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Genetic polymorphism in *Dalbergia sissoo* Roxb. using RAPD markers

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Genetic diversity in forty nine genotypes of *Dalbergia sissoo* Roxb. was analyzed using randomly amplified polymorphic DNA (RAPD) with 10 decamer primers. A total of 129 distinct DNA fragments (bands) were amplified, of which 109 bands were polymorphic ranged from 200 to 1300 base pair. In fact, the number of amplified bands per genotypes varied from 7 to 14 and polymorphism percentage ranged from 63.63 to 93.33 with an average of 84.93. The mean polymorphic information content (PIC) for RAPD primers was recorded to be 0.24 which ranged from 0.24 to 0.33. The Jaccard similarity coefficient ranged from 0.62 to 0.93 showing wide range of variability among the genotypes. The unweighted pair group method with arithmetic averages (UPGMA) and principle coordinate analysis (PCoA) depicted relationship among the genotypes, which were found to be complimentary to each other. The maximum genetic diversity (0.38) was reported between genotypes FRI/DS/7006 and FRI/DS/0059, whereas genotypes FRI/DS/0218 and FRI/DS/1003 were found to be least divergent (0.07). The genotypes were grouped into eight clusters, off which, cluster II contained maximum twenty genotypes, and cluster VII and VIII comprised just one genotypes each.

Keywords: *Dalbergia sissoo,* RAPD, polymorphism, genetic diversity

Introduction

Dalbergia sissoo Roxb. is an important multipurpose tree species distributed between latitude 21.17°N to 32.60°N and longitude 74.80°E to 93.43°E. Twenty-seven species of *Dalbergia* are found in India, of which fifteen are indigenous and three are endemic[1]. Due to its high economic value, the species is not only widely planted throughout its natural environment, but also planted worldwide as an exotic species, such as in China, Cuba, Brazil, Honduras, etc[2]. *Dalbergia sissoo* Roxb. is a nitrogen fixing leguminous multipurpose tree which thrives well upto an altitude of 1000 msl[3] and is extensively used for timber, shelterbelts and fuel wood in the sub-humid and drier areas[4]. In last two decades, species is under tremendous pressure due to over exploitation in both natural forests and man-made plantations[5]. Further, mass scale death of mature trees caused by *Fusarium solani* has become challenging for very existence of the species. Therefore, it was inevitable to formulate appropriate strategies to understand genetic diversity and population genetic structure of the species for optimal use, both in production forestry and conservation. *D. sissoo* exhibits high intra fruit seed abortion as out of four to five ovules in the flower, hardly one or two get mature and rest usually abort. It was observed that seed abortion is a consequence of intense sibling competition for maternal resources which is an inverse function of the genetic relatedness among developing seeds[6]. Therefore, it becomes essential to understand the genetic diversity and natural distribution pattern of the species for further introduction and genetic conservation. Molecular markers provide information that helps to define distinctiveness of the species and the phylogenetic relationship within a species[7]. The DNA markers provide highly efficient and informative way to characterize diversity at population level[8]. RAPD and ISSR molecular markers have been commonly used in population genetics studies and for detecting clonal diversity in many species[9-13]. The RAPDs have been used for evaluating genetic diversity within populations of *D. sissoo, D. latifolia, D. paniculata*, *D. asamica*, and *D. spinosa*[14-15]. The genetic variation of *D. monticola* is also quantified and analyzed using RAPDs. In order to identify level of diversity, DNA based molecular marker techniques have become indispensable. Though the morphological

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markers for identification of elite genotypes and their commercial use have played a significant role, use of DNA-based markers would further strengthen the process particularly for germplasm characterization. The result presented purports on evaluation of genetic diversity among forty-nine clones of *D. sissoo*. These clones were assembled through selection from natural growing ranges of shisham of India and Nepal.

Materials and Methods

Selection of Genotypes

The selection of plus trees was carried out both in natural forests and man-made plantations in different states of India and Nepal using index method[16]. Using the method, three hundred plus trees were selected and propagated clonally to establish in the clone bank cum vegetative multiplication garden (VMG) at the Division of Genetics and Tree Improvement, Forest Research Institute, Dehradun, India (N30^{0}20’04” and E77^{0}52’12”). In VMG, further selections were carried out based on preliminary growth performance and disease incidences. In that way, a total of 49 clones could only be included along with a check for analysis of molecular diversity through RAPD markers (Table 1).

Collection of Plant Material

The young emerging fresh leaves (foliage) from different genotypes and check were collected and tagged separately to extract DNA. The collected foliage was thereafter labeled appropriately, and stored at -80°C. A detail of the origin of different clones is presented in Table 1.

DNA Extraction

The DNA was extracted using protocols basically described by Doyle and Doyle[17] and Stange *et al*[18] , and later modified by Ginwal and Maurya[19]. The leaves (500 mg) were ground to make a fine powder using liquid nitrogen, which was suspended in 1 ml of CTAB extraction buffer (2% CTAB, 100 mM Tris–HCl (pH 8.0), 20 mM EDTA, 5 mM ascorbic acid, 4% PVP 40, 1.4 M NaCl) and 3 µl β-merceptoethanol followed by incubation at 60°C for 40 min in water bath. By adopting this method, approximately 200-600 ng/µl of DNA/500 mg of foliage material was obtained, which was sufficient for RAPD analysis. The DNA was precipitated by adding 500 µl of cold isopropanol and DNA pellet was washed with 998 µl of 76% ethanol and 2 µl of 10 mM ammonium acetate for 45 min. The DNA pellet was further washed with 70% ethanol and finally re-suspended in 100 µl of Tris-EDTA buffer. The quality of DNA was checked on 1% agarose gel and it was quantified using Bio-Photometer (Eppendorf). The DNA was quantified to a concentration of 5 ng/µl using autoclaved ultrapure water.

Primer Screening

Thirty decamer primers corresponding to Operon (Qiagen Operon, Almeda, California, USA) and Mosseler[20] were initially screened on the basis of amplification results according to the ability to detect distinct, clearly resolved and polymorphic amplified products among randomly selected five genotypes of *D. sissoo* Roxb. Keeping the efficacy in view, screening of the primers was conducted so that informative primer with high polymorphism could be screened. Ten informative RAPD primers were used for evaluation of level of genetic diversity among 49 of *D. sissoo.*

PCR Amplifications

The polymerase chain reaction (PCR) conditions including concentration of $MgCl_2$, dNTPs, Primer, *Taq* DNA polymerase and template DNA were standardized for RAPD analysis for the species. The amplification reaction was performed in a total volume of 25 µl (reaction mixture) containing 1 µl template DNA (5 ng/µl), dNTPs (2.5 mM), decanucleotide primer (20 µM), $MgCl_2$ (25 mM), *Taq* buffer (10X), *Taq* DNA polymerase (1U) and autoclaved distilled water. The amplification was carried out in Bio-Rad Thermal Cycler (Mycycler^{TM}) with initial denaturation at 94°C for 2 min, followed by 41 cycles of denaturation at 94°C for 45 sec, annealing at 37°C for 1 min, extension at 72°C for 1 min and final extension for 10 min at 72°C. The amplification products were resolved on 1.5% (w/v) agarose gel using 1X TBE buffer (Tris HCl, pH 8.0), boric acid, ethylenediamine-tetraacetic acid). Amplification with each of the ten primers was repeated at least twice and only those bands, which occurred consistently and found reproducible, were considered for analysis.

Data Analysis

The amplified bands were visualized using UVP-gel documentation system and were manually scored

Table 1 — Details of the origin of the different genotypes of *Dalbergia sissoo* Roxb.

S. No	Genotypes	Sources (Location, District and States of India)	Latitude	Longitude
1.	FRI/DS/0005	Nolowala block Compartt -7, Chiriyapur , Sobalgarh	30° 15' N	79° 15' E
2.	FRI/DS/0006	Nolowala block Compt -7, Chiriyapur , Sobalgarh	30° 15' N	79° 15' E
3.	FRI/DS/0015	Mohand, Saharanpur, Saharanpur, UttarPradesh	29° 58' N	77° 23' E
4.	FRI/DS/0016	Mohand, Saharanpur, Saharanpur, UttarPradesh	29° 58' N	77° 23' E
5.	FRI/DS/0018	Shah Mansorpur, Shahranpur, UttraPradesh	29° 58' N	77° 23' E
6.	FRI/DS/0020	Shah Mansurpur,Khanpur, Saharanpur, UttraPradesh	29° 58' N	77° 23' E
7.	FRI/DS/0023	C.B. Ganj , Bareilly, Uttara Pradesh	28° 22' N	79° 27' E
8.	FRI/DS/0031	Udaipur, Tulsipur , Gonda (north) Uttra Pradesh	27° 55' N	82° 41' E
9.	FRI/DS/0044	Trilokpur, Tulisipur, Gonda, Uttra Pradesh	27° 55' N	82° 41' E
10.	FRI/DS/0046	Beet, Triolakpur,Gonda(North) Uttra Pradesh	27° 28' N	82° 01' E
11.	FRI/DS/0047	Beet, Triolakpur,Gonda(North) Uttra Pradesh	27° 28' N	82° 01' E
12.	FRI/DS/0059	Dinsia, Khalawala, Ambala, Haryana	30° 21' N	76° 52' E
13.	FRI/DS/0080	Hanumangarh range , compat-12-AKola, Rajasthan	29° 35' N	74° 21' E
14.	FRI/DS/0086	54RD Naurn Desal, Hanumangarh, Rajasthan	29° 35' N	74° 21' E
15.	FRI/DS/0090	5-LK Lakhanwali Vill, Hanumangarh, Rajasthan	29° 35' N	74° 21' E
16.	FRI/DS/0094	5-LK Lakhanwali Vill. Hanumangarh, Rajasthan	29° 35' N	74° 21' E
17.	FRI/DS/0099	Compartment -5LK, Hanumangarh (Rajasthan)	29° 35' N	74° 21' E
18.	FRI/DS/0103	120-121RD, Suratgarh, Hanumangarh, Rajasthan	29° 35' N	74° 21' E
19.	FRI/DS/0107	Head Nursery Burdwal, Hanumangarh, Rajasthan	29° 35' N	74° 21' E
20.	FRI/DS/0138	Bassi Jana Campus NSY, Hoshiarpur, Punjab	31° 33' N	75° 49' E
21.	FRI/DS/0218	Birpur-4A ImliaKhondar, Bhambar, Gonda, U.P.	27° 28' N	82° 01' E
22.	FRI/DS/1003	Forest Research Institute, Dehradun, Uttarakhand	30° 19' N	78° 04' E
23.	FRI/DS/1004	Forest Research Institute, Dehradun, Uttarakhand	30° 19' N	78° 04' E
24.	FRI/DS/5001	Forest Research Institute, Dehradun, Uttarakhand	30° 19' N	78° 04' E
25.	FRI/DS/5002	Forest Research Institute, Dehradun, Uttarakhand	30° 19' N	78° 04' E
26.	FRI/DS/5003	Forest Research Institute, Dehradun, Uttarakhand	30° 19' N	78° 04' E
27.	FRI/DS/5004	Forest Research Institute, Dehradun, Uttarakhand	30° 19' N	78° 04' E
28.	FRI/DS/5006	Forest Research Institute, Dehradun, Uttarakhand	30° 19' N	78° 04' E
29.	FRI/DS/5007	Forest Research Institute, Dehradun, Uttarakhand	30° 19' N	78° 04' E
30.	FRI/DS/5008	Forest Research Institute, Dehradun, Uttarakhand	30° 19' N	78° 04' E
31.	FRI/DS/5009	Forest Research Institute, Dehradun, Uttarakhand	30° 19' N	78° 04' E
32.	FRI/DS/5011	Forest Research Institute, Dehradun, Uttarakhand	30° 19' N	78° 04' E
33.	FRI/DS/5012	Forest Research Institute, Dehradun, Uttarakhand	30° 19' N	78° 04' E
34.	FRI/DS/5017	47-48 Km R/S, Ptiala- Sangrur Road, Sangrur, Punjab	30° 12' N	75° 53' E
35.	FRI/DS/5024	73-74 R/D,, Malerkotla, Sangrur, Punjab	30° 12' N	75° 53' E
36.	FRI/DS/5025	Ghagar Branch Canal ,Lehargaga, Sangrur, Punjab	30° 12' N	75° 53' E
37.	FRI/DS/5026	179-180 L/S, Lehargaga, Sangrur, Punjab	30° 12' N	75° 53' E
38.	FRI/DS/5027	Ghagar Branch Canal 194-195, Sangrur, Punjab	30° 12' N	75° 53' E
39.	FRI/DS/5029	DeraBassi, Patiala, Punjab	30° 2' N	76° 25' E
40.	FRI/DS/1009	Forest Research Institute, Dehradun, Uttarakahnd	30° 19' N	78° 04' E
41.	FRI/DS/1010	Forest Research Institute, Dehradun, Uttarakahnd	30° 19' N	78° 04' E
42.	FRI/DS/1013	Forest Research Institute, Dehradun, Uttarakahnd	30° 19' N	78° 04' E
43.	FRI/DS/5045	Moga, Faridkot, Punjab	30° 35' N	75° 15' E
44.	FRI/DS/5053	Sagarnath, Nepal	27° 58' N	86° 55' E
45.	FRI/DS/7002	Birpur, 4 A, ImaliaKhondar,Gonda, Uttar Pradesh	27° 28' N	82° 01' E
46.	FRI/DS/7006	Hasanpur, 2, Tulisipur, Gonda, Uttar Pradesh	27° 55' N	82° 41' E
47.	FRI/DS/9058	Forest Pathology Division, F R I, Uttarakhand	30° 16' N	78° 07' E
48.	FRI/DS/9064	Forest Pathology Division, FRI, Uttarakhand	30° 16' N	78° 07' E
49.	Control/ check	Local selection	-	

for presence and absence of bands as 1 and 0, respectively. Amplified products ranging from 200 bp to 1300 bp were considered for analysis. The pair wise genetic similarities among the sample pairs were employed to obtain similarity coefficient with Jaccard coefficient[21] since it excludes the negative matches while constructing the similarity matrix. The binary data generated from 10 primers was then subjected to cluster analysis using sequential, agglomerative, hierarchical and nested (SAHN) using unweighted pair group method with arithmetic averages (UPGMA). The final dendrogram was then constructed using NTSYS (PC software, version 2.0 e) as described by Rohlf (2000)[22].

The discriminatory power of all the markers was assessed by evaluating the following parameters: (i) polymorphism information content (PIC); (ii) effective multiplex ratio (EMR), (iii) marker index (MI) and (iv) resolving power (Rp).

i. The PIC was calculated for dominant marker system (RAPD) by following formula[23].

$$PIC_i = \{2f_i(1 - f_i)\}$$

Where, PIC_i is the polymorphism information content of marker i, f_i the frequency of marker fragments, which were present and ($1 - f_i$) the frequency of marker fragments, which were absent. PIC was averaged over the fragments for each primer combination.

ii. EMR was estimated as defined by following formula[24]

$$EMR = n.\beta$$

Where, n is total number of loci, β is the fraction of polymorphic markers and is estimated after considering the polymorphic loci (n_p) and non polymorphic loci (n_{np}) as $\beta = n_p/(n_p + n_{np})$.

iii. MI was calculated following formula as described by Taticanda *et al* (2009)[25].

$$MI = PIC \times EMR$$

Where, PIC is polymorphic information content and EMR is effective multiplex ratio.

iv. The Rp is a feature of primer combination that indicates the discriminatory potential of the primer combination. In this study, Rp of each primer was calculated following formula[26]

$$Rp = \Sigma I_b$$

Where, I_b is band informativeness. The I_b can be represented into a 0-1 scale by the following formula

$$I_b = 1 - [2 \times |0.5 - p|]$$

Where, p is the proportion of all the accessions containing the bands

Results and discussion

Banding Pattern

The RAPD primers revealed amplification of total genomic DNA over the genotypes with polymorphism of 84.93%. The number of amplified products obtained per primer varied from as low as 11 to 15 with an average of 12.9 bands per primer (Table 2 & Fig. 1). The maximum number of polymorphic bands (15) were obtained using OPAG-16, whereas, primer

Table 2 — List of RAPD primers used for the study to calculate the genetic diversity of *Dalbergia sissoo* Roxb.

S. No.	Primer code	Primer sequence	NSB	NMB	NPB	PPB	EMR	PIC	MI	Rp	h*
1	M-182	GTT CTC GTG T	12	2	9	83.30	8.33	0.25	1.58	0.34	0.19
2	M-191	CGA TGG CTT T	11	1	10	90.90	9.09	0.04	0.36	0.04	0.05
3	M-198	GCA GGA CTG C	11	1	10	90.90	9.09	0.24	2.00	0.31	0.20
4	OPA-07	GAA ACG GGT G	13	3	10	76.92	7.69	0.33	2.00	0.51	0.26
5	OPG-09	CTG ACG TCA C	13	2	11	84.61	9.31	0.29	2.23	0.42	0.26
6	OPA-12	TCG GCG ATA G	14	1	13	92.85	12.07	0.26	2.90	0.59	0.26
7	OPAG-16	CCT GCG ACA G	15	1	14	93.33	13.07	0.26	3.14	0.38	0.26
8	OPAF-16	TCC CGG TGA G	11	4	7	63.63	4.45	0.30	0.85	0.53	0.20
9	M-33	CCG GCT GGA A	15	3	12	80.00	9.60	0.19	1.44	0.28	0.16
10	M-122	GTA GAC GAG C	14	1	13	92.85	12.07	0.28	3.14	0.46	0.27
TOTAL			129	19	109	-	-	-	-	-	-
AVERAGE			12.9	1.9	11	84.9	9.48	0.24	1.96	0.39	0.21
MAXIMUM			15	4	14	93.33	13.07	0.33	3.14	0.59	0.27
MINIMUM			11	1	7	63.63	4.45	0.04	0.36	0.04	0.05

Where, NSB: No. of scored band, MB: Monomorphic band, NPB: No. of polymorphic band, PPB: Percentage of polymorphic band, EMR: Effective multiplex ratio, PIC: Polymorphic information content, MI: marker index, Rp: Resolving power and h*: Average expected gene diversity

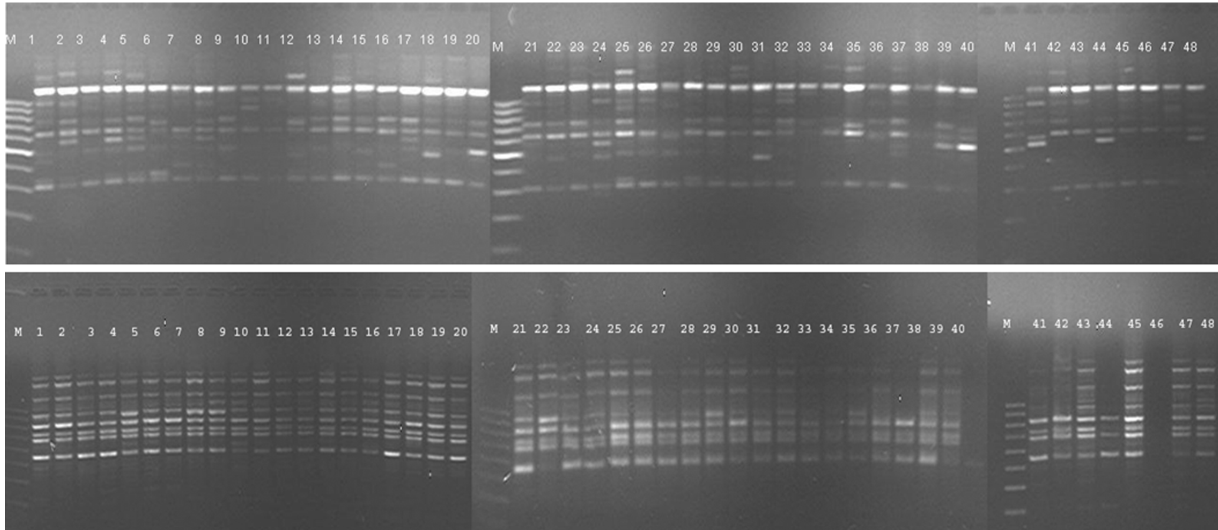

Fig. 1 —.RAPD profile of different clones of *Dalbergia sissoo* (Roxb.) produced with the primer M-182 and M-198 (Lane M is 100 bp ladder)

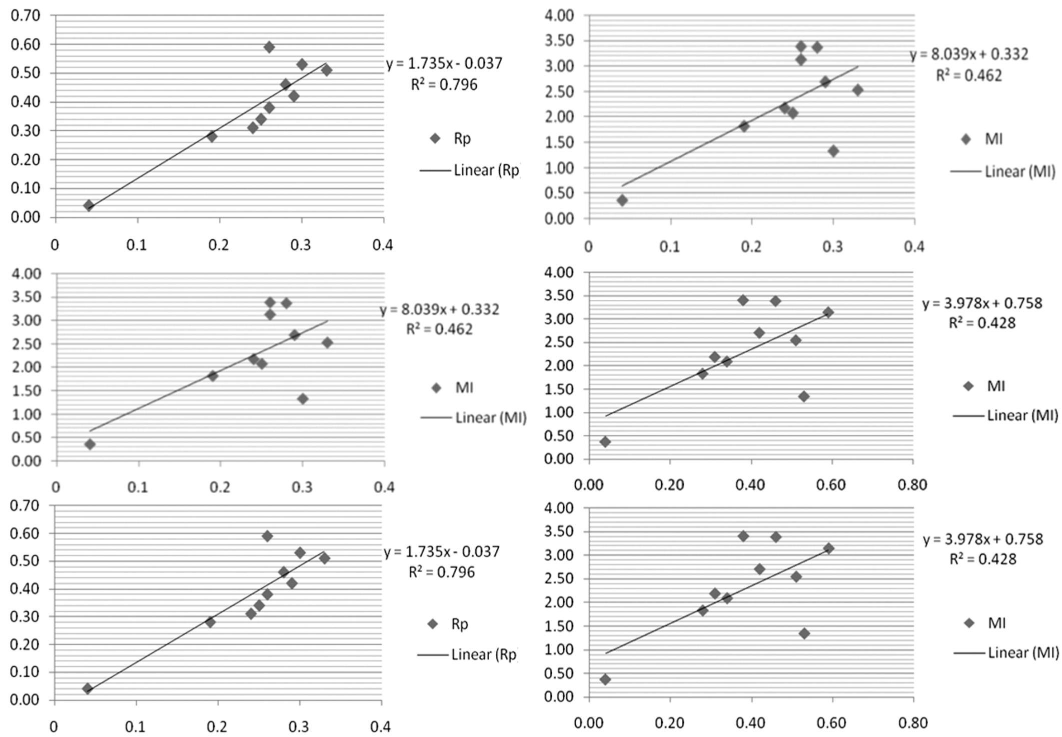

Fig. 2 — Scatter matrix plot of PIC, RP and MI for RAPD assay (above and below diagonally).

OPAF-16 produced least (7) polymorphic bands. The PIC values varied from 0.04 (M-191) to 0.33 (OPA-07), while average expected gene diversity (h*) ranged from 0.05 (M-191) to 0.27 (M-122). The highest PIC (0.33) reported with primer OPA-07 and minimum with primer M-191 (0.04). The Rp values ranged from 0.59 (OPA-12) to 0.04 (M-191) with an average of 0.39 (Table 2). The maximum correlation was thereby reported between PIC and Rp (r^2 = 0.796) followed by PIC and MI (r^2 = 0.462) and the minimum was between Rp and MI (r^2 = 0.428) as demonstrated in Figure 2. Out of total 129 polymorphic bands, 28 polymorphic fragments grouped in 0.40 to 0.50 frequencies. However,

maximum of 51 were found to be in the frequency of 0-0.1 (Fig. 3).

Cluster Analysis

The 48 clones of *D. sissoo* along with one check were subjected to RAPD based markers and analyzed through clustering based on Jaccard similarity coefficient[21] which was used through adopting UPGMA algorithms similarity matrix . The similarity coefficient ranged from 0.62 to 0.93 showing moderate genetic variability among the genotypes. The maximum similarity (0.93) was observed between genotypes FRI/DS/0218 (Gonda, Uttar Pradesh) and FRI/DS/1003 (FRI) and genotypes FRI/DS/0218 and FRI/DS/1004 (FRI), while minimum similarity (0.62) was observed between genotypes FRI/DS/7006 (Gonda, Uttar Pradesh) and FRI/DS/0059 (Ambala, Haryana).

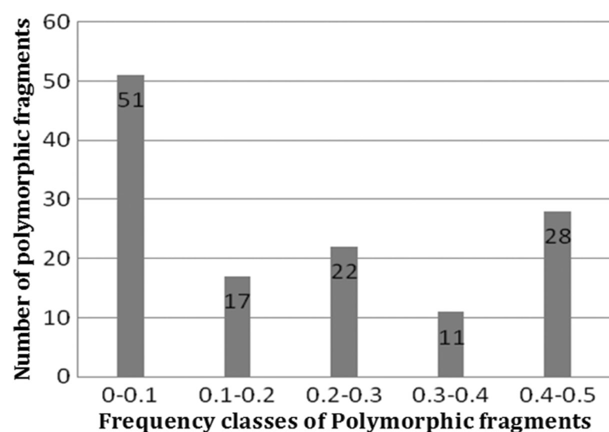

Fig. 3 — Frequency distribution for polymorphic fragments from pooled data in *Delbergia sissoo* genotypes.

The cluster-II comprised maximum twenty genotypes followed by cluster III as detailed in Figure 4 & Table 3. Both the clusters were heterogeneous in nature as contained genotypes originated from different geographical regions. However, all the genotypes from FRI were grouped together only in these two clusters. On the contrary, clusters VII and VIII consisted just one genotypes each as well as demonstrated maximum diversity.

Genetic relationship among genotypes was investigated using different techniques such as

Table 3 — Details of genotypes grouped into the different clusters

Clusters /No. of genotypes	Genotypes	Geographical regions
I (2)	FRI/DS/0005	Nolowala Block Compartt - 7, Chiriyapur, Sobalgarh Range, Uttar Pradesh
	FRI/DS/0018	Shah Mansorpur, Shahranpur, Uttra Pradesh
II (20)	FRI/DS/0006	Nolowala block Compt -7, Chiriyapur , Sobalgarh Range
	FRI/DS/1009	Forest Research Institute, Dehradun, Uttarakahnd
	FRI/DS/5045	Moga, Faridkot, Punjab
	FRI/DS/7002	Birpur, 4 A, Near ImaliaKhondar,Gonda, Uttar Pradesh
	FRI/DS/0020	Shah Mansurpur,Khanpur, Saharanpur, UttraPradesh
	FRI/DS/0094	5-LK Lakhanwali Vill. Hanumangarh, Rajasthan
	FRI/DS/0107	Head Nursery Burdwal, Hanumangarh, Rajasthan
	FRI/DS/0031	Udaipur, Tulsipur , Gonda (north) UttraPradesh
	FRI/DS/0099	Compartment -5LK, Hanumangarh (Rajasthan)
	FRI/DS/0218	Birpur-4A Near ImliaKhondar, Bhambar, Gonda, Uttar Pradesh
	FRI/DS/1003	Forest Research Institute, Dehradun, Uttarakahnd
	FRI/DS/1004	Forest Research Institute, Dehradun, Uttarakahnd
	FRI/DS/0044	Trilokpur, Tulisipur, Gonda, UttraPradesh
	FRI/DS/0023	C.B. Ganj , Bareilly, Uttar Pradesh
	FRI/DS/0103	120-121RD, Suratgarh, Hanumangarh, Rajasthan
	FRI/DS/0138	Bassi Jana Campus NSY, Hoshiarpur, Punjab
	FRI/DS/1013	Forest Research Institute, Dehradun, Uttarakahnd
	FRI/DS/5002	Forest Research Institute, Dehradun, Uttarakahnd
	FRI/DS/5003	Forest Research Institute, Dehradun, Uttarakahnd
	FRI/DS/5026	179-180 L/S, Lehargaga, Sangrur, Punjab

(*Contd*.)

Table 3 — Details of genotypes grouped into the different clusters (*Contd.*)

Clusters /No. of genotypes	Genotypes	Geographical regions
	FRI/DS/0015	Mohand, Saharanpur, Saharanpur, UttarPradesh
	FRI/DS/0016	Mohand, Saharanpur, Saharanpur, UttarPradesh
	FRI/DS/0086	54RD Naurn Desal, Hanumangarh, Rajasthan
	FRI/DS/5012	Forest Research Institute, Dehradun, Uttarakahnd
	FRI/DS/5017	47-48 Km R/S, Ptiala- Sangrur Road, Sangrur, Punjab
	FRI/DS/5024	73-74 R/D,, Malerkotla, Sangrur, Punjab
III (13)	FRI/DS/5004	Forest Research Institute, Dehradun, Uttarakahnd
	FRI/DS/5007	Forest Research Institute, Dehradun, Uttarakahnd
	FRI/DS/5011	Forest Research Institute, Dehradun, Uttarakahnd
	FRI/DS/5006	Forest Research Institute, Dehradun, Uttarakahnd
	FRI/DS/5001	Forest Research Institute, Dehradun, Uttarakahnd
	FRI/DS/5008	Forest Research Institute, Dehradun, Uttarakahnd
	FRI/DS/5009	Forest Research Institute, Dehradun, Uttarakahnd
	FRI/DS/0046	Beet, Triolakpur,Gonda(North) UttraPradesh
	FRI/DS/0047	Beet, Triolakpur,Gonda(North) UttraPradesh
IV (5)	FRI/DS/0059	Dinsia, Khalawala, Ambala, Haryana
	FRI/DS/0080	Hanumaangarh range , compat-12 akola, Rajasthan
	FRI/DS/0090	5-LK Lakhanwali Vill, Hanumangarh, Rajasthan
	FRI/DS/5027	Ghagar Branch Canal 194-195, Sangrur, Punjab
	FRI/DS/5029	DeraBassi, Patiala, Punjab
V (5)	FRI/DS/5053	Sagarnath, Nepal
	FRI/DS/9058	Forest Pathology Division, F R I, Uttarakhand
	FRI/DS/9064	Forest Pathology Division, FRI, Uttarakhand
VI (2)	FRI/DS/5025	Ghagar Branch Canal ,Lehargaga, Sangrur, Punjab
	Control	Local selection
VII (1)	FRI/DS/1010	Forest Research Institute, Dehradun, Uttarakahnd
VIII (1)	FRI/DS/7006	Hasanpur, 2, Tulisipur, Gonda, Uttar Pradesh

UPGMA or Neighbor-Joining clustering algorithm[27-28] and principal coordinate analysis (PCoA)[29]. In this study, principal coordinate analysis (PCoA) based on genetic similarity matrix was used for the understanding of genetic relationships among forty nine genotypes. PCoA revealed that the classification was almost similar to that of UPGMA analysis, and derived a three dimensional scatter plot of genotypes to demonstrate geometrical distances with minimal distortion. The UPGMA based clustering of the genotypes was well supported by PCoA, to resolve different clusters without overlaps (Fig. 5).

The genetic diversity is an essential component for effective implementation of a tree improvement program including synthesis of hybrids. The genetic improvement of the species has not been taken up in a systematic manner and only sporadic works has been carried out mainly limiting to selection of plus trees and propagation. There is a need to conserve species diversity and maintain it at an appropriate level under *ex-situ* conservation repositories like gene banks. In fact, production populations like seed orchards and seed production areas could play important and vital roles in *ex-situ* conservation.

In present study, an attempt was made to ascertain magnitude of genetic diversity among the genotypes of *Dalbergia sissoo* using RAPD markers and results proved to be milestones for future conservation and improvement program of the species. In conclusion, results of this study indicated that RAPDs are sufficiently informative and powerful to assess genetic variability in *D. sissoo* Roxb.

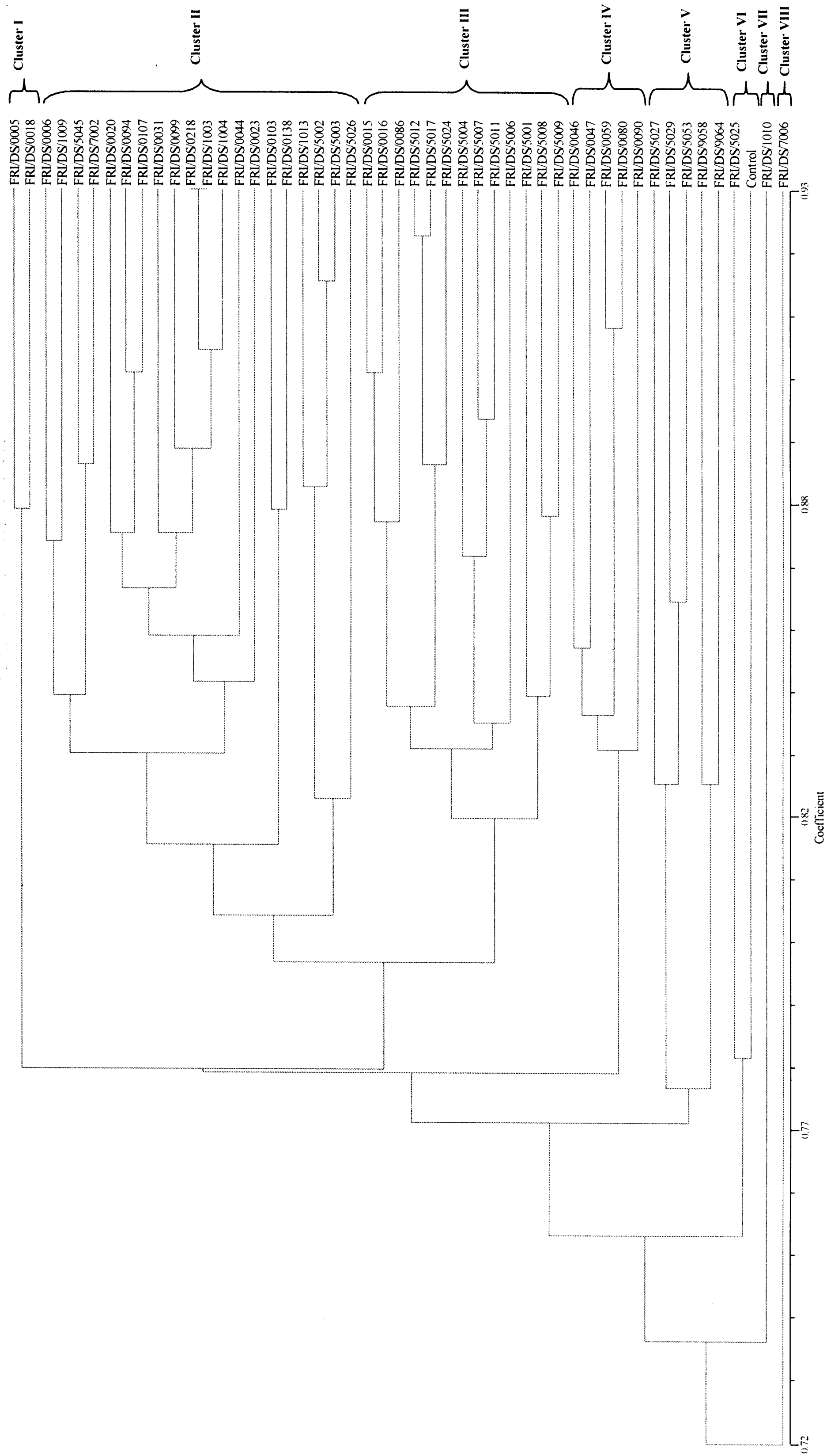

Fig. 4 — Dendrogram obtained from 49 genotypes of *Dalbergia sissoo* Roxb. with UPGMA based Jaccard coefficient.

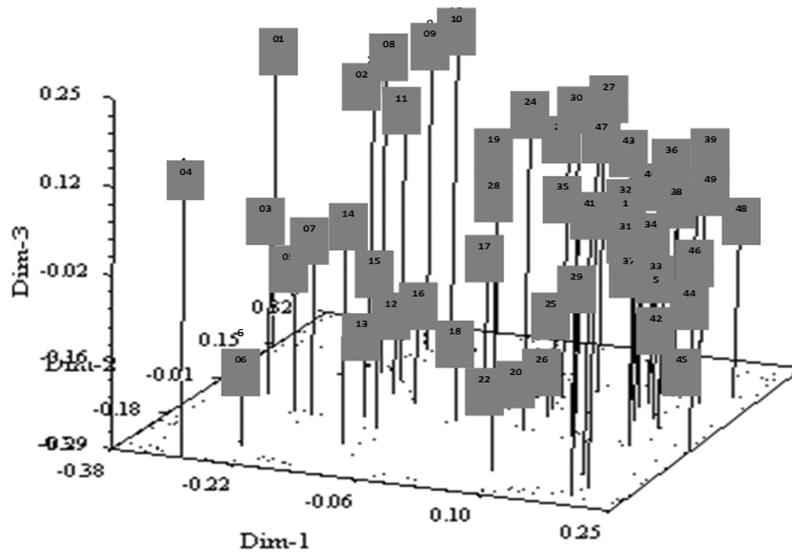

Fig. 5 — Principle co-ordinate analysis (PCoA) map based on RAPD marker for forty nine genotypes of *Dalbergia sissoo* Roxb.

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