African Journal of Biotechnology Vol. 10(35), pp. 6686-6694, 13 July, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB10.2697
ISSN 1684-5315 ©2011 Academic Journals

Full Length Research Paper

Assessment of genetic diversity in different clones of *Dalbergia sissoo* Roxb. by RAPD markers

Ashok Kumar*, Shivani Dobhal and Shruti Sharma

Division of Genetics and Tree Propagation, Forest Research Institute, Dehradun, India.

Accepted 27 May, 2011

Genetic diversity of forty (40) clones of *Dalbergia sissoo* Roxb was analyzed using randomly amplified polymorphic DNA (RAPD) markers by selecting 30 decamer primers, which were later reduced to 10 based on the preliminary PCR amplification. A total of 129 distinct DNA fragments (bands) were amplified, of which 104 bands were polymorphic ranging from 200 to 1300 base pairs. The number of amplified bands per clone varied from 11 to 15 and polymorphism percentage ranged from 18.18 to 100 with an average of 79.23. Jaccard similarity coefficient ranged from 0.66 to 0.95 showing a wide range of variability among the clones. The maximum similarity was found between clone 10 and 12 and the most dissimilar clones were 9032 and 5030 with minimum similarity coefficient of 0.66. The clones were grouped into two clusters which were further divided into sub-clusters. The sub-cluster II consisted maximum of twelve clones followed by cluster I (11 clones) and cluster VI (7 clones) whereas clusters IV, VII and VIII consisted single clone each. In fact, clone 5022 was found to be the most divergent clone which could be used for number of combinations to be established in the seed orchards and as a parent in hybridization programme.

Key words: *Dalbergia sissoo,* genetic divergence, seed orchards, similarity coefficient.

INTRODUCTION

Dalbergia sissoo Roxb. is an important multipurpose tree species distributed between latitude 21.17°N to 32.60°N, longitude 74.80°E to 93.43°E and altitude up to 900 m in the sub- Himalayan tracts of India along the rivers and streams (Tewari, 1994). The species is likely to be indigenous to the sub-Himalayan regions and was introduced elsewhere (Troup, 1921). Twenty-seven (27) species of *Dalbergia* are found in India, of which fifteen (15) are indigenous and three are endemic (Thothathri, 1987). It is also a nitrogen fixing leguminous multipurpose tree that thrives well up to an altitude of 1000 m (Sah et al., 2003) and is extensively used for timber, shelterbelts and fuel wood in the sub-humid and drier areas (Dhakal, 2001). In last two decades, the diversity of the species is under tremendous pressure due to over exploitation both of natural forests and man-made plantations (Newton et al., 2002) and the attack of *Fusarium solani* the cause of dieback disease. Therefore, it is utmost essential to formulate an appropriate plan to understand the genetic diversity and population genetic structure of the species for optimal use both in production forestry and genetic improvement including the conservation plans (Martin and Bermejo, 2000). *Dalbergia sissoo* exhibits high intra fruit seed abortion. Of the four to five ovules in the flower, generally one and occasionally two or three develop to maturity. It has been proposed that the seed abortion is a consequence of intense sibling competition for maternal resources and that this competition occurs as an inverse function of the genetic relatedness among the developing seeds. Accordingly, developing seeds compete intensely when they are genetically less related but tend to develop together when genetically more related (Mohana et al., 2001) detected the diversity by randomly amplified polymorphic DNA (RAPD) and isozymes in Shisham trees. Genetic diversity and natural distribution pattern are very important for the introduction and conservation. Molecular markers provide information that helps to define the distinctiveness of the species and the phylogenetic

*Corresponding author. E-mail: ak_meena@yahoo.co.in or ashok@icfre.org Tel: +91-9411173675 or +91-135-2224379.Fax: +91-135-2756865.

Abbreviations: PIC, polymorphism information content; **CPTs,** candidate plus trees.

relationship within a species (Scheepers et al., 1997). Due to its high economic value, the species is not only widely planted throughout its natural distribution, but also planted worldwide as an exotic species, such as in China, Cuba, Brazil, Honduras, etc (Wang et al., 2011). Extensive studies have shown that the DNA markers provide highly efficient and informative way to characterize the diversity at population level (Karp and Edwards, 1998). Among various markers used, random amplified polymorphic DNA (RAPD) is the simplest, fast and most cost effective molecular markers. RAPD markers provide a larger number of polymorphic bands that are useful for preliminary analysis of genetic diversity (Chunyang, 2000). In order to identify the level of diversity, DNA based molecular marker techniques have become indispensable. Though, the morphological 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 of germplasm characterization. A wide array of molecular techniques has been used in the studies of forest trees environment relationship and variations. Random amplified polymorphism DNA (RAPD) is one of the most popular DNA-based approaches (Bekessy et al., 2002). It is the least technically demanding and offers a fast method of providing information from a large number of loci. Though, lack of reproducibility is considered to be its limitation, but studies show that the results of RAPD can be reproduced in a stable polymerase chain reaction (PCR) system (Wang et al., 2003). The result presented in this study purport to evaluate clonal diversity among the forty (40) individuals of *Dalbergia sissoo*. In fact, the selected clones used in the study were assembled from a wide range of shisham growing regions of India. DNA fingerprinting thus play an important role in advanced breeding, particularly in clonal forestry in providing a role in the release of new genotypes by retaining the identity at the molecular level.

MATERIALS AND METHODS

Selection of clones

The selection of candidate plus trees (CPTs) was carried in both natural forests and plantations in the states of Haryana, Punjab, Uttarakhand and Uttar Pradesh. The index method of selection was adopted to select the plus trees from the CPTs as described by Cotterill and Dean (1990). In this manner, 280 plus trees were selected and propagated clonally to establish the clone bank of the Forest Research Institute, Dehradun, India (Kumar and Luna, 2007). The plus trees were selected using standard method (Cotterill and Dean, 1990) in different states of North India and Nepal. A minimum of 100 m distance was maintained between two plus trees so that sufficient variability could be retained. Their progenies and clonal trials were raised in different geographical locations and on the basis of preliminary growth performance and disease incidences, a total of 40 clones were further screened for understanding the genetic diversity (Table 1).

Collection of plant material

The young emerging fresh leaves (foliage) from all the clones were collected and tagged separately to extract the DNA. The collected foliage was thereafter labeled and stored at -80°C for further experimentation.

DNA extraction

The DNA was extracted using protocol described by Doyle and Doyle (1990) and Stange et al. (1998) modified by Ginwal and Maurya (2009). The leaves (500 mg) were ground to 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 iso-propanol and the DNA pellet was washed with 998 µl of 76% ethanol and 2 µl of 10 mM ammonium acetate for 45 min. Furthermore, rewashing of DNA pellet was done with 70% of ethanol to be re-suspended in 100 µl of Tris-EDTA buffer. The DNA quality was checked by 1% agarose gel electrophoresis visualization for DNA quantity and subsequently it was quantified by Eppendroff Bio-Photometer at 260 nm. The quantitated DNA was then diluted in sterile distilled water to 5 ng/µl concentration for further use in amplification by polymerase chain reaction (PCR).

Primer screening

Thirty (30) decamer primers corresponding to Operon (Qiagen Operon, 1000 Atlantic Avenue, Almeda, California 94501, USA) and Mosseler (1992) 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 clones of *Dalbergia sissoo* (Roxb.). Keeping the efficacy in view, screening of the primers was conducted so that most informative primer with high polymorphism could be screened. Further studies thereafter were carried out only with ten screened RAPD primers, as described (Table 2). A dynamic process was therefore adopted so that genetically diverse genotype could be identified for further tree improvement and breeding programme.

PCR amplifications

The polymerase chain reaction (PCR) conditions including Mg concentration, *Taq* polymerase concentration and extension time were standardized for RAPD analysis for the species. The amplification reactions were performed in a total volume of 25µl (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 BioRad Thermal Cycler (Mycycler™ Thermal Cycler) with initial denaturation at 94°C for 2 min, followed by 41 cycles of denaturing 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 separated on 1.5% (w/v) agarose gel using 1X TBE buffer (Tris HCl pH 8.0, Boric Acid, Ethylenediamine-tetraacetic Acid) at pH 8. 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.

Table 1. Details of the clones used for the analysis of genetic diversity.

S/N	Clone number	Source (Location, District and States of India)	Latitude	Longitude
1	10	Sabalgarh, Pathri, Haridwar, Uttarakhand	29º 58' N	78° 13' E
2	12	Sabalgarh, Pathri, Haridwar, Uttarakhand	29º 58' N	78° 13' E
3	66	Chihrauli, Yamunanagar, Haryana	30º 06' N	77° 17' E
4	218	Birpur-4A,Near,Imlia Khondar, Bhambar, Gonda, Uttar Pradesh	27° 28' N	82° 01' E
5	237	Bankatwa 2, Gonda, Uttar Pradesh	27° 08' N	81° 56' E
6	5010	Forest Research Institute, Dehradun, Uttarakhand	30° 19' N	78°04' E
7	5012	Forest Research Institute, Dehradun, Uttarakhand	30° 19' N	78° 04' E
8	5013	Forest Research Institute, Dehradun, Uttarakhand	30° 19' N	78° 04' E
9	5016	2-3 L/S, Dhuri- Bagaria Road, Malerkotla, Sangrur, Punjab	30° 12' N	75° 53' E
10	5018	65-66 L/S, Sangrur- Barnala Road, Sangrur, Punjab	30° 12' N	75° 53' E
11	5019	6-7, Amargarh-Chaunda Road, Sangrur, Punjab, India	30° 12' N	75° 53' E
12	5020	70-71 L/S, Bhatinda Branch Canal, Malerkotla, Sangrur, Punjab	30° 12' N	75° 53' E
13	5021	Bhatinda Branch Canal 70-71 L/S, Malerkotla, Sangrur, Punjab	30° 12' N	75° 53' E
14	5022	Bhatinda Branch Canal 70-71 L/S, Malerkotla, Sangrur, Punjab	30° 12' N	75° 53' E
15	5023	72-73 L/S, Bhatinda Branch Canal, Malerkotla, Sangrur, Punjab	30° 12' N	75° 53' E
16	5028	Ghagar Branch Canal 200-201, Sangrur, Punjab	30° 12' N	75° 53' E
17	5030	Forest Research Institute, Dehradun, Uttarakhand	30° 19' N	78° 04' E
18	5031	Forest Research Institute, Dehradun, Uttarakhand	30° 19' N	78° 04' E
19	5032	Forest Research Institute, Dehradun, Uttarakhand	30° 19' N	78° 04' E
20	5036	Forest Research Institute, Dehradun, Uttarakhand	30° 19' N	78° 04' E
21	5037	Forest Research Institute, Dehradun, Uttarakhand	30° 19' N	78° 04' E
22	5043	Mukutsar, Faridkot, Punjab	30° 40' N	74°45' E
23	5044	Abohar, Firozepur, Punjab	30° 55' N	74° 40' E
24	5046	Abohar, Firozepur, Punjab	30° 55' N	74° 40' E
25	5050	Abohar, Firozepur, Punjab	30° 55' N	74° 40' E
26	5057	Forest Research Institute, Dehradun, Uttarakhand	30° 19' N	78° 04' E
27	5063	Forest Research Institute, Dehradun, Uttarakhand	30° 19' N	78° 04' E
28	7001	Birpur, 4 A, Near Imalia Khondar, Bhamber, Gonda, Uttar Pradesh	27° 28' N	82° 01' E
29	7003	Sibalgarh, Chiriapur, Haridwar, Uttarakhand	29° 58' N	78° 13' E
30	7004	Bhainsasur, Tulisipur, Gonda, Uttar Pradesh	27° 28' N	82° 01' E
31	7005	Hasanpur, 2, Tulisipur, Gonda, Uttar Pradesh	27° 28' N	82° 01' E
32	7007	Hasanpur, 2, Tulisipur, Gonda, Uttar Pradesh	27° 28' N	82° 01' E
33	9032	Forest Pathology Division, FRI, Dehradun, Uttarakhand	30° 19' N	78° 04' E
34	9049	Forest Pathology Division, FRI, Dehradun, Uttarakhand	30° 19' N	78° 04' E
35	9050	Forest Pathology Division, FRI, Dehradun, Uttarakhand	30° 19' N	78° 04' E
36	9058	Forest Pathology Division, FRI, Dehradun, Uttarakhand	30° 19' N	78° 04' E
37	9063	Forest Pathology Division, FRI, Dehradun, Uttarakhand	30° 19' N	78° 04' E
38	9065	Forest Pathology Division, FRI, Dehradun, Uttarakhand	30° 19' N	78° 04' E
39	9077	Forest Pathology Division, FRI, Dehradun, Uttarakhand	30° 19' N	78° 04' E
40	9093	Forest Pathology Division, FRI, Dehradun, Uttarakhand	30° 19' N	78° 04' E

Scoring of bands and data analysis

The amplified bands were visualized using UVP-Gel Documentation System and were manually scored for presence and absence of bands with 1 and 0, respectively. Amplified products ranging from the 200 to 1300 bp were considered in the analysis. The pair wise genetic similarities among the sample pairs were employed to obtain the similarity coefficient with Jaccard coefficient (Jaccard, 1908) since it excludes out 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 un-weighted pair group method with arithmetic averages (UPGMA). The final dendrogram was then constructed (Figure 1) using the NTSYS (PC Software, version 2.0 e) as described by Rohlf (2000). Genetic diversity was estimated using Nei genetic diversity (Nei, 1973) and resolving power (Rp) of a primer as 1-[2X (0.5-p)] described by Vaishali et al. (2008). The polymorphic information content (PIC) for each marker was determined when allele frequencies are known, which could be calculated statistically on the loci, including

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

S/N	Primer code	Primer sequence	Total band	Mono-morphic band	Poly-morphic	% polymorphism	Average expected gene diversity	PIC value	Resolving power
1	M-33	CCG GCT GGA A	15	2	13	86.67	0.19	0.19	7.36
2	M-122	GTA GAC GAG C	14	2	12	85.71	0.29	0.27	8.95
3	M-182	GTT CTC GTG T	12	0	12	100.00	0.25	0.25	3.90
4	M-191	CGA TGG CTT T	11	9	2	18.18	0.03	0.03	9.80
5	M-198	GCA GGA CTG C	11	4	7	63.64	0.10	0.10	7.60
6	OPA-07	GAA ACG GGT G	13	3	10	76.92	0.27	0.27	8.56
7	OPA-12	TCG GCG ATA G	14	0	14	100.00	0.20	0.19	4.05
8	OPAF-16	TCC CGG TGA G	11	1	10	90.91	0.22	0.22	6.10
9	OPAG-16	CCT GCG ACA G	15	1	14	93.33	0.21	0.21	8.08
10	OPG-09	CTG ACG TCA C	13	3	10	76.92	0.21	0.20	9.41
Total		-	129	25	104	-	-	-	-
average		-	12.9	2.5	10.4	79.3	0.2	0.2	7.38

number of alleles and PIC using thefollowing equation:

Polymorphism information content (PIC) = $1-\sum_{i=1}^{n} p_i^2 - 2\left[\sum_{i=1}^{n-1}\sum_{j=i+1}^{n} p_i^2 p_j^2\right]$

Where, p_i is the frequency of the i^{th} allele, and n is the number of alleles (Botstein et al., 1980). The PIC value ranges from 0 to 1.

The PIC of each marker provides an estimate of the discriminatory power of a locus or loci was calculated by taking into account not only the number of alleles that are expressed but also the relative frequencies of the alleles (Kumar et al., 2003).

RESULTS

RAPD banding pattern

Ten polymorphic RAPD primers were used for the evaluation of level of genetic diversity among forty (40) clones of *Dalbergia sissoo.* These primers generated 129 reproducible RAPD bands with fragments size ranging from 200 to 1300 bp, of which 104 were polymorphic. The percent of polymorphism ranged from 18.18 (M-191) to 100% (M-182 and OPA-12) with average of 79.28 (Table 2). Polymorphic information content (PIC) varied from 0.10 (M-198) to 0.27 (M-122 and OPA-07), while average expected gene diversity ranged from 0.033 (M-191) to 0.285 (M-122) for the clones under investigation (Table 2). The number of amplified products obtained per primer varied from as low as 11 to a maximum of 15 with an average of 12.9 bands per primer (Figure 1).

Cluster analysis

The similarity matrix representing Jaccard similarity coefficient was used for clustering forty clones adopting UPGMA algorithms similarity matrix. The clustering of different clones based on *Jaccard* coefficient is presented in Figure 1. The maximum similarity coefficient was observed between clone 10 and 12 (0.95) both collected from Sabalgarh, Pathri, Haridwar, Uttarakhand while minimum similarity (0.66) was observed between 5030 and 9032 clones. The cluster-1 (C-1) comprised thirty nine (39) of the forty (40) clones and was found to be a major cluster while cluster 2 (C-2) clone (5022) originated from Sangrur, Punjab, India. The C-1 was further sub-divided into seven distinct sub-clusters and named as SC-I to SC-VII. The clones mainly from Forest Research Institute, Uttarakhand in SC-I, except for two clones from Uttar Pradesh (218 and 237) and one from Haryana (66). The two most similar clones (10 and 12) were grouped together in this cluster with maximum similarity of 0.95 (Figure 2). The sub-cluster II similarly, consisted of twelve clones four each from Punjab, Uttar Pradesh and Uttarakhand. Interestingly, sub-clusters III and V consisted of three and four clones, respectively, and they were selected based on disease incidences by the Division of Forest Pathology, FRI, Dehradun. Similarly, sub-

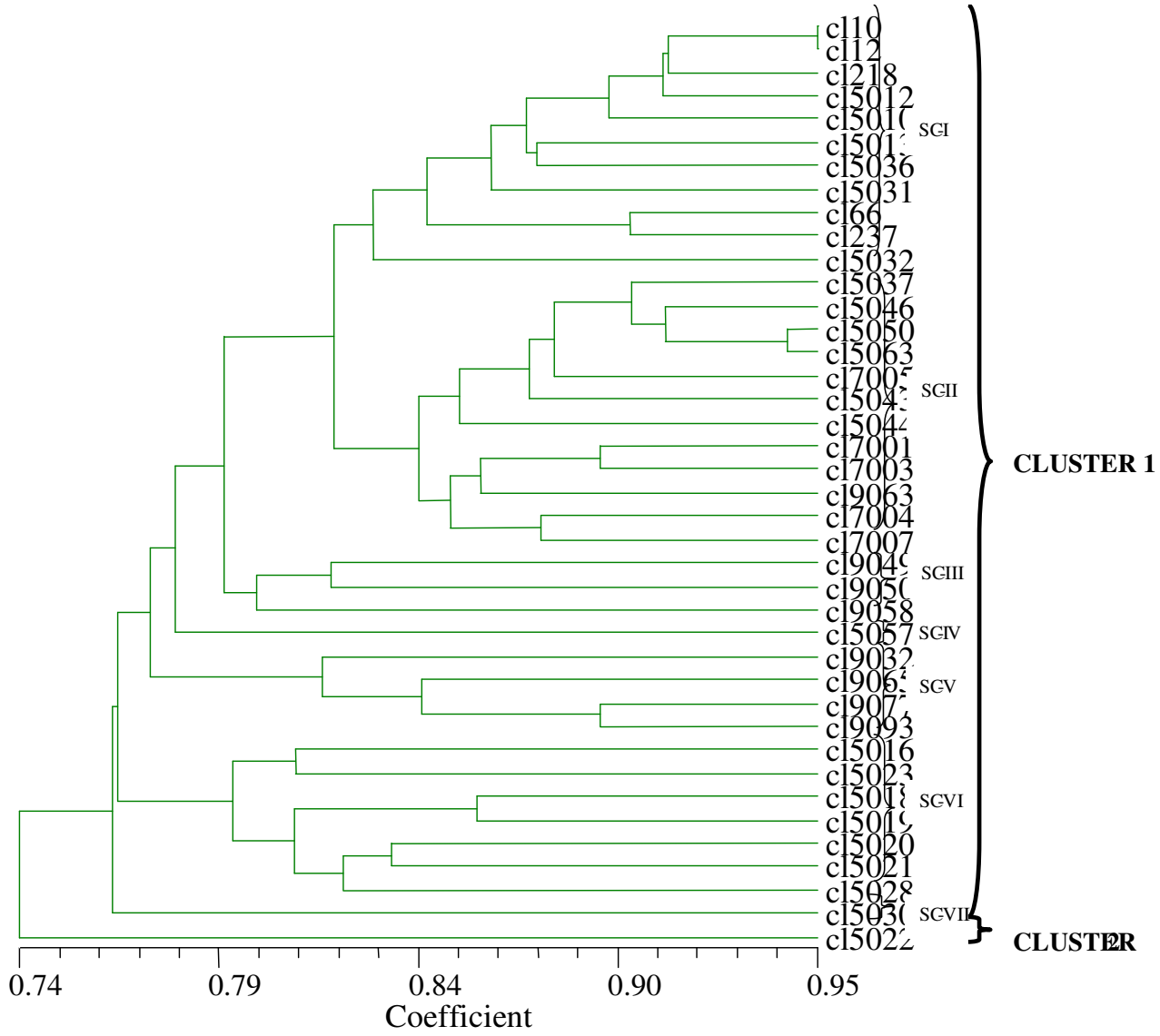

Figure 1. Dendrogram obtained from 40 clones of *Dalbergia sissoo* (Roxb.) with UPGMA based on Jacccard coefficient.

cluster VI consisted of seven clones originated from a single source that is, Sangrur, Punjab. Clones *viz*. 5057 (Uttarakhand) and 5030 (Uttarakhand) clustered into two independent groups (sub-cluster IV and VII), respectively. In fact, clone 5030 originated from Forest Research Institute, Uttarakhand was found to be the most distinct and genetically divergent clone of C-1 and retained minimum similarity (0.66) with clone 9032 (Table 3). Genetic diversity of each individual clone for each primer and average values with respect to all the primers were calculated and are presented in Table 2.

The RAPD primers varied in detecting the genetic diversity of different clones. The highest average expected genetic diversity (0.285) was obtained with primer M-122 while the lowest (0.101) with primer M-198 (Table 2). The highest polymorphism (100%) was reported with primer M-182 and the lowest (18.18%) with primer M-191. Nonetheless, the average polymorphism for ten primers was calculated to 79.30 % (Table 2).

DISCUSSION

The genotypic diversity is an essential component for the effective implementation of a tree improvement program. Keeping both factors in view, 300 plus trees were selected from the natural forests and the man-made plantations in the states of Haryana, Punjab, Rajasthan, Uttar Pradesh and Uttarakhand. On the basis of growth pattern and disease incidences, the selection process was further carried out to select 40 most promising clones that were established in randomized block design (RBD) at different locations to understand the genotypic and environmental interactions. In order to analyze the genetic diversity, forty (40) selected clones were subjected

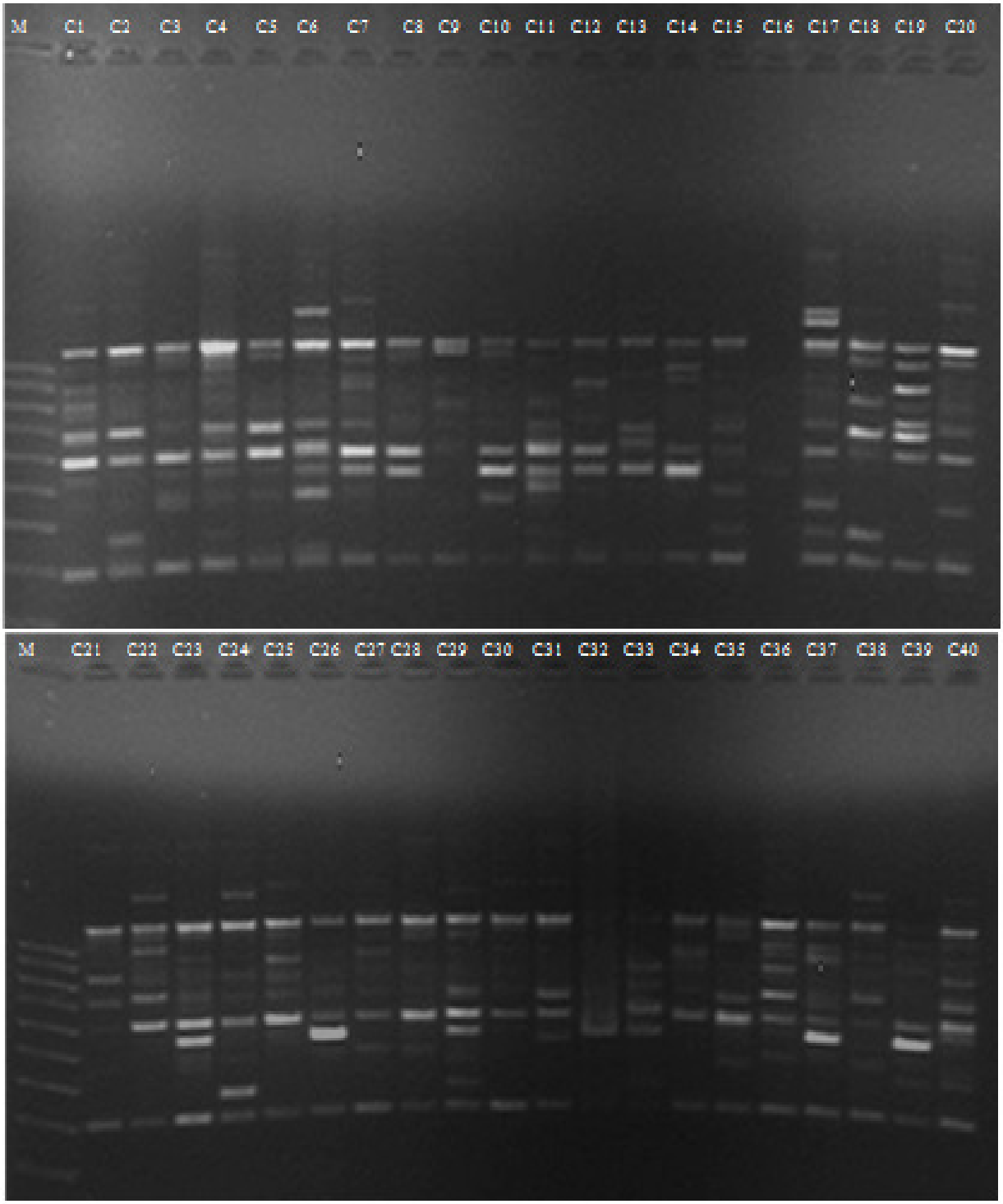

Figure 2. RAPD profile of different clones of *Dalbergia sissoo* (Roxb.) produced with the primer M-182 (Lane M is 100-bp ladder and lanes for Clone 1 to 20 and 21 to 40).

to RAPD markers investigation and analyzed by clustering based on Jaccard Similarity Coefficient (Jaccard, 1908). The tested RAPD primers allowed the discovery of high level of polymorphism (79.30%).

The PIC value for RAPD primers ranged from 0.03 to 0.27 for different primers and concluded that the primers used for this study are informative enough to assess the genetic diversity. In fact, the PIC values not only

Table 3. Similarity matrix based on Jaccard's Coefficient for 40 clones of *Dalbergia sissoo*.

CLONES	C0010	C0012	C0066	C0218	C0237	C5010	C5012	C5013	C5016	C5018	C5019	C5020	C5021	C5022	C5023	C5028	C5030	C5031	C5032	C5036	C5037	C5043	C5044	C5046	C5050	C5057	C5063	C7001	C7003	C7004	C7005	C7007	C9032	C9049	C9050	C9058	C9063	C9065	C9077	C9093
C0010	1.00																																							
C0012	0.95	1.00																																						
C0066	0.84	0.84	1.00																																					
C0218	0.92	0.90	0.88	1.00																																				
C0237	0.86	0.85	0.90	0.88	1.00																																			
C5010	0.89	0.88	0.82	0.91	0.81	1.00																																		
C5012	0.92	0.89	0.85	0.91	0.86	0.90	1.00																																	
C5013	0.88	0.86	0.89	0.90	0.85	0.88	0.85	1.00																																
C5016	0.82	0.81	0.80	0.82	0.77	0.78	0.79	0.84	1.00																															
C5018	0.84	0.82	0.85	0.86	0.81	0.82	0.84	0.87	0.80	1.00																														
C5019	0.80	0.80	0.85	0.78	0.85	0.77	0.78	0.80	0.79	0.86	1.00																													
C5020	0.79	0.77	0.77	0.81	0.77	0.78	0.81	0.79	0.80	0.83	0.83	1.00																												
C5021	0.78	0.77	0.78	0.81	0.76	0.78	0.80	0.81	0.78	0.80	0.77	0.84	1.00																											
C5022	0.73	0.70	0.78	0.76	0.78	0.73	0.75	0.79	0.71	0.75	0.80	0.77	0.76	1.00																										
C5023	0.74	0.77	0.78	0.74	0.77	0.74	0.77	0.78	0.81	0.76	0.79	0.77	0.84	0.75	1.00																									
C5028	0.82	0.82	0.80	0.85	0.78	0.80	0.80	0.78	0.81	0.81	0.83	0.82	0.83	0.78	0.82	1.00																								
5030	0.81	0.82	0.80	0.82	0.74	0.84	0.84	0.79	0.76	0.77	0.75	0.75	0.76	0.75	0.74	0.82	1.00																							
C5031	0.89	0.87	0.84	0.87	0.82	0.82	0.89	0.86	0.78	0.82	0.77	0.79	0.79	0.78	0.78	0.84	0.80	1.00																						
C5032	0.84	0.80	0.83	0.84	0.85	0.81	0.87	0.83	0.75	0.79	0.79	0.80	0.78	0.77	0.78	0.76	0.72	0.85	1.00																					
C5036	0.88	0.86	0.82	0.86	0.81	0.88	0.88	0.88	0.77	0.80	0.81	0.77	0.74	0.78	0.75	0.81	0.82	0.84	0.81	1.00																				
C5037	0.85	0.84	0.82	0.83	0.80	0.83	0.83	0.84	0.77	0.80	0.77	0.73	0.74	0.74	0.81	0.77	0.76	0.78	0.79	0.82	1.00																			
C5043	0.85	0.81	0.77	0.84	0.78	0.84	0.84	0.80	0.73	0.79	0.74	0.72	0.74	0.78	0.77	0.80	0.77	0.82	0.80	0.83	0.90	1.00																		
C5044	0.86	0.86	0.81	0.85	0.78	0.83	0.84	0.79	0.80	0.77	0.73	0.73	0.74	0.68	0.76	0.82	0.76	0.78	0.76	0.81	0.85	0.82	1.00																	
C5046	0.89	0.89	0.81	0.88	0.81	0.88	0.86	0.82	0.79	0.77	0.75	0.71	0.72	0.70	0.74	0.81	0.81	0.84	0.78	0.85	0.89	0.88	0.89	1.00																
C5050	0.85	0.83	0.79	0.83	0.80	0.85	0.84	0.81	0.78	0.77	0.71	0.73	0.73	0.71	0.78	0.75	0.74	0.79	0.82	0.83	0.89	0.87	0.85	0.91	1.00															
C5057	0.78	0.77	0.74	0.78	0.75	0.77	0.80	0.73	0.78	0.76	0.70	0.76	0.80	0.67	0.78	0.75	0.73	0.76	0.74	0.74	0.80	0.76	0.85	0.81	0.82	1.00														
C5063	0.88	0.84	0.79	0.84	0.82	0.86	0.88	0.84	0.77	0.77	0.73	0.74	0.74	0.76	0.78	0.76	0.75	0.82	0.83	0.87	0.91	0.86	0.86	0.90	0.94	0.82	1.00													
C7001	0.85	0.83	0.81	0.87	0.82	0.85	0.87	0.80	0.77	0.80	0.79	0.81	0.76	0.77	0.77	0.82	0.78	0.82	0.78	0.85	0.87	0.88	0.85	0.87	0.87	0.84	0.88	1.00												
C7003	0.84	0.85	0.80	0.84	0.79	0.84	0.84	0.81	0.75	0.79	0.74	0.75	0.71	0.69	0.74	0.78	0.77	0.82	0.77	0.82	0.87	0.80	0.84	0.87	0.85	0.81	0.87	0.89	1.00											
C7004	0.85	0.80	0.79	0.86	0.80	0.85	0.84	0.80	0.77	0.77	0.73	0.73	0.72	0.71	0.71	0.76	0.79	0.82	0.76	0.81	0.83	0.82	0.81	0.88	0.86	0.81	0.85	0.88	0.85	1.00										
C7005	0.83	0.80	0.78	0.83	0.78	0.83	0.81	0.84	0.74	0.75	0.72	0.70	0.72	0.74	0.74	0.77	0.76	0.83	0.79	0.84	0.86	0.85	0.86	0.88	0.88	0.77	0.90	0.84	0.84	0.88	1.00									
C7007	0.81	0.77	0.76	0.80	0.76	0.77	0.78	0.77	0.79	0.77	0.78	0.76	0.74	0.71	0.73	0.83	0.76	0.81	0.74	0.80	0.80	0.79	0.80	0.85	0.83	0.78	0.82	0.85	0.84	0.88	0.85	1.00								
C9032	0.74	0.71	0.71	0.71	0.72	0.71	0.71	0.73	0.70	0.68	0.74	0.71	0.71	0.71	0.75	0.72	0.66	0.77	0.78	0.71	0.79	0.75	0.71	0.73	0.74	0.73	0.78	0.75	0.75	0.78	0.78	0.82	1.00							
C9049	0.77	0.79	0.78	0.77	0.81	0.75	0.77	0.77	0.76	0.70	0.75	0.73	0.74	0.74	0.79	0.73	0.73	0.78	0.78	0.78	0.81	0.76	0.80	0.81	0.84	0.80	0.86	0.84	0.82	0.81	0.83	0.83	0.79	1.00						
C9050	0.79	0.76	0.75	0.78	0.80	0.78	0.80	0.77	0.70	0.73	0.76	0.72	0.73	0.75	0.75	0.74	0.69	0.77	0.80	0.81	0.81	0.81	0.81	0.79	0.79	0.71	0.84	0.83	0.77	0.76	0.82	0.76	0.75	0.82	1.00					
C9058	0.81	0.81	0.80	0.81	0.81	0.74	0.79	0.75	0.72	0.73	0.77	0.74	0.70	0.75	0.71	0.79	0.74	0.82	0.83	0.76	0.79	0.77	0.79	0.81	0.78	0.74	0.79	0.80	0.80	0.79	0.81	0.82	0.78	0.82	0.78	1.00				
C9063	0.88	0.83	0.82	0.86	0.83	0.86	0.88	0.84	0.77	0.79	0.77	0.76	0.77	0.73	0.74	0.78	0.78	0.83	0.79	0.86	0.85	0.82	0.81	0.86	0.84	0.81	0.89	0.87	0.85	0.86	0.85	0.83	0.76	0.78	0.81	0.78	1.00			
C9065	0.76	0.74	0.75	0.76	0.74	0.74	0.76	0.75	0.72	0.73	0.77	0.72	0.70	0.75	0.75	0.78	0.75	0.75	0.74	0.79	0.85	0.81	0.78	0.84	0.79	0.76	0.83	0.85	0.83	0.81	0.79	0.84	0.78	0.82	0.78	0.80	0.84	1.00		
C9077	0.79	0.77	0.77	0.78	0.78	0.74	0.79	0.73	0.73	0.73	0.76	0.72	0.73	0.69	0.78	0.79	0.72	0.76	0.77	0.76	0.85	0.78	0.81	0.79	0.81	0.79	0.83	0.81	0.85	0.79	0.79	0.81	0.85	0.79	0.77	0.78	0.85	0.85	1.00	
C9093	0.74	0.75	0.72	0.73	0.74	0.73	0.74	0.72	0.72	0.71	0.71	0.71	0.70	0.67	0.80	0.74	0.69	0.73	0.75	0.72	0.85	0.81	0.78	0.79	0.82	0.81	0.82	0.81	0.80	0.76	0.78	0.78	0.83	0.81	0.77	0.78	0.81	0.85	0.89	1.00

significantly helped in enabling the authors to narrow down to ten primers from thirty in this study but also identified primers M-122, M-182, OPAF-16 and OPAG-16 which had higher PIC value and amplified products that were more than the average polymorphic (Table 2). In fact, it is expected that these primers with high level of PIC values and polymorphism could potentially be used for large scale screening of the *Dalbergia sissoo* germplasm. In fact, the technique has effectively been used in checking the genetic identity of ramets in the clonal seed orchard of *Pinus thunbergii* (Goto et al., 2001). The RAPD markers were found to be relatively more efficient than ISSR markers in *Dalbergia sissoo* (Arif et al., 2009). The *Jaccard* similarity coefficient varied from 0.66 to 0.95, and in UPGMA based dendrogram the clustering pattern effectively depicts clonal diversity.

The clustering pattern under study revealed considerable genetic differences among the clones (Figure 1). Among different clusters, Cluster 2 showed higher level of diversity in comparison to Clusters 1 including all the sub-clusters. Hence the clone (5022) of this cluster was the most divergent which could easily be exploited for hybridization programme in different combinations. It is utmost essential to select most divergent parents so that maximum heterosis is obtained in the shortest possible time, which could easily be done by selecting the parents from two divergent clusters used in a variety of combinations. It is also important to obtain immediate gain of high diversity by establishing these clones in the clonal seed orchards.

The genetic improvement of this versatile species has not been taken up in a systematic manner and only sporadic works has been carried out mainly directed towards selection of plus trees and clonal propagation. It is a known fact that analysis of genetic diversity is the most important component for any of the breeding and genetic improvement programme that basically deals with ecosystem stability and forest sustainability (Libby, 1973). The analysis of genetic diversity becomes still more valuable in tree improvement programme (Zobel, 1971) owning to the long generation time of trees. Obviously, the analysis of genetic diversity enables selection of divergent parents for hybridization either to exploit the gain due to heterosis or to synthesize new recombinants for subsequent generations. In this study, an attempt was made to ascertain the magnitude of genetic diversity among the clones of *Dalbergia sissoo* using RAPD markers.

In conclusion, results of this study indicate that RAPDs are sufficiently informative and powerful to assess genetic variability in *D. sissoo* Roxb. The focus now need to divert on designing suitable strategies for the conservation of the variation observed within the clones and use in screening / developing disease resistant genotypes.

REFERENCES

Arif M, Zaidi NW, Singh YP, Haq QMR, Singh US (2009). A comparative analysis of ISSR and RAPD Markers for the Study of genetic diversity in Shisham (*Dalbergia sissoo*). Plant Mole. Biol. Reporters., 27: 488-495.

Bekessy SA, Allnutt TR, Premoli AC, Lara A (2002). Genetic variation in the vulnerable and endemic Monkey Puzzle tree, detected using RAPDs. Heredity, 88: 243-249.

Botstein D, White RD, Skolnick M, Davis RW (1980). Construction of a linkage map in man using restriction fragment polymorphism. Am. J. Hum. Genet., 32: 314-331.

Chunyang L (2000). RAPD Analysis of genetic variation in *Eucalyptus microtheca F. Muell* populations. Heredity, 132: 151-156.

Cotterill PP, Dean CA (1990): Successful tree breeding with index selection. Pp 79, CSIRO, Melbourne.

Dhakal L (2001). Sissoo dieback: Is tree improvement a probable solution? In Proc., Workshop on Sissoo Dieback, Kathmandu, Nepal (1991). Reprint available from HMG/DANIDA. Tree Improvement and Silviculture Component / Natural Resources Managements Sectors Assistant Program, Hattisar, Naxal, Kathmandu, Nepal.

Doyle JJ, Doyle JL (1990): Isolation of plant DNA from fresh tissue. Focus, 12: 13-15.

Ginwal HS, Maurya SS (2009). Evaluation and optimization of DNA extraction method for *Dalbergia sissoo* leaf. Indian J. Biotechnol., 9: 69-73.

Goto S, Miyahara F, Ide Y (2001). A fast method of checking the genetic identity of ramets in a clonal seed orchard by RAPD analysis with a Bulking Procedure. Silvae Genetica, 50: 271-275.

Jaccard P (1908). Nouvelles recherches sur la distribution florale. Bull. Soc. Vaud. Sci. Nat., 44: 223-270.

Karp A, Edwards K (1998). DNA markers: A global overview in DNA Markers: Protocols, Applications and Overviews, G. C. Anolles and P. M. Gresshoff, Willy-Liss Inc., New York.

Kumar A, Luna RK (2007). Conservation of first generation seed orchards to advance generation orchard for higher genetic gains in *Dalbergia sissoo* (Roxb.). The Indian Forester, 133: 80-89.

Kumar P, Singh K, Vikal Y, Randhawa LS, Chahal GS (2003). Genetic diversity studies of elite cotton germplasm lines using RAPD markers and morphological characteristics. Indian J. Gen., 63 (1): 5-10

Libby WJ (1973). Domestication strategies for forest trees. Can. J. For. Res., 3: 265-276.

Martin JP, Bermejo JEH (2000). Genetic variation in the endemic and endangered *Rosmarinus tomentosus* Huber-Morath and Maire (Labiatae) using RAPD markers. Heredity, 85: 434-443.

Mohana GS, Uma Shaanker R, Ganeshaiah KN, Dayanandan S (2001). Genetic relatedness among developing seeds and intra fruit seed abortion in *Dalbergia sissoo* (Fabaceae). Am. J. Bot., 88: 1181-1188.

Mosseler A, Egger KN, Hughes GA (1992). Low levels of genetic diversity in red pine confirmed by Random Amplified Polymorphic DNA markers. Can. J. For. Res., 22: 1332-1337.

Nei M (1973). Analysis of genetic diversity in subdivided populations. In: Proc., National Academy of Sciences, USA, 70: 3321-3323.

Newton AC, Allnutt TR, Dvorak WS, Castillo RFD, Ennos RA (2002). Patterns of genetic variation in *Pinus chiapensis,* a threatened Mexican pine, detected by RAPD and mitochondrial DNA RFLP markers. Heredity, 89: 191-198.

Rohlf FJ (2000). NTSYS-pc Numerical Taxonomy and Multivariate Analysis System (Version 2.1). Exeter Publishing Ltd., Setauket, New York.

Sah SP, Sharma CK, Sehested F (2003). Possible role of the soil in the sissoo forest (*Dalbergia sissoo* Roxb.) decline in the Nepal Terali. Plant Soil, 49: 378-385.

Scheepers D, Eloy MC, Briquet M (1997). Use of RAPD patterns for clone verification and in studying provenance relationships in Norway Spruce (*Picea abies*). Theoretical Appl. Gen., 94: 480-485.

Stange C, Prehn D, Arce–Johnson P (1998). Isolation of *Pinus radiata* genomic DNA suitable for RAPD analysis, Plant Mol. Biol. Reporter,

16: 1-8
Tewari DN (1994): A monograph on *Dalbergia sissoo (*Roxb.). International Book Distributor, Dehradun. pp. 316.
Thothathri K (1987). Taxonomic revision of the Dalbergieae in the Indian sub-continent in Indus Publishing Company, Botanical Survey of India, Howrah, India, p. 244
Troup RS (1921). The Silviculture of Indian Trees 1(Rev. Edition 1980). Oxford Press, Oxford.
Vaishali, Khan S, Sharma V (2008). RAPD based assessment of genetic diversity of *Butea monosperma* from different agro ecological regions of India. Indian J. Biotechnol., 7: 320-327.
Wang BY, Shi L, Ruan ZY, Deng J (2011). Genetic diversity and differentiation in *Dalbergia sissoo* (Fabaceae) as revealed by RAPD. Gen. Mole. Res., 10: 114-120.
Wang BY, Sun JX, Liu KQ, Yang XQ (2003). The study on the stability of RAPD-PCR. J. Yunnan Univ. 25: 386-372.
Zobel BJ (1971). The Genetic Improvement of Southern Pines. Sci. Am., 225: 94-103.