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Assessment of Genetic Diversity in Drek *(Melia azedarach)* Using Molecular Markers

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ABSTRACT

RAPD molecular markers were used to evaluate the genetic diversity in populations of *Melia azedarach* Linn. from different locations of Himachal Pradesh and Punjab. Out of the 17 primers used, 13 yielded polymorphic banding patterns. In total, 97 different DNA bands were reproducibly obtained and all showed 100% polymorphism. The polymorphisms were scored and used in band-sharing analysis to identify genetic relationships. Cluster analysis based on Jaccard's similarity coefficient using UPGMA grouped all the 19 populations into three major groups. Similarity indices ranged from 0.24 to 0.92. The highest similarity coefficient detected between progenies of selected mother trees from S_{17} (Dhaulakuan) and S_{18} (Ghagas) and lowest in the populations of S_8 (Bharmar) and S_{12} (Bharatgarh), indicated that drek germplasm within Himachal Pradesh and Punjab constitutes considerably of broad genetic base.

Key words:

Genetic variation, *Melia azedarach*, Mother tree, polymorphism, Progeny, RAPD

INTRODUCTION

Trees of Meliaceae are more useful to human beings for their high quality timbers and for the ease with which they can be grown in plantations. Drek *(Melia azedarach Linn.)* is a deciduous tree and grows up to an elevation of 2000 m in Himalayas. It is also found in Iran, Philippines, Hawaiian Islands, Cuba and China. It is a highly valuable species for its multipurpose importance and recognition as a species of agroforestry. The species is endowed with a high degree of climatic adaptability. It thrives well in hot weather where maximum temperatures are as high as 45^{o}C and tolerates cold below freezing point during winter. The wide geographical and climatic distribution is indicative of the fact that there exists a tremendous genetic diversity which needs to be identified and catalogued.

Relative genetic diversity among the individuals or populations can be determined using morphological and molecular markers. Molecular markers, based on DNA sequence polymorphism, are independent of environmental conditions and show high levels of polymorphism. Of the various kinds of DNA markers characterized so far, restriction fragment length polymorphisms (RFLPs) were the first to provide the means to directly detect variations present at the DNA level. RFLPs give a much higher degree of polymorphism and stability. RFLPs have been used to document genetic diversity in many cultivated plant species. Although highly specific, performing RFLPs is quite laborious and expensive since it requires large amount of pure

DNA and needs an expertise in handling radioactivity. As an alternative, randomly amplified polymorphic DNAs (RAPDs), a PCR based technique, resolved most of the technical impediments owing to its cost- effective and easy to perform approach. This efficient technique obviates the need to work with radioisotopes and gives good results even with crude DNA preparations. RAPDs have therefore been used in assessing genetic variation in several agricultural crops and forest tree species. We report here the application and reliability of RAPD markers to investigate the extent and distribution of genetic diversity in *M. azedarach* from different locations of Himachal Pradesh and Punjab.

MATERIALS AND METHODS

Plant material

Seeds from mother trees of drek were collected/ obtained from 19 different locations in Himachal Pradesh and Punjab. Progenies of these selected sites were observed for molecular studies. A comprehensive list of the drek populations used in the present study along with their latitude and longitudinal position is tabulated in Table 1. The progeny of individual tree was raised in polythene bags filled with equal proportions of sand, soil and vermicompost. The fresh young leaves from the growing tips of seedlings of each tree progeny formed the basic plant material for the present study.

Table 1. Details of trees and their collection regions

Code No.	Site	District	Latitude (N)	Longitude (E)	Altitude (m)
S_1	Bulkhar	Hamirpur	$31^0$67′54″	$76^0$71′59″	1189
S_2	Palu (upper)	Hamirpur	$31^0$68′62″	$76^0$52′12″	375
S_3	Palu (lower)	Hamirpur	$31^0$68′62″	$76^0$52′12″	375
S_4	Bhota	Hamirpur	$31^0$36′26″	$76^0$33′56″	805
S_5	Saloni	Hamirpur	$31^0$34′44″	$76^0$29′39″	375
S_6	Nagrota Surian	Kangra	$32^0$05′58″	$76^0$03′04″	874
S_7	Nagrota Bagwan	Kangra	$32^0$06′36″	$76^0$22′35″	846
S_8	Bharmar	Kangra	$32^0$10′04″	$75^0$57′17″	470
S_9	Jawali	Kangra	$32^0$08′46″	$76^0$0′30″	455
S_{10}	Jwala ji	Kangra	$31^0$52′31″	$76^0$19′27″	610
S_{11}	PAU Campus Ludhiana	Ludhiana	$30^0$54′02″	$75^0$48′37″	244
S_{12}	Bharatgarh	Ropar	$31^0$99′09″	$76^0$61′41″	277
S_{13}	Ropar	Ropar	$30^0$57′59″	$76^0$31′59″	260
S_{14}	Dhanol	Sirmour	$30^0$36′36″	$77^0$27′30″	932
S_{15}	Purwala	Sirmour	$30^0$49′92″	$77^0$68′91″	932
S_{16}	Renuka	Sirmour	$30^0$36′36″	$77^0$27′30″	645
S_{17}	Dhaulakuan	Sirmour	$30^0$33′0″	$77^0$18′0″	468
S_{18}	Ghagas	Bilaspur	$31^0$31′35″	$76^0$80′95″	478
S_{19}	Peer garden	Bilaspur	$32^0$44′0″	$74^0$52′0″	670

DNA Extraction

Nineteen progenies were raised in nursery selected from nineteen different sites. Molecular characterizations of these progenies, after evaluation in the nursery trial, were investigated using RAPD primers. Nuclear DNA was extracted from young leaves using the protocol described by using CTAB method. A total of seventeen decamer primers were used for amplification. Among them 13 primers were polymorphic, which are listed in Table 2.

Table 2. Nucleotide sequences of 13 decamer random primers which showed polymorphism

S. No	Name of Primers	Base Sequences (5'-3')
1	OPA - 01	CAGGCCCTTC
2	OPA - 05	AGGGGTCTTG
3	OPA - 14	TCTGTGCTGG
4	OPA - 20	GTTGCGATCC
5	OPB - 10	CTGCTGGGAC
6	OPC - 06	GAACGGACTC
7	OPC - 08	TGGACCGGTG
8	OPF - 07	CCGATATCCC
9	OPF - 15	CCAGTACTCC
10	OPO - 17	GGCTTATGCC
11	OPS - 03	CAGAGGTCCC
12	OPS - 10	ACCGTTCCAG
13	OPAC - 20	ACGGAAGTGG

DNA Amplification

Arbitrary decamer primers from M/S Bangalore Genei, India Limited were used in the present study. Seventeen primers from Operon Kits were used for RAPD amplification. Amplification was carried out in 50 ml of reaction, 10 ml of genomic DNA, 8 ml of each amplification primers, 15 ml Master-Mix (with $MgCl_2$ 2 mmol/L) and 17 ml deionized water. The PCR cocktail with a total volume of 50.0 ml/ reaction was placed in costar 96 micro-well PCR plates, spinned briefly in a micro centrifuge before it was run using the DNA PCR thermocycler. The 96 well plates were inserted on the top of the machine and then the required temperature profile (Table 3) was programmed and run according to the primer temperature specifications. After amplification the PCR products were stored at 4°C until further analyzed using gel electrophoresis. The PCR products were resolved by running in 1.5% agarose gel. The agarose gel was prepared using 1.5g Agarose, 2 ml 50X TAE Buffer, 98 ml deionized water then heated with Microwave oven for 2 minutes. Ethidium bromide of about 2 μl was added to the gel and then mixed properly. The gel was poured and allowed to settle for 15 to 20 minutes. About 2ml of 10 X loading dye was added to 2-3 ml of each PCR product and loaded on the agarose gel. Electrophoresis was carried out at 90 volts for 1 hour and 30 minutes. After electrophoresis, the amplified DNA fragments were visualized under UV light in Alphaimager.

Table 3. Conditions of PCR reaction

Sr. No.	Reactions		Temperature and Time specifications	Number of cycles
1.	Initial Denaturation		94°C - for 3 min	1
2.	A	Denaturation	92°C - for 45 sec	
	B	Annealing of Primer	36°C - for 1 min	45
	C	Primer amplification	72°C - for 2 min	
3.	Final amplification		72°C - for 10 min	1
	After completion		4°C - till electrophoresis	

Data Analysis

The RAPD products were scored as present (1) or absent (0) for each primer- genotype combination. The data entry was done into a binary data matrix as discrete variables. Jaccard's coefficient of similarity was measured and a dendrogram based on similarity coefficients was generated by using Unweighted Pair Group Method with Arithmetic Mean (UPGMA). The computer package NTSYS-PC was used for cluster analysis.

RESULTS AND DISCUSSION

Out of the 17 decamer RAPD primers screened, only 13 primers were found to produce intensely stained (Table 2) and reproducible polymorphic bands among 19 progenies of selected trees, while the rest of the primers resulted in either no amplification or smeared profiles. The banding pattern of each primer is presented in Table 4. Each primer generated a unique set of amplification products. RAPD analysis revealed high levels of genetic diversity within the reference set of progenies (Table 1). Amplification of genomic DNA of 19 progenies from 13 different random decamer primers generated a total of 97 RAPD markers and all were found polymorphic in nature. Number of amplification products per primer ranged from 4 (OPC- 08, OPO-17 and OPS- 03) to 10 (OPA- 01 and OPS- 10), average number of bands per primer being 7.46. The size of the amplified DNA products separated by electrophoresis in 1.5% agarose gel ranged from 100 bp - 2000 bp. Some primers produced conspicuous unique bands of varying sizes (Table 5).

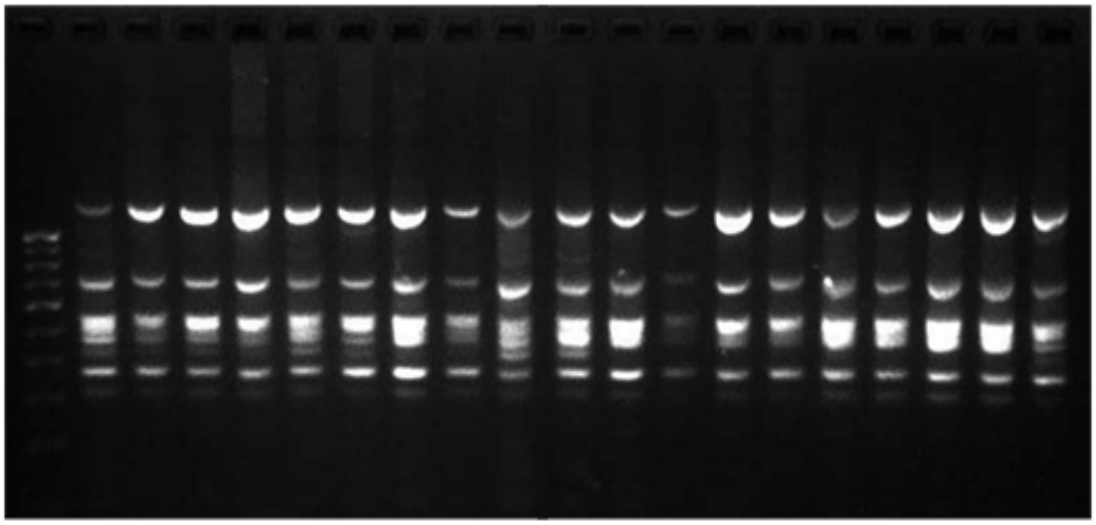

Fig. 1 RAPD fingerprints of 19 progenies revealed by OPA-01

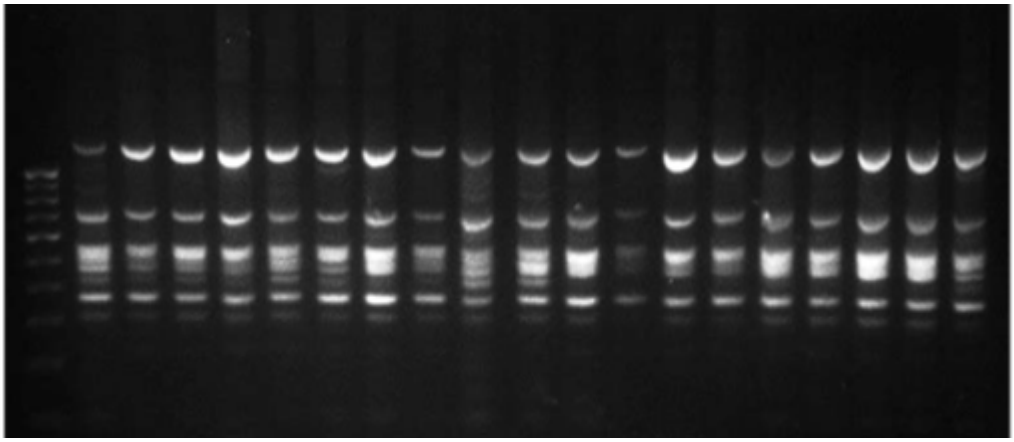

Fig. 2 RAPD fingerprints of 19 progenies revealed by OPB-10

The pair-wise comparison of the RAPD profiles based on both shared and unique amplification products was made to generate a similarity matrix. The RAPD fragments obtained after the amplification of genomic DNA from 19 progenies. The scoring of bands for presence as 1 (band present) and absence as 0 (band absent) for each progeny was done by using Alpha Imager software. The data matrix so obtained was analyzed with NTSYS- PC (Ver. 2.2) software to obtain the Jaccard's similarity correlation coefficient. The coefficient values ranged from 0.24 to 0.92. Such a wide range in similarity coefficient values suggests that the drek germplasm collection represents a genetically diverse population. One of the major contributory factor to the high degree of polymorphism observed in drek may be on account of its evolutionary status as an out-crossing angiosperm. The high diversity revealed by RAPD is in agreement with the fact that out-breeding woody plants retain considerable variability. This was further supported by molecular marker studies. The highest value of similarity coefficient (0.92) was detected between progenies, S17 (Dhaulakuan) and S18 (Ghagas). Our molecular studies, based on Fig.3, showed that at similarity index value of 0.48, S8 (Bharmar) and S12 (Bharatgarh) were separated from the rest of all progenies giving information about their most diverse nature and hence they should be used for hybridization and improvement programmes.

Table 4. Total number of amplified and polymorphic fragments generated by PCR using RAPD primers

S. No	Primer name	Total no. of scorable bands	Total no. of polymorphic bands	Total no. of monomorphic bands	Size range of amplified products (bp)
1	OPA - 01	10	10	0	200-1200
2	OPA - 05	9	9	0	200-1,100
3	OPA - 14	9	9	0	150-1,350
4	OPA - 20	8	8	0	200-1,000
5	OPB - 10	9	9	0	300-1,300
6	OPC - 06	8	8	0	250- 800
7	OPC - 08	4	4	0	200-1,000
8	OPF - 07	8	8	0	150-1,250
9	OPF - 15	5	5	0	400-1,000
10	OPO - 17	4	4	0	550-2,000
11	OPS - 03	4	4	0	300-1,200
12	OPS - 10	10	10	0	200-1,000
13	OPAC - 20	9	9	0	100-1,000
	TOTAL	**97**	**97**	**0**	**100-2,000**

Table 5. Informative RAPD markers specific for a particular progeny

Primer	Approximate size of DNA band	Progeny/ Site
OPA-14	450bp	(Bhota) S_4
	350 bp and 1000 bp	(Salooni) S_5
OPA-20	250 bp and 300 bp	(Nagrota Surian) S_6
	200bp	(Nagrota Bagwan) S_7
OPC-06	800bp	(Bharmar) S_8
OPF-07	500bp	(Bhota) S_4
OPO-17	800bp	(Nagrota Bagwan) S_7
OPS-10	350bp	(Nagrota Bagwan) S_7
	500bp	(Purwala) S_{15}

Cluster analysis based on similarity values classified drek population into three major groups (Fig. 3). Cluster analysis of the drek genotypes employing UPGMA led to the segregation of the accessions into the distinct groups, which reflected their geographical distribution. Ecological and geographical differentiation are important factors which influence breeding and sampling strategies of tree crops, which further helps in understanding the population structure. Variation in genetic diversity is usually related with geographic range, mode of reproduction, mating systems, seed dispersal and fecundity. Muller-strack et al in their review of results of studies on genetic variation in various coniferous and angiospermic tree species observed that species with geographically distinct ranges tend to show a moderate to high- inter and intra-population genetic variation. High genetic diversity detected in the present study may be due to all these prevalent background factors as populations of selected trees studied are widely distributed in different eco-geographic regions. Similar conclusions were reached by Singh et al. while assessing genetic diversity in 37 neem accessions from different agroclimatic regions of India using AFLP and SAMPL molecular markers. They reported that neem germplasm within India constitutes a broad genetic base. Thus the results of present study confirmed that drek being relative of neem germplasm has wide genetic base.

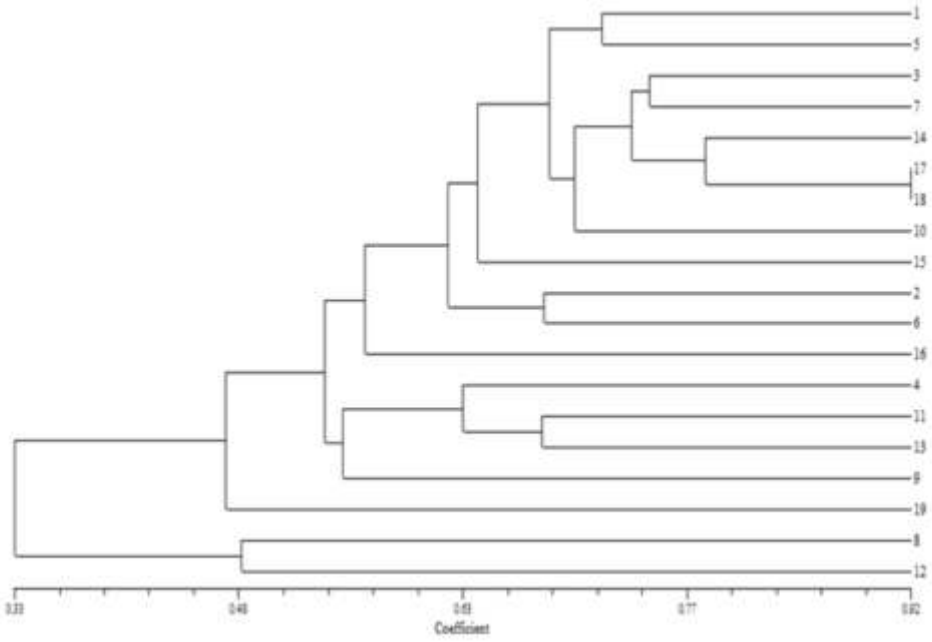

Fig. 3. Dendrogram based on UPGMA analysis of 19 progenies of *Melia azedarach* using RAPD markers

CONCLUSION

Molecular techniques have been found to be more useful and accurate for determination of both interspecific and intraspecific genetic variation in plants. Randomly amplified polymorphic DNA (RAPD) markers, in particular, have been successfully employed for determination of intraspecies genetic diversity in several plants. Besides this, the RAPD technology has received a great deal of attention for genetic diversity studies because of its simplicity and rapidity. The RAPD profile usually represent available portion of the genome. The result thus highlights the utility of RAPD marker in providing information on the genetic structure of *M. azedarach* progenies. Markers were also helpful in characterizing progenies. The clustering pattern exhibited that the geographic distribution provides true index of genetic diversity in progenies of this species.

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