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Full Length Research Paper

Genetic diversity of *Santalum album* using random amplified polymorphic DNAs

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Santalum album **is a parasitic, evergreen tree growing primarily in south Central India, in the dry forest of the Decan plateau. Sandalwood is found distributed all over the country with over 90% of the area in Karnataka and Tamil Nadu. It is of great economic importance because of its fragrant heartwood and oil. In the present study Random Amplified Polymorphic DNA (RAPD) technique was used to accesses the genetic diversity among 30 accessions of** ***S. album*** **collected from different parts of South India. A total of 248 polymorphic amplicons were obtained from 30 primers. The value of Jaccard coefficient ranged from 0.66 to 0.99. Similarity results indicated the high level of genetic diversity existing in** ***S. album*****. Cluster analysis using UPGMA separated the accession into 2 major groups. These results promote the initiative to integrate this RAPD markers in the discrimination of genotypes which can be exploited in the improvement of** ***S. album*****.**

Key words: *Santalum album,* RAPD, Molecular Markers, DNA, Genetic Diversity.

INTRODUCTION

Sandalwood is one of the most economically important species belonging to the family santalaceae (Bhattacharaya and Lakshmi, 1999). Out of the 16 species, the most important commercial species today is *Santalum album.* In India, the genus is represented by *S. album* L. Sandalwood is found distributed all over the country (9600 km^2) with over 90% of the area in Karnataka (5245 km^2) and Tamil Nadu (3040 km^2); (Nelson et al., 1996) The forests once protected due to inaccessibility, have now been expos-ed to severe biotic factors including human interference leading to heavy exploitation and massive clearance, grazing, fire and the spike disease. Several causes have been attributed to the depletion of sandalwood population mainly amongst which theft is causing dysgenic effect on the quality of species by constant removal of superior trees. Therefore, assessment of genetic diversity is vital in developing effective conservation strategies (Loveless, 1992) and sustainable management guidelines (Radomiljac, 1999).

Recently various molecular techniques have been developed as powerful tools for diversity analysis in the establishing relationship between cultivars. Among this RAPD technique is technically simplest, less expensive and doesn't require huge infrastructure to start with (Willams et al., 1990; Adetula, 2006). A systematic study is thus, essential in *S. album,* in order to examine the existing genetic diversity among populations and also to gain knowledge on the impact of disturbance on genetic variability of this species. Hence the objective of the present study was to assess the diversity at the DNA level using RAPD technique.

MATARIALS AND METHODS

Plant material

A total of 30 accession of *Santalum album* (Table 1) were obtained obtained from germplasm block maintained by the Institute of Wood Science and Technology (IWST), Bangalore, where sandalwood genotypes procured from different geographical regions of India are conserved.

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Table 1. Accession of *Santalum album* L. used in the present study with their Place of collection.

S. No.	Genotype	Place of collection
1	T1	Kumbakonam
2	T2	Coimbatore
3	T3	Hosur
4	T4	Vellore
5	T7	Vellore
6	T9	Salem
7	T15	Theosophical socity, Madras
8	T21	Tirunelveli
9	T24	Dharmapuri
10	T25	Thombakal, shanimadu, Dharmapuri
11	T26	Dharmapuri
12	K3	Hallihatti, Rani bennur, Gadag
13	K7	Bangalore
14	K9/	Bangalore
15	K14	Kolar
16	K25	Kuderekonda ayanur Shimoga
17	KL1	Munnar
18	KL2	Anackelpetymaryur, Munnar
19	KL3	Munnar
20	AP4	Nehru Zoological Park, Hyderabad
21	K6	Bangalore
22	K8	Bangalore
23	K10	Bangalore
24	K11	Chamarajnagar
25	K13	Chamarajnagar
26	K16	Chamarajnagar
27	K27	Sagar
28	T12	Salem
29	T13	Salem
30	T19	Tirunelveli

DNA extraction

The leaf sample for DNA extraction was prepared by following Porebski et al. (1997), method and approximately 8-10 g of dried leaves were powdered by using a homogenizer for 45-60 s and sieved to obtain a fine powder. And DNA was extracted following the method of (Abdul azeez, 2008). The purity of sandalwood genomic DNA was evaluated by measuring absorbance (A_{260} nm/A_{280} nm ratio) with a Double Beam UV spectrophotometer. The size, purity and integrity of DNA isolated were determined by agarose gel (0.8%) electrophoresis using λ phage DNA cleaved with *Hind*III as a size standard.

PCR amplification

PCR reaction was carried out with MJ Cycler machine. Preliminary analysis varying with the concentration of DNA from 10 to 50ng revealed that 25 ng of DNA gave the maximum number of reproducible bands, therefore 25 ng was used in all the analyses. Different concentration of *Taq* DNA polymerase, $MgCl_2$ and primer were used to optimize the RAPD reaction (Williams et al., 1990). One unit of *Taq* DNA polymerase, 3.0 mM $MgCl_2$, 1.00 µl mixture of 215 µM dNTPs (dATP, dCTP, dGTP and dTTP) and 0.5 µM primer were found to be the best and used in all subsequent PCR reactions. The RAPD profile consisted of an initial denaturation at 94°C for 3 min, followed by 45 cycles consisting of 1 min at 94°C, 1 min at 35°C annealing and 2 min at 72°C (primer extension) and final extention for 7 min at 72°C The amplification products were analysed on 1.2% agarose gel containing ethidium bromide (0.5 µg/ml) using 1x TBE buffer with 5 µl of loading buffer run at constant voltage of 120 v for 2 h (Sambrook et al., 1989). The agarose gel was visualized under UV light and photographed under UV light by using Total Lab Gel Documentation System (New Zealand).

Data scoring

Fragments that were clearly resolved on the gels were scored as 1 for the presence and 0 for the absence across the 30 *S. album* genotypes. The similarity matrix was computed using Squared Euclidean Distance (SED) that estimated all pair wise difference similarity matrices in the amplification product, using sequential, hierarchical clustering option of the NTSYS-pc version 2.02j software package (Rohlf, 1993). The program

Table 2. List of primers, the amplified fragments obtained in *Santalum album* and percent of polymorphic bands.

S. No.	Primer code	Nucleotide sequence(5'-3')	Polymorphism %	Number of polymorphic Bands
1	OPK12	TGGCCCTCAC	36.6	11
2	OPAL08	GTCGCCCTCA	36.6	12
3	OPJ 10	AAGCCCGAGG	66.6	20
4	OPB 12	CCTTGACGCA	60	18
5	OPE20	AACGGTGACC	73.3	22
6	OPF 12	ACGGTACCAG	13.3	4
7	OPP 20	GACCCTAGTC	40	12
8	OPF5	CCGAATTCCC	36.6	11
9	OPF 10	GGAAGCTTGG	50	15
10	OPJ 20	AAGCGGCCTC	30	9
11	OPF 02	GAGGATCCCT	53.3	16
12	OPE 15	ACGCACAACC	60	18
13	OPX 02	TTCCGCCACC	56.6	17
14	OPV 17	ACCGGCTTG	20	6

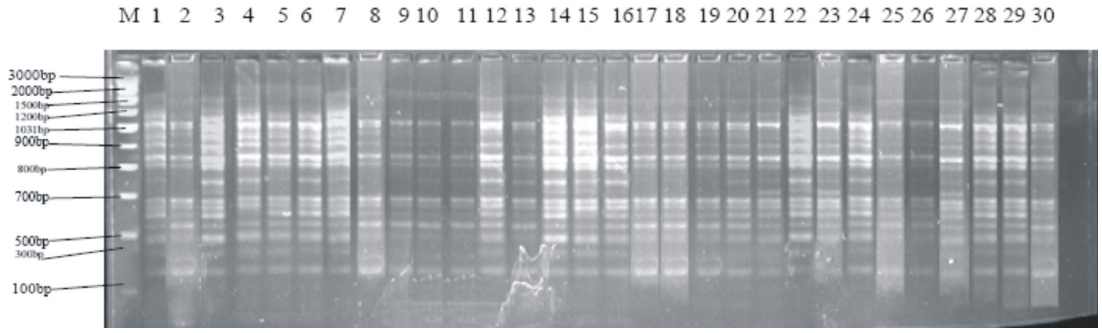

Figure 1. RAPD gel profile of sandalwood clones generated using 10-mer random primer OPK 12. Lane M: 100 bp DNA Ladder, Lanes 1-30 Sandalwood clones (lanes 1: T1, 2: T2, 3: T3, 4: T4, 5: T7, 6: T9, 7: T15, 7: T7, 8: T21, 9: T24, 9: T9, 10: T25, 11: T26, 12: K3, 13: K7, 14: K9, 15: K14, 16: K25, 17: KL1, 18: KL2, 19: KL3, 20: AP4, 21: K6, 22: K8, 23: K10, 24: K11, 25: K13, 26: K16, 27: K27, 28: T12, 29: T13, 30: T19.

also generated a dendogram which grouped the accessions and species on the basis of Nei Genetic distance (Nei, 1972) using unweighted pair group method with arithmetic average (UPGMA) cluster analysis (Sneath and Sokal, 1973).

RESULTS AND DISCUSSION

The total number of DNA bands amplified with the thirty accession of *S. album* as well as the number of polymorphic bands among these is presented in Table 2. Figure 1 shows the RAPD profile for the 30 accession screened by the OPK12 primer. The number of amplification products obtained were in the range 4 to 22, with the primer OPF12 producing the minimum number and OPE20 producing the maximum number of bands. Cluster analysis based on 248 RAPD markers revealed that the 30 genotypes examined, clustered at 0.66 similarity coefficient on dendrogram with T1 (Tamil Nadu, Kumbakonam) and T7 (Vellore, Tamil Nadu) spanning the extremes. All the accessions were grouped into two major groups at about 0.70 coefficient. The resultant dendrogram grouped all the exotic genotypes in a separate distinct sub cluster at about 0.85 coefficient on the dendrogram (Figure 2). Within each major group, two distinct sub groups could be visualized. The genetic similarity value ranged from 1 to 45% suggesting a wide genetic base within the sandalwood genotypes used in the present investigation. The highest similarity level 45% was observed between genotypes of Tamil Nadu, HosurT3 and the least 1% between T24 and T12 (Figure 3).

To visualize the genetic relatedness among the sandalwood genotypes in detail, principal component analysis (PCA) was made for 248 RAPD markers generated by fourteen decamer random primers. The description of the data was done using three dimensions and the same is presented in (Figure 2). From the graph, it was evident that sandalwood genotypes were more dispers-

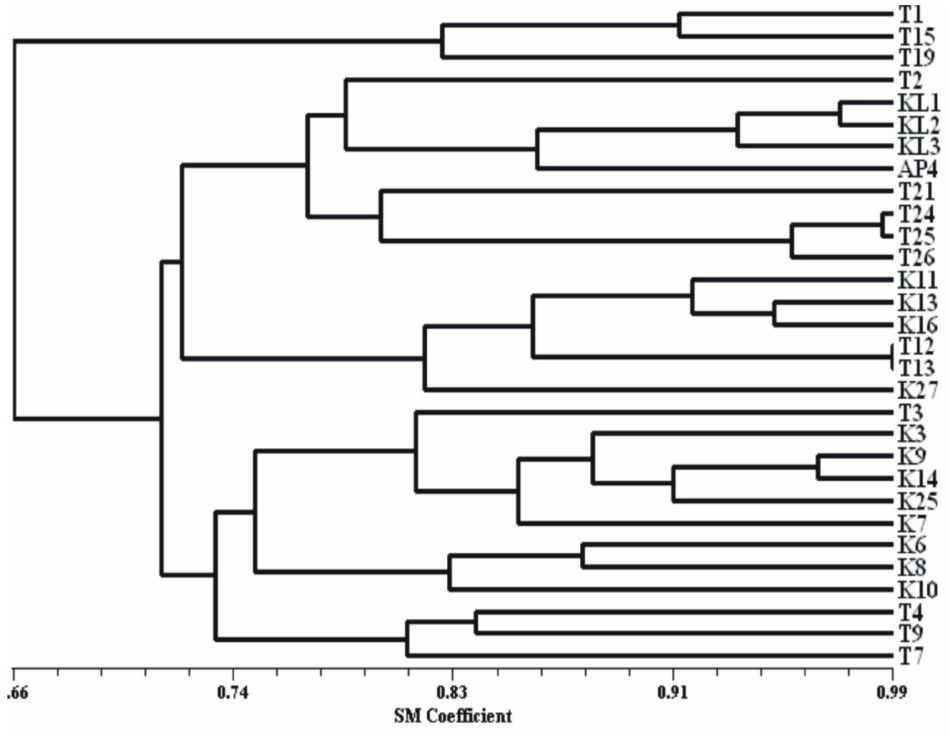

Figure 2. Dendogram showing the genetic relationship among sandalwood genotypes.

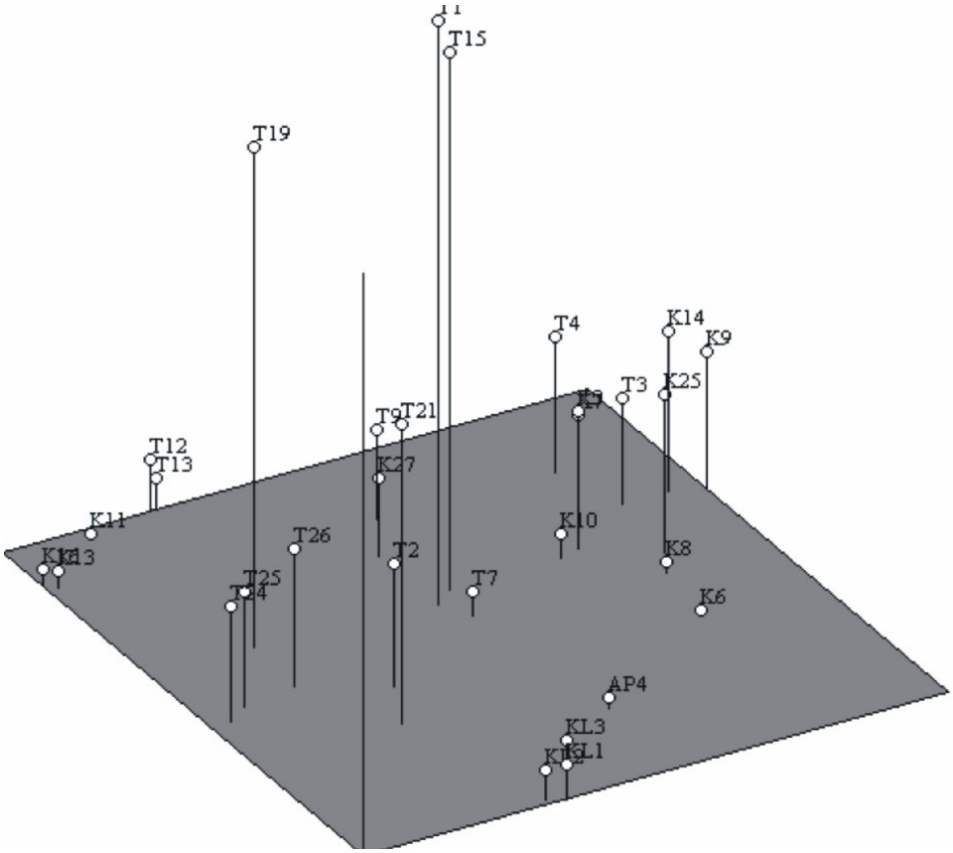

Figure 3. 3D principal component analysis of sandalwood genotypes generated by NTSYSpc.

ed on the PCA plot, which is a reflection of wider genetic base. Some of the Indian genotypes appear to be overlapping with each other depicting redundancy in these genotypes (Figure 3). Among the Indian genotypes, K6-Bangalore and AP4-Nehru Zoological park, Hyderabad, Andhra Pradesh, were quite distinct from the others forming a separate entity. T19, T24, KL1, K9, T4 and T9 are some of the genotypes found at the periphery of the PCA. In general, the result obtained from PCA was in agreement with the dendrogram generated by Nei method. Interestingly collections originating from various parts of the country did not form well-defined distinct groups and were interspersed with each other indicating no accession association between RAPD pattern and the geographic origin of accessions. Similar results have been observed in Capsicum (Adetula, 2006); Brassica (Divaret et al., 1999); lettuce (Tardin et al., 2003) and solanum (Singh et al., 2006).

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