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MOLECULAR VARIATIONS AMONG DIFFERENT ACCESSIONS OF *JATROPHA CURCAS* L.

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ABSTRACT

Molecular variations among different accessions 15 *Jatropha curcas* accessions were carried out. The RAPD analysis of accessions showed that the five decamer primers produced a total of 27 scorable bands. Out of them 18 were polymorphic and 9 were monomorphic. The percentage of polymorphism ranged from maximum 77.78 % showed by OPF-08 to a minimum of 60.00 % by OPF-3, OPF-5 and OPF-10. Lowest genetic similarity (0.333) was observed between accessions No. 9 and 1 and 9 and 3 and the highest genetic similarity (1.000) was among various accessions including 2, 4, 5, 6, 7, 10, 11, 12, 13, 14 and 15. The dendrogram of fifteen accessions reflected that at a similarity level of 70.00 %, the accessions were divided in two clusters. The accession No. 9 was found in second cluster having minimum similarity within other accessions whereas rests of accessions were laid in first cluster.

Key Words: RAPD, Accessions, *Jatropha curcas* L.

Introduction

Jatropha curcas L. (Family - *Eurphobiaceae*), known as 'Ratanjyot', has immense potential of producing jatropha oil which finds large scale industrial uses. Although the physic-nut is of Mexican and Central American origin, it is cultivated in many other Latin American, Asian and African countries as a live hedge. The genus *Jatropha* belongs to tribe Joanneisieae of crotonideae in the Euphorbiaceae family and contains approximately 170 known species (Heller, 1996). Nine species of *Jatropha* have been reported in India (Joshi, 2005).

Molecular markers have been looked upon as tools for a large number of applications ranging from localization of a gene to improvement of plant varieties by marker-assisted selection. If we look at the history of the development of these markers, it is evident that they have been improved over the last two decades to provide easy, fast and automated assistance to scientists and breeders. Genome analysis based on molecular markers has generated a vast amount of information and a number of databases are being generated to preserve and popularize it (Joshi *et al.*, 1999).

Most of the early theories of evolution were based on morphological and geographical variations between organisms. However, it is becoming more and more evident that the techniques from molecular biology hold a promise of providing detailed information about the genetic structure of natural population, than what we have been able to achieve in the past (Slatkin, 1987). Furthermore, molecular diversity analysis hold a great promise for revealing more about the pattern of genetic variation within species (Avise, 1994).

As crop improvement programmes have taken due attention on *Jatropha curcas* L. breeding, we should have ample knowledge of its population genetic makeup. We have good amount of germplasm conserved for its improvement, which can be utilized for developing new lines of *Jatropha curcas* L. But before that we must find out the existing homology between the genomes that are compatible, if not what is the reason? The plants having evolutionary relationship should be explored so that we can design plant for intergenetic crop improvement programmes. Keeping in view the above facts and economic importance of the species, present investigation of "molecular variations among different accessions of *Jatropha curcas* L." was undertaken.

Material and Methods

Under the NOVOD sponsored project on "National Network on Integrated Development of *Jatropha*" we received seeds of 16 numbers of accessions of *Jatropha* from 7 centers (States). Six accessions were locally collected by our own centre. Hence, the total number of accessions was 22. From 22 accessions 15 accessions were selected for the study. Their details is given in Table 1. The genomic DNA extraction protocols was

The RAPD analysis of 15 *Jatropha curcas* accessions showed 27 scorable bands which include 18 polymorphic and 9 monomorphic.

standardized and subjected for analyzing existing molecular variations among *Jatropha* accessions. Number of monomorphic band, number of polymorphic band and genetic similarity were recorded. Molecular profile to be subjected to Jaccard's similarity coefficient analysis using NTSYSpc ver 2.2 software.

The leaf samples of *Jatropha curcas* L. from field plants were collected and stored at -20 °C. The genomic DNA was extracted following the CTAB (Cetyldimethylethyl Ammonium Bromide) method of Keim *et al.* (1988) with some modifications. The genomic DNA samples extracted from *Jatropha curcas* L. leaves were subjected to PCR amplification. Amplification was carried out in a 200 µl thin walled PCR tube containing a 25 µl reaction mix volume.

The reaction volume of 25 µl was subjected to amplification through PCR in a thermal cycler (Eppendorf) along with a control (without genomic DNA). Prior to amplification, reaction mixture was gently tapped and spun briefly.

The genomic DNA amplified using random primers of OPL, OPM, OPF and OPG series (Operon Tech., California, USA). The PCR reactions for RAPD were carried out in a 25 µl of reaction mixture as described by William *et al.* (1990).

The PCR amplification was carried out under following thermal cycling regime :

1)	Initial Denaturation	:	94° C for 5 minutes	
2)	Annealing	:	94° C for 1 minute	30 times
			35° C for 45 seconds	
			72°C for 1 minute	
			94° C for 1 minute	30 times
			36° C for 50 seconds	
			72° C for 1.30 minute	
3)	Final Extension	:	72° C for 7 minutes	
4)	Hold	:	at 4° C	

The amplified product was loaded on to 1.5 percent (w/v) agarose gel containing 5 µl ethidium bromide prepared in 0.5X TBE (pH 8.0). The required volume of 0.5X TBE (pH 8.0) was used as running buffer. Whole of the 25 µl PCR amplified product was mixed with 6X gel loading dye of which 15 µl was loaded in well. Alongwith the samples 'O'Range Ruler 500bp ladder ready to use molecular weight premix DNA ladder was also loaded. A potential difference of 5-6 V/cm was provided till the bands resolved properly.

The band profiles were visualized and documented using gel documentation system (Syngene). For each locus the presence and absence of the polymorphic band was recorded as 0 and 1 respectively.

Band positions for each *Jatropha* accessions and primer combination were scored as either present (1) or absent (0). The scores were entered into a database programme (Microsoft Excel) and compiled in a binary matrix for phylogenetic analysis using NTSYS-pc (Numerical Taxonomy and Multivariate analysis) system version 2.2 by Exeter Software (Rohlf, 2004). The SIMQUALK programme was used to calculated Jaccard's similarity coefficient and a graphical phenogram (dendrogram) of the genetic relatedness among the 15 accessions was produced by means of the unweighted pair group method with arithmetic average (UPGMA) analysis (Sneath and Sokal, 1973).

Results and Discussion

The genomic DNA extracted from each accession was subjected to polymerase chain reaction using random decamers. Initially, a total of 40 primers belonging to OPL, OPM, OPF and OPG series of universal primers set, each consisting of 10 decamers were screened. However, primers from OPL and OPM and OPG showed no amplification may be due to absence of complementary sequence in the genome. Finally, 5 primers *viz.*, OPF-1, OPF-3, OPF-5, OPF-8 and OPF-10 were selected for evaluating molecular differences.

A perusal of data (Table 2) revealed that five decamer primers produced a total of 27 scorable bands in the 15 accessions of *Jatropha curcas* L. out of which 18 were polymorphic and 9 were monomorphic. The percentage of polymorphism ranged from a maximum 77.78 % by OPF-08 to a minimum of 60.00 % by OPF-3, OPF-5 and OPF-10. The RAPD primer OPF-08 can be further used for molecular diversity analysis of accessions of *Jatropha curcas* L.

Genetic similarity

It is evident from Table 3 that the lowest genetic similarity (0.333) was between the accessions 9 and 3 and 9 and 1. The highest genetic similarity (1.000) was between the accessions 4 and 6; 2 and 5, 7, 12, 13, 14, and 15; 5 and 7, 12, 13, 14, and 15; 7 and 12, 13, 14, and 15; 10 and 11; 12 and 13, 14, and 15; 13 and 14, and 15 and 14 and 15.

According to dendrogram (Fig. 1), at a similarity level of 70.00 % the accessions were divided in two clusters. The accession no. 9 found in second cluster was having minimum similarity with other accessions while rests of accessions were laid in first cluster. Moreover, first sub-cluster was again divided into two sub-sub-cluster. The first sub-sub-cluster was consisting 13 accessions and the second one only the accession no. 8. The results showed that accession no. 1, 2, 3, 4, 5, 6, 7, 10, 11, 12, 13, 14 and 15 were found in the same cluster. The

Table 1 : List of accessions selected for the present study

Provenances Name	Name of accessions	State	Latitude	Longitude
Rajpipla	NAUJ-6	Gujarat	21° 53'N	73° 31'E
Navsari	NAUJ-7	Gujarat	20° 57'N	72° 56'E
Sardarkrushi Nagar	SKNJ – 7	Gujarat	24° 13'N	72° 27'E
Sardarkrushi Nagar	SKNJ-11	Gujarat	24° 13'N	72° 27'E
Jabalpur	TFRI-1	Madhya Pradesh	23° 11'N	79° 57'E
Jabalpur	TFRI-2	Madhya Pradesh	23° 11'N	79° 57'E
Dhawad	PKVJ DHW-1	Maharashtra	19° 45'N	75° 55'E
Shirpur Jain	PKVJ SJ-1	Maharashtra	20° 30'N	74° 34'E
Chittorgarh	MPC-CH-B	Rajasthan	14° 13'N	76° 24'E
Jhalawar	MPC-UD-J	Rajasthan	25° 22'N	72° 38'E
Kota	MPC-UD-K	Rajasthan	25° 12'N	75° 52'E
Pantnagar	PANT J SEL-1	Uttranchal	29° 05'N	79° 06'E
Pantnagar	PANT J SEL-2	Uttranchal	29° 05'N	79° 06'E
Ludhiana	PAUJ-1	Punjab	30° 53'N	75° 58'E
Bawal	CCSH-1	Haryana	29° 10'N	75° 43'E

Table 2 : Details of amplification obtained with different RAPD primers in different accessions of *Jatropha curcas* L.

Primer	Number of monomorphic Band	Number of polymorphic Band	Total number of bands	Percentage of polymorphism
OPF-01	1	2	3	66.67
OPF-03	2	3	5	60.00
OPF-05	2	3	5	60.00
OPF-08	2	7	9	77.78
OPF-10	2	3	5	60.00
Total	**9**	**18**	**27**	

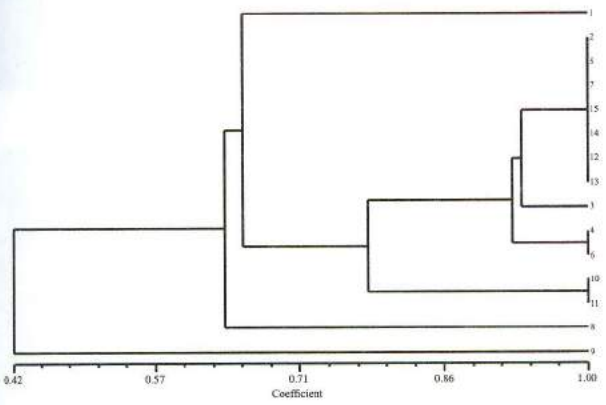


Fig. 1 : Dendrogram depicting the genetic relationship among different accessions of *Jatropha curcas* L. based on RAPD data

results showed that RAPD can be used for evaluating the molecular variation existing among different accessions of *Jatropha curcas* L. Our results showed very less polymorphism among tested accessions. Only accession no. 1, 8 and 9 were found to be diverse. Still a screening of more number of primers is recommended to evaluate the present set of accessions. Moreover, screening of more accessions may also give some diverse accessions. Several workers have used RAPD markers to detect genetic variation *viz.,* Behera *et al.* (2008) in 38 diverse Indian bitter gourd (*Mormordica charantia* var. *charantia* and var. *muricata*) accessions, Shiran *et al.* (2007) in 39 cultivars and species of almond, Syamkumar and Sasikumar (2007) in 15 curcuma species, Faseela and Joseph (2007) in amaranth landraces belonging to three species *viz., Amranthus tricolor, A. dubius* and *A. hypochondriacus,* Zoghlami *et al.* (2007) in 36 Tunisian *Opuntia ficus indica* L. Mill. ecotypes and Surkhosh *et al.* (2006) in 24 genotypes of Iranian pomegranate (*Punica granatuon* L.).

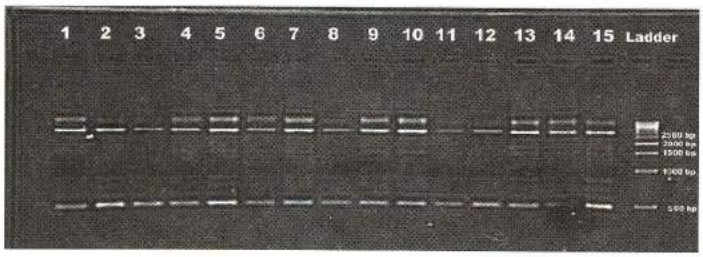
Conclusion

The *Jatropha curcas L.* is an important bio-diesel plant which can be grown on wasteland. On the basis of results obtained from experimentation, it could be concluded that, the five decamer primers produced a total of 27 scorable bands in the fifteen accessions of *Jatropha curcas* L. Out of them 18 were polymorphic and 9 were monomorphic. The percentage of polymorphism ranged from a maximum 77.78 % showed by OPF-08 to a minimum of 60.00 % showed by OPF-3, OPF-5 and OPF-10. The genetic similarity index showed that the lowest genetic similarity (0.333) was between the accessions 9 and 3 and the highest genetic similarity (1.000) was between the accessions 4 and 6; 2 and 5, 7, 12, 13, 14, and 15; 5 and 7, 12, 13, 14, and 15; 7 and 12, 13, 14, and

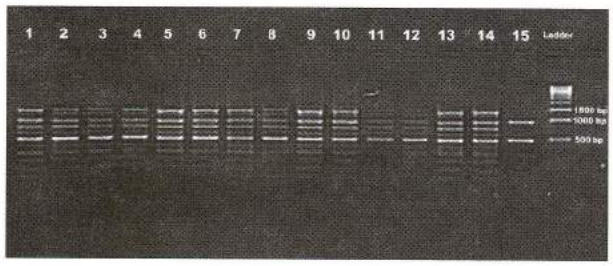
Table 3 : Jaccard's similarity coefficient among different accessions of *Jatropha curcas* L. based on the RAPD data

Accessions	NAUJ-6	NAUJ-7	SKNJ-7	SKNJ-11	TFRI-1	TFRI-2	PKVJ-DHW-1	PKVJ-SJ-1	MPC-CH-B	MPC-UD-J	MPC-UD-K	PANT-J-SEL-1	PANT-J-SEL-2	PAUJ-1	CCSH-1
NAUJ-6	1.000														
NAUJ-7	0.667	1.000													
SKNJ - 7	0.600	0.933	1.000												
SKNJ-11	0.600	0.933	0.867	1.000											
TFRI-1	0.667	1.000	0.933	0.933	1.000										
TFRI-2	0.600	0.933	0.867	1.000	0.933	1.000									
PKVJ-DHW-1	0.667	1.000	0.933	0.933	1.000	0.933	1.000								
PKVJ-SJ-1	0.429	0.667	0.600	0.714	0.667	0.714	0.667	1.000							
MPC-CH-B	0.333	0.400	0.333	0.429	0.400	0.429	0.400	0.600	1.000						
MPC-UD-J	0.692	0.800	0.733	0.733	0.800	0.733	0.800	0.571	0.500	1.000					
MPC-UD-K	0.692	0.800	0.733	0.733	0.800	0.733	0.800	0.571	0.500	1.000	1.000				
PANT-J-SEL-1	0.667	1.000	0.933	0.933	1.000	0.933	1.000	0.667	0.400	0.800	0.800	1.000			
PANT-J-SEL-2	0.667	1.000	0.933	0.933	1.000	0.933	1.000	0.667	0.400	0.800	0.800	1.000	1.000		
PAUJ-1	0.667	1.000	0.933	0.933	1.000	0.933	1.000	0.667	0.400	0.800	0.800	1.000	1.000	1.000	
CCSH-1	0.667	1.000	0.933	0.933	1.000	0.933	1.000	0.667	0.400	0.800	0.800	1.000	1.000	1.000	1.000

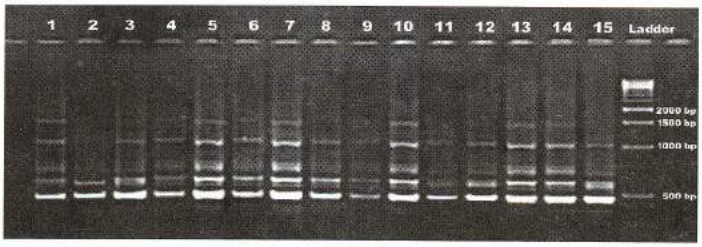
15; 10 and 11; 12 and 13, 14, and 15; 13 and 14, and 15 and 14 and 15. The dendrogram of fifteen accessions reflected that at a similarity level of 70.00 %, the accessions were divided in two clusters. The accession No. 9 found in second cluster was having minimum similarity within other accessions whereas rest of accessions were laid in first cluster. Our results showed very less polymorphism among tested accessions. Only



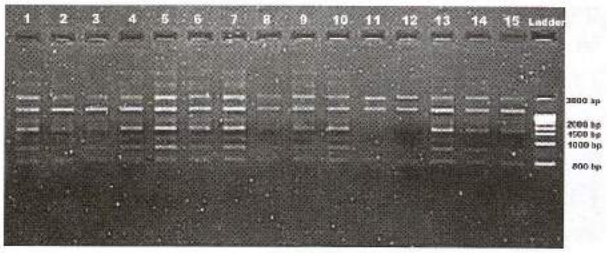
OPF- 1



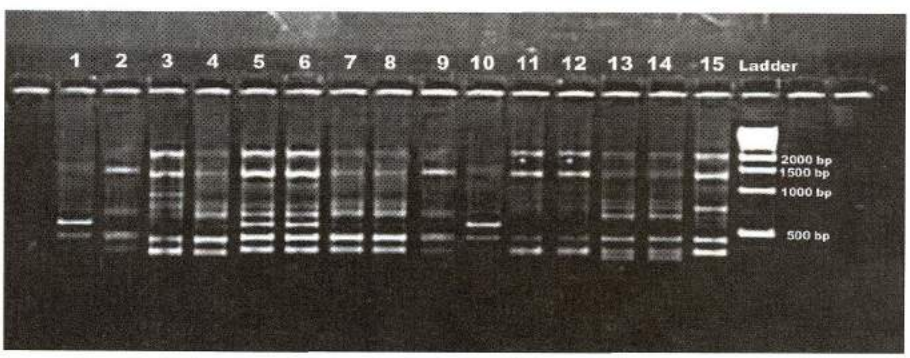
OPF- 3



OPF- 5



OPF- 8



OPF- 10

Plate I : RAPD amplification pattern of different accessions of *Jatropha curcas* L. using primer OPF-1, 3, 5 8 and 10

accession no. 1, 8 and 9 were found to be diverse. Still a screening of more number of primers is recommended to evaluate the present set of accessions. Since, accessions 9, 8 and 1 recorded higher molecular divergence therefore it can be further used for population improvement through breeding programmes.

जैट्रोफा करकास एल. के विभिन्न वर्धकों में आणविक वैविध्य

एम.बी. टंडेल, एम.यू. कुकाड़िया, एम.आर. परमार तथा एन.के. पटेल

सारांश

विभिन्न *जैट्रोफा करकास* मे से 15 वर्धकों में आण्विक वैविध्यों को चयनित किया गया। आर.ए.पी.डी. विश्लेषकों से पता चला कि पांच डिकेमर प्राईमर्स ने कुल 27 स्कोरेबल बैन्ड्स उत्पन्न किये जिनमें से 18 पॉलीमार्फिक तथा 9 मोनोमार्फिक थे। पॉलीमार्फिज्म की प्रतिशतता अधिकतम 77.78 थी जो कि ओ.पी.एफ.-08 में ओ.पी.एफ.- 3, ओ.पी.एफ.-5 तथा ओ.पी.एफ.-10 में न्यूनतम 60% थी। न्यूनतम आनुवंशीय समानता (0.333) वर्धक सं0, 9 और 1 तथा 9 और 3 में थी। जबकि उच्चतम आनुवंशीय समानता (1.000) विभिन्न वर्धकों यथा: 2, 4, 5, 6, 7, 10, 11, 12, 13, 14 और 15 में थी। 15 वर्धकों के डेन्ड्रोग्राम से पता चला कि 70.00% समानता स्तर में वर्धक दो समूहों में विभक्त थे। वर्धक सं0-9 को द्वितीय समूह में पाया गया। जिसकी अन्य वर्धकों के साथ न्यूनतम समानता थी। जबकि शेष वर्धक पहले समूह में थे।

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