of RAPD, ISSR, and DAMD Markers for Genetic Diversity Assessment between Accessions of *Jatropha curcas* L. and Its Related Species

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### ABSTRACT

Molecular characterization of 19 *Jatropha* accessions that included 15 accessions of *J. curcas* and 4 different species was carried out using 3 different markers systems. Highest polymorphism (96.67%) was recorded by RAPD followed by DAMD (91.02%) and ISSR (90%). Polymorphism Information Content (PIC) was higher for DAMD (0.873) and almost equal for RAPD (0.863) and ISSR (0.862) markers, whereas Resolving Power (Rp) was found to be higher for RAPD as compared to the other two marker systems. Marker Index (MI) values varied greatly with highest (19.07) in RAPD. Shannon index (i), observed number of alleles (na), effective number of alleles (ne) and Nei's genetic diversity (h) values were found to be significantly higher for ISSR as compared to RAPD and DAMD markers. Thus, all the markers proved to be equally efficient for diversity studies in *Jatropha*. Several alleles in all the markers indicated *J. gossypifolia* as one of the parents of *J. tanjorensis*. Dendrograms and PCA plots generated based on RAPD showed three major clusters with *J. integerrima* and *J. podagrica* falling in group I, fifteen *J. curcas* accessions in group II, and *J. gossypifolia* as an outlier in group III. DAMD markers also showed similar clustering pattern whereas ISSR showed last cluster of *J. gossypifolia* and *J. tanjorensis*. These results may provide a future base for conservation and characterization of available *Jatropha* genetic resources.

**Keywords:** Genetic diversity, *Jatropha*, Molecular markers, Polymorphism.

### INTRODUCTION

The genus *Jatropha* belongs to tribe Joannesieae of Crotonoideae in the Euphorbiaceae family and contains approximately 170 known species (Heller, 1996). The approximate genome size of *J. curcas* is 416 Mbp, which is close to that of rice (430 Mbp) (Carvalho *et al.*, 2008). The true center of origin of *J. curcas* is still controversial, but several group of scientists argue it to be a part of flora of Mexico and, probably, of northern central America as its original center (Wilbur, 1954). Aponte(1978) stated central America as well as Mexico, where it is mostly found in the coastal forests as its origin. It is a drought resistant species widely cultivated in tropics as a living fence.

The plant is monoecious and flowers are unisexual. Mostly, it is an insect pollinated plant and its life span is approximately 50 years (Henning *et al.*, 2003; Putten *et al.*, 2010)

It is a multipurpose plant with many attributes and considerable potential that can be grown in low to high rainfall areas and can be used to reclaim land, as a hedge and/or as a commercial crop. Thus, growing it could provide employment, improve the environment, and enhance the quality of rural life (Openshaw, 2000). In today's world, it has attained an important position as an oil bearing crop. In spite of best nutritional composition, seed cake obtained from the *J. curcas* remains unutilized as an animal feed due to its toxic nature and no successful attempts have been made till now for completely eliminating the

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toxicity (Makkar *et al.*, 1998; Makkar *et al.*, 2009; Ahmed *et al.*, 2009). Several attempts have been made to improve the oil content through biochemical means for biodiesel production (Su and Wei, 2008).

Apart from *Jatropha curcas*, several other species of *Jatropha* include *J. podagrica*, *J. multifida*, *J. paendurifolia*, *J. tanjorensis*, *J. glandulifera*, and *J. gossypifolia* are widely distributed in India. *J. tanjorensis* Ellis and Saroja, reported to be a native to India, appears in only few districts of Tamil Nadu. It is generally grown as a hedge plant and reported as a natural interspecific hybrid between *J. curcas* L. and *J. gossypifolia* L (Prabhakaran *et al.*, 1999). New ornamental hybrids have also been developed between *J. curcas* and *J. integerrima* using interspecific hybridization (Sujatha and Prabhakaran, 2003).

Germplasm characterization is necessary to enhance germplasm management and utilization. Information regarding the extent and pattern of genetic variation in *J. curcas* population is limited (Basha and Sujatha, 2009). Diversity studies, based on their morphological traits, are not reliable as they are highly influenced by environment. Molecular diversity assessed by using molecular markers is independent of the influence of environment and estimated by using DNA from any growth stage. Moreover, a large number of polymorphic markers are required to measure genetic relationships and genetic diversity in a reliable manner. This limits the use of morphological characters and isozymes as useful markers because they lack polymorphism. Molecular genetic markers could aid the long term objective of identifying diverse parental lines to generate segregating populations for tagging important traits, such as gene(s) for high content of specific fatty acids like oleic, linolenic, etc (Gupta *et al.*, 2008). Also, DNA-based diagnostics are now well established as a means to assay diversity at the locus, chromosome, and whole genome levels. Moreover, the use of low cost molecular markers like RAPD (Bardacki, 2001) and ISSR for the identification of species and interspecific hybrids can lead to the genetic improvement of the species and genetic resource management (Bornet and Branchard,

2001; Pamidimarri *et al.*, 2009a). Several studies pertaining to genetic diversity assessment in the genus *Jatropha* using RAPD (Pamidimarri *et al.*, 2009a; Iqbal *et al.*, 2010; Basha and Sujatha, 2007; Ranade *et al.*, 2008; Pamidimarri *et al.*, 2009b; Ganesh Ram *et al.*, 2008; Subramaniam *et al.*, 2009), AFLP (Pamidimarri *et al.*, 2009a; Pamidimarri *et al.*, 2009b; , Tatikonda *et al.*, 2009; Sun *et al.*, 2008)), ISSR (Gupta *et al.*, 2008; Basha and Sujatha, 2007; Cai *et al.*, 2010; Vijayanand *et al.*, 2009; Senthil Kumar *et al.*, 2008; Tanya *et al.*, 2011; Umamaheshwari *et al.*, 2010) as well as SSR (Pamidimarri *et al.*, 2009a; Sun *et al.* 2008; Pamidimarri *et al.*, 2010) have been reported.

By keeping in view the above mentioned reasons, it seemed necessary to carry out diversity analysis among the 15 *Jatropha curcas* genotypes, four *Jatropha* species viz. *J. podagrica*, *J. gossypifolia* and *J. integerrima* and one naturally occurring interspecific hybrid, *J. tanjorensis* (hybrid of *J. curcas* and *J. gossypifolia*) using RAPD, ISSR and DAMD markers. Moreover, there are very few reports pertaining to multiple marker comparison studies in *Jatropha*. In addition to the above mentioned points, the objectives of the present study included the identification of some species specific markers, comparison of all the three markers and thereby testing their reliability of strength for diversity analysis and finally genetic purity testing and confirmation of hybrid nature of *J. tanjorensis*, which is reported to be a natural interspecific hybrid between *J. curcas* and *J. gossypifolia*.

## MATERIALS AND METHODS

### Experimental Material

In total, 19 accessions were collected from experimental plantations raised at *Jatropha* farm, Anand Agricultural University. These included 15 of *Jatropha curcas* L. from different geographical regions of India, four species of *Jatropha* genus viz. *Jatropha gossypifolia* L., *Jatropha podagrica* Hook, *Jatropha integerrima* Jacq. and *Jatropha tanjorensis*, which is reported to be a naturally occurring male sterile interspecific hybrid of

*Jatropha curcas* L. and *Jatropha gossypifolia* L. (Table 1).

### Genomic DNA Extraction

Total genomic DNA was extracted by using the CTAB method as described by Doyle and Doyle (1990) with some minor modifications. The spectrophotometric readings showed the purity of DNA in the range of 0.8-2.0.

### Molecular Marker Analysis

Three different markers *viz.* RAPD (Table 2), ISSR (Table 3), and DAMD (Table 4) were used in the study. Amplification of RAPD fragments was performed according to standardized methods described by Williams *et al.* (1990). Total of 100 primers from OPA to OPH series (MWG biotech, Germany) were randomly screened out of which 22 were selected based on the resolution and those having more than five bands. The reaction was performed in a 25  $\mu$ l volume containing 2.5  $\mu$ l Taq buffer with MgCl<sub>2</sub> (Bangalore Genei, India), 0.5  $\mu$ l Taq polymerase (3 U  $\mu$ l<sup>-1</sup>) (Bangalore Genei India), 0.5  $\mu$ l dNTPs (2.5 mM each) (Fermentas, USA), 1.5  $\mu$ l primer (10 picomoles  $\mu$ l<sup>-1</sup>), 2.5  $\mu$ l template DNA (20 ng  $\mu$ l<sup>-1</sup>) and the volume was finally made up with 17.5  $\mu$ l nuclease free water (Amresco, USA). Amplification was performed in a thermal cycler (Biometra, Germany) with program of initial denaturation at 94°C for 4 minutes, 42 cycles of denaturation at 94°C for 1 minute, annealing at 38°C for 1 minute, extension at 72°C for 2 minutes, and final extension at 72°C for 6 minutes. The amplification of genomic DNA for ISSR analysis was performed using the primers of Gupta *et al.* (2008) and two primers of UBC (University of British Columbia) series (Table 3). The amplification of genomic DNA for DAMD analysis (Heath *et al.*, 1993) was performed using four DAMD primers (Ranade *et al.*, 2008) (Table 4). All the amplicons generated were resolved on 1.8 to 2% Agarose gel prepared in 1X TBE. The gels were stained with ethidium bromide and

**Table 1.** List of accessions used in the present study.

Sr. No	Accession Name	Pedigree/Source	Latitudes/Longitudes	Sr. No	Accession Name	Pedigree/Source	Latitudes/Longitudes
1	Chharodi 5-1	Chharodi, Gujarat	23°2'N /72°16'E	11	C14	Jhambugoda, Gujarat	22°39'N/73°70'E
2	RRL-MON-1105-C1	Assam	26°N /93°E	12	NBPGR-RAJ-UDJ -905-C1	Udaipur, Rajasthan	27°72'N/75°48'E
3	SKN big			13	C52	Jhambugoda, Gujarat	22°39'N/73°70'E
4	Hansraj		81°36'N /23°46'E	14	<i>Jatropha tanjorensis</i> (Ranchi-1-24)	Ranchi, Jharkhand	23°23'N/85°23'E
5	Urulikanchan			15	Ranchi -1-22	Ranchi, Jharkhand	23°23'N/85°23'E
6	Chhatrapathi	SK Nagar, Gujarat		16	CSMCR1-OR-GANJ-1205-C4	CSMRI, Gujarat	21°46'N/72°11'E
7	C-65	Godhra, Gujarat	22°45'N/73°40'E	17	<i>Jatropha gossypifolia</i>		22°32'N/73°E
8	AFRI- KER-Palak-206-C5	Jodhpur, Rajasthan	26°18'N/73°04'E	18	<i>Jatropha podagrica</i>		
9	SKN-J-2	SK Nagar, Gujarat	81°36'N /23°46'E	19	<i>Jatropha integerrima</i>	Anand, Gujarat	
10	MP seeds	Bhopal, MP	23°16'N/77°36'E				

**Table 2.** RAPD marker analysis data.

Primer name	No. of polymorphic loci	Polymorphism (%)	PIC	Resolving power	Marker Index (MI)
OPA4	11	100	0.901	5.95	
OPA7	9	100	0.818	5.26	
OPA9	9	100	0.755	6.79	
OPA18	14	87.5	0.881	8.45	
OPB10	13	100	0.889	7.95	
OPB11	8	100	0.886	3.15	
OPC8	10	100	0.864	4.95	
OPC15	13	100	0.830	5.90	
OPC18	12	100	0.862	7.16	
OPD5	14	100	0.868	9.37	
OPD14	12	100	0.872	6.85	
OPD17	12	92.3	0.830	3.90	19.07
OPE4	12	92.3	0.877	5.58	
OPE6	12	100	0.783	4.42	
OPF4	9	90	0.803	6.94	
OPF10	15	100	0.892	9.0	
OPG10	12	100	0.875	5.95	
OPG12	8	72.72	0.892	3.69	
OPG14	16	94.11	0.906	11.10	
OPH12	17	100	0.920	7.63	
OPH13	17	100	0.916	10.48	
OPH14	11	100	0.838	5.31	
Total	266	96.72%			
Average	12.09	96.76%	0.862	6.62	

documented using gel documentation system (Bio-Rad, California).

### Data Analysis

Clear and distinct bands amplified by the primers were scored for the presence and absence (0 and 1) of the corresponding band among the genotypes. By comparing the banding patterns of all the accessions, specific bands were identified and genetic purity of *J.tanjorensis* was also confirmed. Various genetic parameters viz., Polymorphism Information Content (PIC) (Bootstein *et al.*, 1980), Resolving power (Rp) (Prevost and Wilkinson, 1999), Marker Index (Nagraju *et al.*, 2001, Powell *et al.*, 1996), Shannon index (i) (Shannon and Weaver, 1949), Observed (na) and effective (ne) no. of alleles, Nei's genetic diversity (h) (Nei, 1973) were calculated.

$$PIC = 1 - \sum f^2$$

Where,  $f$  is the frequency of  $i^{\text{th}}$  allele.

Marker Index (MI) = EMR X DI (av) p, where EMR= Effective Multiplex Ratio= the product of the fraction of polymorphic loci and the number of polymorphic loci for an individual assay. EMR=  $np(np/n)$ ., DI=Diversity Index for genetic markers =  $1 - \sum p_i^2$  where  $p_i$  is the allele frequency of the  $i^{\text{th}}$  allele.  $Di(av)$  =Arithmetic mean heterozygosity =  $\sum Di/n$  where  $n$  is the markers analysed.  $Di$  for polymorphic markers is  $(Diav)p = \sum Di/np$  where ' $np$ ' is the number of polymorphic loci and  $n$  is the total number of loci.

$$Rp = \sum Ib$$

Where,  $Ib$ = Band informativeness and  $Ib = 1 - 2|0.5 - p|$ , where  $p$ = Proportion of genotypes containing the band.

Genetic similarity matrices were generated by Jaccard's coefficient of similarity (Jaccard, 1908) by using the SIMQUAL module of NTSYS-pc 2.02 (Rohlf, 1998). Cluster analysis was performed by agglomerative technique using the Un-weighted Pair Group Method with

**Table 3.** ISSR marker analysis data.

Primer name	Sequence	No. of polymorphic bands	Polymorphism (%)	PIC	Resolving power	Marker Index (MI)
ISSR 2	AGAGAGAGAGAGAGT	9	100%	0.855	3.9	
ISSR 4	GAGAGAGAGAGAGAT	8	88.88	0.831	4.74	
ISSR 7	CTC'TCT CTC'TCT CTC'TT	19	100	0.928	6.00	
ISSR 8	ACA CAC ACA CAC ACA CT	7	77.77	0.844	4.73	
ISSR12	TGT GTG TGT GTG TGT GA	12	100	0.890	6.52	
ISSR 21	AGA GAG AGA GAG AGA GYT	13	86.6	0.911	7.92	
ISSR 22	GAG AGA GAG AGA GAG AYT	10	70	0.915	8.31	
ISSR 23	CTCTCTCT TCT CTC'IRA	20	95	0.928	10.00	7.76
ISSR24	GAGAGAGAGAGAGAGAC	3	50	0.694	2.95	
UBC 841	GAGAGAGAGAGAGAYC	12	100	0.827	3.68	
UBC 826	ACACACACACACACACC	13	92%	0.866	10.00	
Total		126	90%			
Average		11.45	71%	0.863	6.25	

**Table 4.** DAMD marker analysis data.

Primer name	Sequence	No. of polymorphic loci	Polymorphism (%)	PIC values	Resolving power	Marker Index (MI)
33.6	GGTGTAGAGAGGGGT	19	100	0.914	9.32	
HBV	CCTCCTCCCTCCT	9	81.8	0.841	3.16	
HVR	GGAGGTTTICA,	12	92.3	0.898	7.05	
M13	GAGGGTGGCGGTCT	9	90	0.837	3.48	3.46
Total		49	92.45	3.49		
Average		12.25	91.02	0.872	5.75	



Arithmetic Mean (UPGMA) by SAHN clustering function of NTSYSpc 2.02.

Cophenetic correlation and Mantel's tests were carried out by using the COPH and MXCOMP modules of the same software.

PCA analysis was carried out using the EIGEN module and results were expressed as 2D and 3D plots. These plots were constructed by extracting the first three most informative *EIGEN* values that showed the maximum variation.

All the above mentioned variables were calculated individually for all the three markers as well as for RAPD+ISSR, ISSR+DAMD and RAPD+DAMD+ISSR for testing the combined ability of the markers for genetic diversity assessment. Comparison study was made between all the markers for their efficiency in diversity analysis.

## RESULTS AND DISCUSSION

### RAPD Results

The data collected from random amplification of polymorphic DNA with 22 arbitrary primers produced 275 total loci with 2,112 amplicons. Out of the 275 loci produced, 266 were polymorphic, amounting to a total polymorphism percentage of 96.67 (Table 2). Sixteen primers out of the 22 analyzed produced 100% polymorphism. Moreover, 15 out of the 22 primers produced fragments that were specific to

some of the accessions of *Jatropha curcas*. Eleven primers amplified fragments that were common to *J. tanjorensis* and *J. gossypifolia*. The primer OPG-10 amplified a fragment of mol wt 2.5 Kb that was common to many accessions but was intense in *J. tanjorensis* and *J. gossypifolia*, which may indicate its high copy number in the particular accession. Examples of RAPD profile OPG 14 is presented in Plate 1.

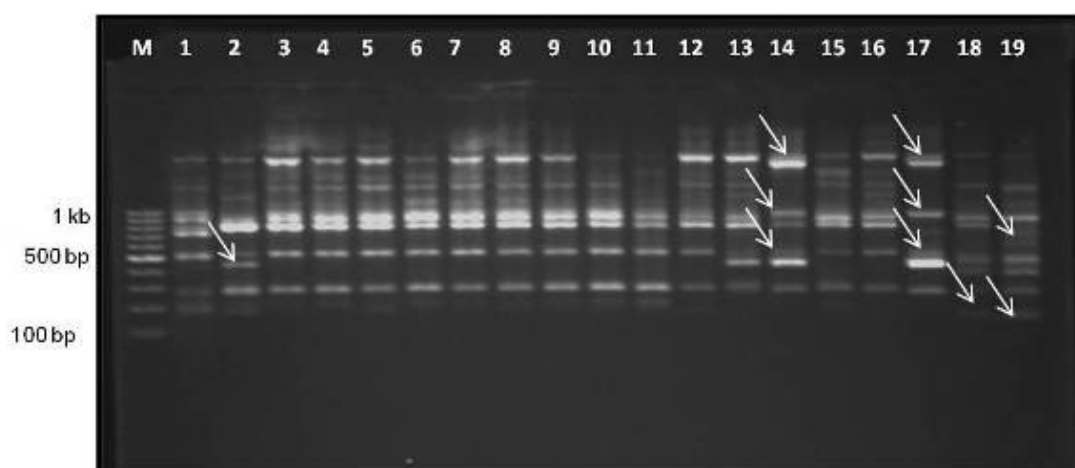
The PIC values ranged from 0.755 to 0.920, indicating hypervariability among the accessions studied. *R<sub>p</sub>* values ranged from 3.15 to 11.1, indicating the variability in the discriminating capacity of the primer.

### Genetic Relationship

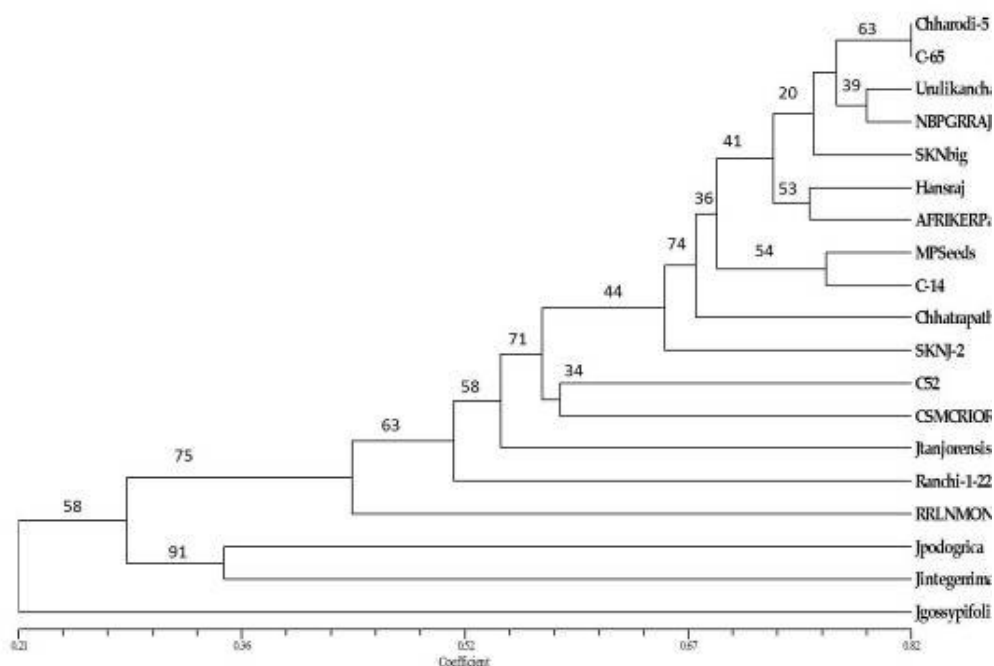
Genetic similarity (GS) matrix generated based on Jaccard's similarity coefficient was found to be in the range of 0.14 (*J. podagrica* and *J. gossypifolia*) to 0.82 (C-65 and Chharodi-5). Within the *Jatropha curcas* accessions, GS value observed were in the range of 0.41 (*J. curcas* cv. CSMCRI-ORGANJ-12 and RRL-MON-1105-C1) to 0.82 (C-65 and Chharodi-5).

### Cluster and PCA Analysis

Clustering pattern revealed three major clusters. *J. integerrima* and *J. podagrica* were included in the same cluster, indicating high



**Plate 1.** RAPD profile of OPG 14 depicting species specific amplicons in all the 19 accessions studied. Some common bands in accession numbers 14 and 17 prove the hybrid nature of *J. tanjorensis*.



**Figure 1.** Dendrogram showing the relationships among the 19 *Jatropha* accessions based on 257 bands of RAPD using Jaccard's coefficient and UPGMA clustering method. X axis values indicate divergence scale coefficient.

similarity between the two, and the remaining accessions were included in the cluster I. *J. gossypifolia* remained as an outlier and formed a separate identity (Figure 1). Thus, due to the high amount of morphological distinctness among the various species of *Jatropha*, the distribution of all the three wild species as separate clusters can be truly justified. Moreover, greater morphological variability of *J. gossypifolia* in comparison to the other two can be attributed to its separate cluster formation (Pamidimarri *et al.* 2009b). *J. tanjorensis*, which is reported to be a naturally occurring male sterile hybrid of *J. curcas* and *J. gossypifolia*, was included in the group with *J. curcas* accessions, indicating its closeness to *J. curcas*. Within the *J. curcas* accessions, accessions from Gujarat and its neighboring regions exhibited lower genetic diversity, whereas those of Ranchi and Assam showed greater variability as deduced from the dendrogram results.

The Principal Component Analysis (PCA) results almost coincided with the results of cluster analysis and the first three components, calculated through EIGEN module of

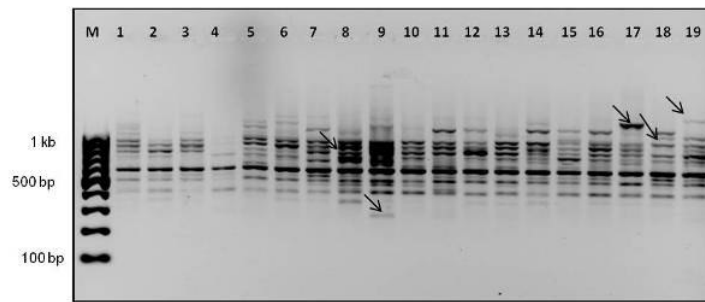
NTSYSpc 2.02, revealed the maximum variation of 83%.

### ISSR Results

ISSR results (Table 3) showed the polymorphism percentage in the range of 50% to 100%. Minimum polymorphism percentage of 50% was recorded by the primer ISSR 24, whereas 100% polymorphism was observed by the primers ISSR 7, ISSR 12, ISSR 2 and UBC 841. Out of the 140 loci observed, 126 were polymorphic and showed the average polymorphism percentage of 90%.

The PIC values for ISSR markers in the present investigation ranged from 0.827 to 0.928, reflecting a very high allelic diversity among the accessions. *R<sub>p</sub>* values ranged from 2.95 to 10.0, which indicated a considerable variation in accession discriminating power of a primer. An example of ISSR 21 banding pattern is shown in Plate 2.

Total of 80 unique alleles were observed in all the 19 accessions of which 35 alleles were specific to *J. curcas* accessions and the remaining belonged to the wild species.



**Plate 2.** ISSR profile of ISSR 21 depicting species specific amplicons in all the 19 accessions studied.

M: 100bp marker, 1: Chharodi 5, 2: RRL-MON-1105-C1, 3: SKN big, 4: Hansraj, 5: Urulikanchan, 6: Chhatrapathi, 7: C65, 8: AFRI-KER-Palak-206-C5, 9: SKN-J-2, 10: MP seeds, 11: C14, 12: NBPGR-RAJ-UDI -905-C1, 13: C52, 14: *Jatropha tanjorensis*, 15: Ranchi -1-22, 16: CSMCRI-OR-GANJ- 1205-C4, 17: *Jatropha gossypifolia*, 18: *Jatropha podagrica*, 19: *Jatropha integerrima*.

Maximum number of alleles in *J. curcas* accessions was observed in Ranchi-1-22 and RRL-MON-1105-C1. RAPD also showed maximum alleles in RRL-MON-1105-C1. Six alleles were observed which were common to *J. tanjorensis* and *J. gossypifolia*, hinting the possibility of *J. gossypifolia* as a second parent of *J. tanjorensis*. Only one allele of 254 bp was observed in *J. tanjorensis* by the primer UBC 841.

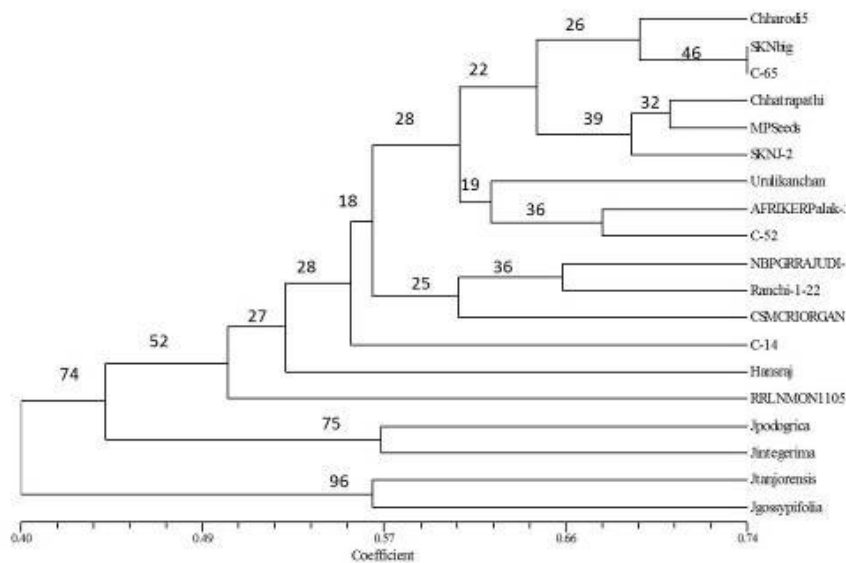
### Genetic Relationships

The values obtained ranged from 0.29 to 0.74, reflecting a high genetic diversity

between the accessions. Genetic similarity values between *J. gossypifolia* and *J. tanjorensis* (0.55) was higher than that observed between *J. tanjorensis* and *J. curcas* accessions which showed average value of 0.48.

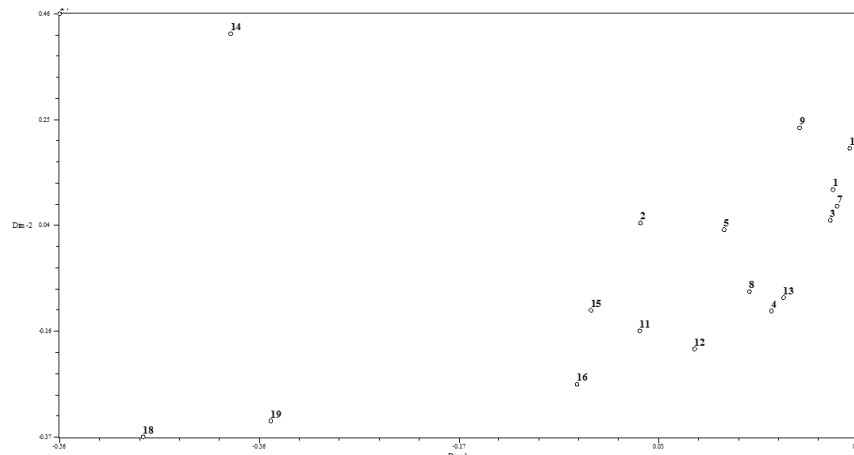
### Cluster and PCA Analysis

The cluster analysis (Figure 2) using UPGMA method revealed three major clusters consisting of *J. curcas* accessions (I), *J. podagrica* and *J. integerrima* (II) and the third cluster of *J. gossypifolia* and *J. tanjorensis*.



**Figure 2.** Dendrogram showing relationships among 19 *Jatropha* accessions using 156 ISSR bands based on Jaccard's coefficient and UPGMA clustering method. X axis values indicate divergence scale coefficient.





**Figure 3.** Two dimensional plot showing relationships among 19 *Jatropha* accessions using 156 ISSR bands and extracting first three PCA components. 1: Chharodi 5, 2: RRL-MON-1105-C1, 3: SKN big, 4: Hansraj, 5: Urulikanchan, 6: Chhatrapathi, 7: C65, 8: AFRI-KER-Palak-206-C5, 9: SKN-J-2, 10: MP seeds, 11: C14, 12: NBPGR-RAJUDI-905-C1, 13: C52, 14: *Jatropha tanjorensis*, 15: Ranchi-1-22, 16: CSMCRI-OR-GANJ- 1205-C4, 17: *Jatropha gossypifolia*, 18: *Jatropha podagrica*, 19: *Jatropha integerrima*.

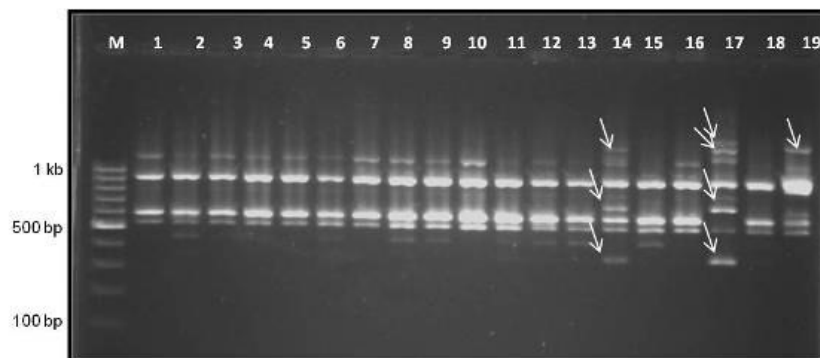
No particular relation pertaining to cluster resolvance and geographical distribution was observed, but RRL-MON-1105-C1 formed a separate cluster as in RAPD. Hansraj and C-14 also formed a separate a cluster, the reason for which could not be ascertained. Hence, some other studies pertaining to morphological characters and quantitative characters need to be carried out which may lead to some better conclusions. *J. gossypifolia* and *J. tanjorensis* were included in the same group. The cluster consisting of *J. podagrica* and *J. integerrima* was found to be similar to that observed in RAPD.

Total variation exhibited by all the three

PCA components was 67%. The results obtained through PCA (Figure 3) produced separate clusters for *J. curcas* accessions and other species. *J. tanjorensis* was closer to *J. gossypifolia*, pointing to its possibility as one of the parents and the result was in accordance with cluster analysis. Similar studies have also been reported in cucurbitacea (Dje *et al.*, 2006), gossypium (Dongre *et al.* 2007) and castor (Gajera *et al.*, 2010).

### DAMD Results

The total number of loci amplified by



**Plate 3.** DAMD profile of HBV depicting species specific amplicons along with some amplicons, which prove the hybrid nature of *J. tanjorensis*.

M: 100bp marker, 1: Chharodi 5, 2: RRL-MON-1105-C1, 3: SKN big, 4: Hansraj, 5: Urulikanchan, 6: Chhatrapathi, 7: C65, 8: AFRI-KER-Palak-206-C5, 9: SKN-J-2, 10: MP seeds, 11: C14, 12: NBPGR-RAJUDI -905-C1, 13: C52, 14: *Jatropha tanjorensis*, 15: Ranchi-1-22, 16: CSMCRI-OR-GANJ- 1205-C4, 17: *Jatropha gossypifolia*, 18: *Jatropha podagrica*, 19: *Jatropha integerrima*.



DAMD primers was 53, with the highest observed in 33.6 and the lowest in M13. Out of the 53 loci amplified, 49 were polymorphic and the highest polymorphic loci were observed in 33.6 and lowest in M13 and HBV (9). The polymorphism percentage obtained ranged from 81.8 to 100%, whereby the highest was obtained for 33.6 and lowest for HBV (Plate 3). Average polymorphism percentage was found to be 92.02.

The PIC values ranged from 0.837 to 0.914 with the lowest observed with primer M13 and the highest with 33.6. The Rp values ranged from 3.16 to 9.32, which indicated a moderate to very high resolving capacity of a primer for all the 19 accessions. The MI value was 3.46.

In total, 36 species specific markers were obtained by using all the four DAMD markers out of which 26 were specific to the wild species including the hybrid and the remaining ten were specific to *J. curcas* accessions (Table 7).

### Genetic Relationship

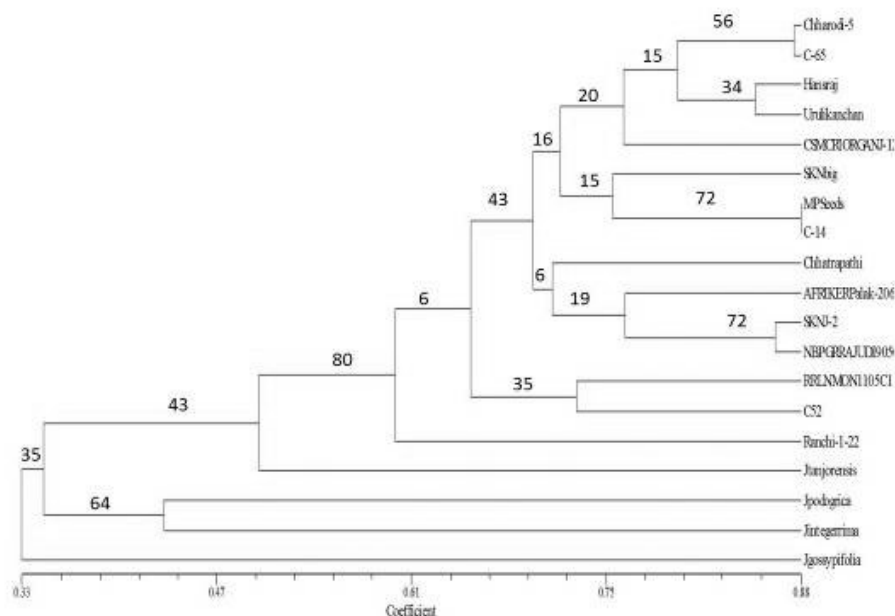
The similarity matrix generated on the basis of Jaccard's coefficient produced values ranging from 0.24 (between *J. gossypifolia*

and *J. podagrica*) and 0.88 (between Chharodi-5 and C-65 and between MP seeds and C-14). The high variation in the values indicated a good amount of variation between the accessions. The average similarity value between *J. curcas* and *J. tanjorensis* was found to be 0.50, whereas between *J. tanjorensis* and *J. gossypifolia*, it was 0.45, pointing to almost-equal contribution of both parents to its hybrid nature.

### Cluster and PCA Analysis

The dendrogram (Figure 4) generated through UPGMA method was resolved into three major groups including group I of *J. curcas* accessions, group II of *J. podagrica* and *J. integerrima*, and group III of *J. gossypifolia* alone. There was no specific relationship between the geographical distribution and clustering pattern, but Ranchi-1-22 and *J. tanjorensis* were resolved into separate clusters as in RAPD. RRL-MON-1105-C1, which was resolved as a separate cluster in RAPD, was clustered with C-52.

Total variation exhibited by all the three components of PCA was 75% (Figure 5). The results obtained through PCA produced



**Figure 4.** Dendrogram showing relationships among 19 *Jatropha* accessions using 56 DAMD bands based on Jaccard's coefficient and UPGMA clustering method.

separate clusters for *J. curcas* accessions and other species. Ranchi-1-22 formed a separate entity as in RAPD.

### Combined Analysis of RAPD, ISSR and DAMD Markers

Combined analysis of all three markers considered for the present study was performed in order to judge the best marker, either individually or in combination for diversity studies in *Jatropha*. Hence, a combined analysis of RAPD+ISSR, ISSR+DAMD and RAPD+DAMD+ISSR was carried out. Cophenetic correlation values indicated very good correlation between all the markers, except between RAPD and ISSR and ISSR and DAMD (Tables 5 and 6).

### Genetic Variability Parameters' Comparison

RAPD marker showed the highest polymorphism of 96.76%, whereas ISSR and DAMD showed almost equal polymorphism of 92.85 and 90.72% (Table 5). In combination studies, RAPD+ISSR and RAPD+ISSR+DAMD showed almost equal polymorphism of approximately 94%, whereas ISSR+DAMD showed approximately 90% polymorphism. Highest species specific markers were found in DAMD i.e.6.5 and highest *J. curcas* accession specific markers were observed in ISSR i.e.3.18. Thus, DAMD marker can be considered better for identification of species specific diagnostic markers (Heath *et al.*, 1993). The combined analysis of RAPD+ISSR, ISSR+DAMD and RAPD+ISSR+DAMD revealed the efficiency of RAPD+ISSR to be better as compared to other combinations (Table 5).

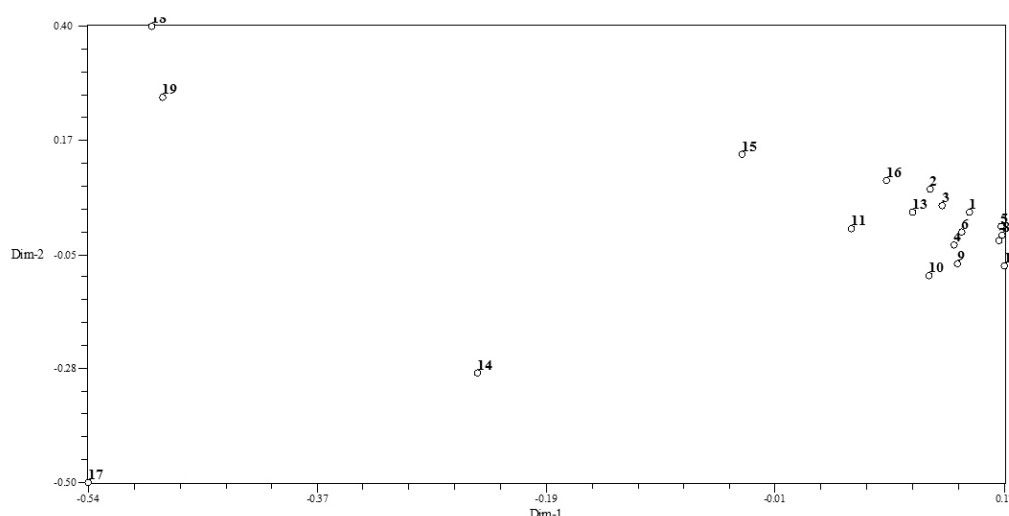
Thus, from the present analysis, it can be concluded that the maximum number of parameters need to be evaluated to judge the efficiency of a marker for diversity analysis.

## DISCUSSION

High variation in *Jatropha* accessions within the species is usually related with geographic

**Table 5.** Combined RAPD, ISSR and DAMD marker data.

Marker	RAPD	ISSR	DAMD	RAPD+ISSR	ISSR+DAMD	RAPD+DAMD+ISSR
Primers used	22	11	4	32	15	36
Total amplicons obtained	2112	1222	461	3215	1683	3676
Total loci analysed	275	140	56	399	196	455
Total No. of monomorphic loci	9	15	4	22	19	26
Total No. of polymorphic loci	266	127	52	377	177	429
Polymorphism (%)	96.76	90.71	92.85	94.48	90.30	94.28
Average No. of bands/primer	12.50	12.72	14	12.46	13.06	12.63
Average No. of polymorphic bands/primer	12.09	11.36	13	11.78	11.8	11.91
Range of band molecular weight	142bp-3.8Kb	110bp-3.0Kb	163bp-2.0Kb	-	-	-
Average Polymorphism Information Content (PIC value)	0.862	0.862	0.873	0.861	0.867	0.865
Resolving power (Rp)	6.62	6.25	5.75	6.63	6.0	6.20
Marker Index	19.17	8.15	3.86	13.09	5.81	10.01
Shannon Diversity Index	0.441	0.444	0.379	0.442	0.398	0.412
Observed no of alleles (na)	1.937	1.987	1.928	1.985	1.918	1.964
Effective no of alleles (ne)	1.455	1.474	1.367	1.489	1.445	1.453
Nei's genetic diversity (h)	0.284	0.288	0.237	0.301	0.274	0.282
No. of species specific bands obtained	72	45	26	-	-	-
	(Average: 3.27)	(Average: 4.09)	Average (6.5)	-	-	-
No. of <i>J. curcas</i> accession specific bands	45	35	10	-	-	-
	(Average: 2.04)	(Average: 3.18)	(Average: 2.5)	-	-	-



**Figure 5.** Two dimensional plot showing relationships among 19 *Jatropha* accessions using 56 DAMD bands and extracting the first three PCA components. 1: Chharodi 5, 2: RRL-MON-1105-C1, 3: SKN big, 4: Hansraj, 5: Urulikanchan, 6:Chhatrapathi, 7: C65, 8: AFRI-KER-Palak-206-C5, 9: SKN-J-2, 10: MP seeds, 11:C14, 12: NBPGR-RAJUDI-905-C1, 13:C52, 14: *Jatropha tanjorensis*, 15: Ranchi-1-22, 16: CSMCRI-OR-GANJ- 1205-C4 , 17:*Jatropha gossypifolia*, 18: *Jatropha podagrica*, 19: *Jatropha integerrima*.

**Table 6.** Cophenetic correlation r values of RAPD, DAMD and ISSR.

Markers	RAPD	ISSR	DAMD
RAPD	<b>0.96</b>	0.777	0.916
ISSR	0.727	<b>0.912</b>	0.822
DAMD	0.889	0.764	<b>0.961</b>

- Below diagonal : Values based on original similarity matrix.
- Above diagonal: Values showing the comparison of cophenetic matrices.
- Diagonal: Values in bold showing the correlation the correlation of cophenetic and original similarity matrices on which the dendrograms were based

**Table 7.** Correlation r values for various marker combinations.

Markers	RAPD+DAMD	ISSR+DAMD	RAPD+DAMD+ISSR	RAPD+ISSR
RAPD	0.995	0.964	0.995	0.755
DAMD	0.901	0.994	0.994	0.875
ISSR	0.776	0.994	0.995	0.847

range, mode of reproduction, mating system, seed dispersal, and fecundity. The genetic diversity detected in the present study may be due to all these prevalent factors. Moreover, the accessions studied were distributed in different geographical regions. The heterozygous and heterogeneous structure of *Jatropha* population driven by its out breeding behavior can also be attributed as one of the major reasons for high variability (Umamaheshwari et al., 2010). The ISSR results obtained in the present study portray

slightly less polymorphism percentage when compared to Vijayanand et al. (2009) and Senthil Kumar et al. (2008), but showed very high polymorphism when compared to Tanya et al. (2011) and Basha and Sujatha (2007). The high Rp values obtained in all the three markers indicates good accession discriminating power of a primer. The species specific markers could be potentially useful in order to identify a *Jatropha* species from any mixed population comprising other members of *Jatropha* complex. These species specific

markers could be a useful target for the development of SCAR markers which will be useful for large scale screening of *Jatropha* accessions (Basha and Sujatha, 2007). Cophenetic matrix comparison studies carried out to compare the genetic similarity and clustering patterns showed a very good fit between RAPD and DAMD markers but comparatively less fit between RAPD and ISSR markers. This may be due to the different genome target sites of the two markers. The combined correlation analysis revealed a very good correlation for all the combinations which all the combinations which included RAPD with RAPD+DAMD and ISSR+DAMD, DAMD with RAPD+DAMD, ISSR+DAMD and ISSR with ISSR+DAMD. All the combinations of RAPD, ISSR and DAMD with RAPD+DAMD+ISSR revealed a very good correlation.

### CONCLUSIONS

It can be concluded from the present study that all three markers were equally efficient for diversity studies. Moreover, it can also be concluded that large number of parameters need to be calculated to judge the best marker as polymorphism percentage, marker index, *PIC*, and *R<sub>p</sub>* values were higher for RAPD marker but the remaining parameters i.e. Shannon index, Observed and effective number of alleles, and Nei's diversity were highest for ISSR marker followed by DAMD. All the three markers *viz.* RAPD, ISSR, and DAMD proved to be the potential tools to carry out future population genetic studies in *Jatropha* germplasm. Also, the phylogenetic and PCA analysis based on RAPD data generated region specific clustering patterns that revealed geographical variation, which may be due to selection pressure exerted upon the accessions due to the differences in the environmental conditions. Such kind of specificity was not observed for accessions from Gujarat and its neighboring regions but only for distant regions like Assam and Ranchi. Thus, to achieve better conclusions, still wider geographic regions with more number of accessions need to be investigated.

The prior investigations that indicated the possibility of *J. tanjorensis* to be a naturally occurring interspecific hybrid between *J. gossypifolia* and *J. curcas* were confirmed by all the three markers. Results of the present investigation can be helpful for future researchers to define the inter- and intra-specific genetic diversity and, also, to detect the hybrids among these species.

The unique alleles obtained can be further investigated through cloning and sequencing approaches and thereby developing even more efficient species specific markers (SCARs) for amplification. These markers along with SSRs can be used for further breeding programs through Marker Assisted Selection and also in selective cultivation of specific variety for species improvement.

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## مقایسه نشانگر های رایید (RAPD)، ISSR، و DAMD برای ارزیابی تنوع ژنتیکی در میان نمونه های *Jatropha curcas* L. و گونه های وابسته به آن

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### چکیده

در این مطالعه، تشخیص ملکولی ۱۹ نمونه *Jatropha* که شامل ۱۵ نمونه *J. curcas* و ۴ گونه متفاوت بود با استفاده از سه سامانه نشانگر مختلف به انجام رسید. حد اکثر "چند شکلی" (پلی مرفیزم) به میزان ۹۶.۶٪ برای رایید ثبت شد و بعد از آن DAMD (۹۱.۰۲٪) و ISSR (۹۰٪). محتوای اطلاعاتی چندشکلی (PIC) برای DAMD بیشترین بود (۰.۸۷۳) و برای RAPD (۰.۸۶۳) و ISSR (۰.۸۶۲) تقریباً یکسان بود. اما، توان تمیز (Rp) برای RAPD بیشتر از دو سامانه نشانگر دیگر بود. مقدار شاخص نشانگر (MI) بسیار متغیر بود و بیشترین آن برابر ۱۹.۰۷ برای RAPD به دست آمد. برای نشانگر ISSR، شاخص شانون ( $i$ )، تعداد آلل مشاهده شده ( $na$ )، تعداد آلل موثر ( $ne$ ) و عدد تنوع ژنتیکی نای ( $h$ ) به طور معنی داری بیشتر از نشانگرهای RAPD و DAMD بود. به این قرار، در مطالعه تنوع ژنتیکی در *Jatropha* همه نشانگر ها به گونه ای برابر کارآمد بودند. چندین آلل در همه نشانگر ها حاکی از آن بودند که *J. gossypifolia* یکی از والد های *J. tanjorensis* است. نمودار شجره ای (دندروگرام) و نمودارهای PCA ترسیم شده بر مبنای RAPD، سه خوشه اصلی را نشان میدادند که *J. integerrima* و *J. podagrica* در گروه I، پانزده نمونه *J. curcas* در گروه II، و *J. gossypifolia* به عنوان مشاهده پرت در گروه III قرار داشتند. نشانگرهای DAMD نیز گروه بندی مشابهی را نشان دادند ولی نشانگر ISSR خوشه آخر *J. gossypifolia* و *J. tanjorensis* را نشان میداد. این نتایج می توانند مبنایی را برای حفاظت و شناسایی منابع ژنتیکی موجود *Jatropha* در آینده تامین نمایند.