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RESEARCH ARTICLE

RAPD PROFILING ASSESSMENT OF GENETIC DIVERSITY ON *TECTONA GRANDIS*

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ABSTRACT

The present study was made an attempt to differentiate clonal variation of teak clone samples by using RAPD technique. A total of 10 different primers were used to identify the polymorphic variations between the clones. The results of the phylogenetic tree showed the highest genetic diversity (0.93) between the clones of Kerala and Tamil Nadu teaks and the lowest genetic diversity (0.53) was identified between Thaunakadavu clones of TNT 1 and TNT 2 and the results of the present findings could be further useful for the long term breeding of potential teak plants.

INTRODUCTION

India is a known mega-diversity centre for harboring a multitude of plant species each presumably studded with as yet unknown genetic and chemical variations of economic importance. Therefore, the rich and varied plant diversity is one of India's important strengths and is the bedrock for all future industrial developments (Ayala and Kiger 1980; Lewin *et al.,* 1994; Norishige Yotsukura *et al.,* 2006). Moreover, teak plant is distributed widely throughout the southeast side. Few studies such as isozyme marker for the identification of the clonal variation. But, studies related with the genetic diversity is too limited. Hence, the present study was initiated to identify the genetic variation by using RAPD techniques.

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MATERIALS METHODS

Sample Collection

Teak samples were collected from three different states viz., Tamilnadu (Thunakadavu), Kerela (Karulai, Nilambur and Sungam) and Andhra Pradesh (Bhadrachalam). A total of 20 samples were collected from Thunakadavu (TNT01-TNT25), 8 samples from Karulai (KLS01-KLS08), 7 samples from Nilambur (KLN01-KLN07), 6 samples from Sungam (KLS01-KLS06) and 5 samples from Bhadrachalam (SBL01-SBL05).

DNA isolation and PCR amplification

Genomic DNA of 15 genotypes was extracted from 200 g fresh individual teak leafs with CTAB method (Khanuja *et al.,*1999). After purification, DNA was quantified by the

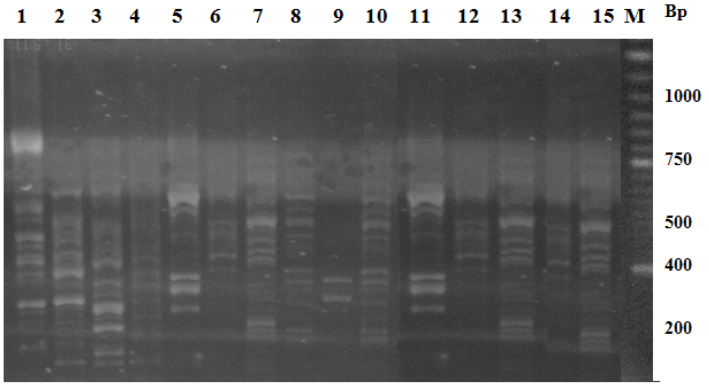
Table 1: Primers used for the amplification of Genomic DNA

Code	Sequence of the Primers	GC %	Molecular Weight	pmoles
OPB-O4	5' GGACTGGAGT 3'	60	3108	4894
OPB-15	5' GGAGGGTGTT 3'	60	3139	4990
OPB-18	5' CCACAGCAGT 3'	60	2997	5194
OPE-04	5' GTGACATGCC 3'	60	3028	5032
OPE-06	5' AAGACCCCTC 3'	60	2957	5415
OPE-15	5' ACGCACAACC 3'	60	2966	5090
OPM 02	5' ACAACGCCTC 3'	60	2957	5415
OPM-05	5' GGGAACGTGT 3'	60	3108	4894
OPM-06	5' CTGGGCAACT 3'	60	3028	5302
OPM-13	5' GGTGGTCAAG 3'	60	3108	4894

Table 2: Nucleotide sequences of RAPD primers showing amplification status with fifteen clones of *T. grandis*

Primer code	Sequence (5'- 3')	Amplification status	Polymorphism
OPB 04	GGACTGGAGT	+	++
OPB 15	GGAGGGTGTT	+	++
OPB 18	CCACAGCAGT	+	++
OPE 04	GTGACATGCC	+	-
OPE 06	AAGACCCCTC	+	++
OPE 15	ACGCACAACC	+	++
OPM 02	ACAACGCCTC	+	++
OPM 05	GGGAACGTGT	+	++
OPM 06	CTGGGCAACT	+	++
OPM 13	GGTGGTCAAG	+	++

+ Amplification present; ++ Polymorphic bands present; - No polymorphic bands present



M – 1 kb Marker (Fermentas Inc.); 1 – KLK 1 (OPM 5); 2 – KLK 2 (OPM 2); 3 – KLN 1 (OPB 18); 4 – KLN 2 (OPM 6); 5 – KLN 4 (OPE 15); 6 – SBL 1 (OPM13); 7 – TNT 1 (OPB 4); 8 – TNT 3 (OPE 15); 9 – TNT 4 (OPE 6); 10 – TNT 10 (OPM 6); 11 – TNT 11 (OPB 18); 12 – TNT 20 (OPM2); 13 – KLS 2 (OPB 15); 14 – KLS 3 (OPM13); 15 - KLS 4 (OPB 15).

Fig 1. RAPD profiling of selected clones with different primers

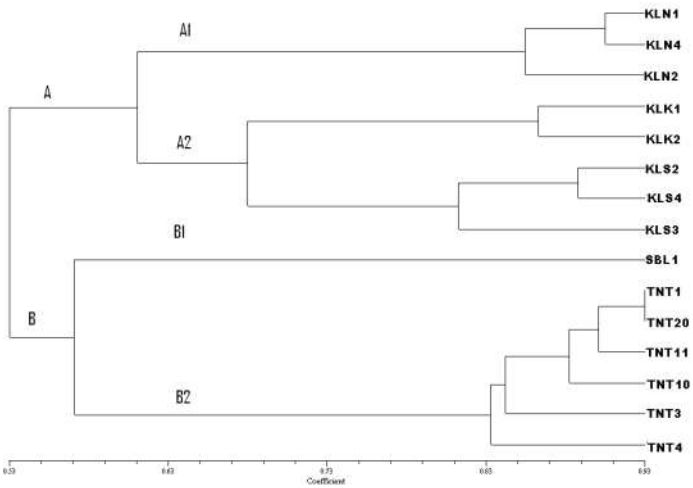


Fig 2. Clustering of Teak Clones using SAHN Coefficient (NTSYSpc v2.02)

spectrophotometricaly and visualized under UV light after electrophoresis on 0.8% (w/v) agarose gel. In spectrophotometric method, suitably diluted DNA in distilled water was read at A_{260} and A_{280}. A_{260}/A_{280} of 1.8 - 2.0 to indicate a good DNA preparation. A_{260} of 1 corresponds to 50 µg/ml of dsDNA in a 1cm quartz cuvette. The unknown DNA concentration can then be calculated. PCR amplification were carried out in a thermal cycler in a final volume of 25µl, containing 25 ng template DNA, 100µM of each of the four deoxynucleotide triphosphate, 20 ng of primer (Table 1), 1.5 mM $MgCl_2$, 10x Tag buffer (10mM Tris HCl pH 9.0, 50 mM KCl) and 0.5 U Taq DNA polymerase. The samples were further subjected for initial denaturation for 5 min at 94°C, followed by 39 cycles of 1 min at 94°C, 1 min at 39°C for RAPD and 42-64°C for ISSR and extension for 1 min at 72°C with a final extention of 7 min at 72°C. 10µl of amplified PCR product was separate through gel electrophoresis on 2% agrose gel stained with ethidium bromide and photographed with gel documentation system (Sambrook *et al.,* 1989).

RAPD profiling

The samples which showed the amplification with the selected primers were selected further for RAPD profiling. DNA fragment size on agrose gel was estimated by comparing with 1kb DNA ladder. The bands were scored '1' for presence and 'o' for absence in DNA samples amplified to create a binary data matrix. The data obtained by scoring the RAPD and ISSR profiles with different primers individually as well as collectively were subjected to the construction of similarly matrics using Jaccards coefficients (Jaccard 1908).

Data analysis

The data obtained by scoring RAPD profile with different primers were collectively were used to the construction of the similarity matrix using Jaccords coefficients. The similarity values were used for cluster analysis. Sequential hierarchical agglomerative non over lapping (SHAN) clustering was done using UPGMA method (Lanham *et al.,* 1995). The similarity matrix was obtained after multivariative analysis using the Dice co efficient similarity (Nei and Li, 1979).

RESULTS

The results of the RAPD amplification showed that, all the 10 primers were showed the pure amplification for the entire selected clonal genomic DNA. Further, all the primer amplification are also showed the polymorphic variations between the clones, except the OPE4 primer. Of all the primers used, OPB 18 showed maximum polymorphism among the fifteen clones. Out of 144 amplified products observed in 9 primers, 107 products were polymorphic which accounted to be 74%. Moreover, the primer OPE 4 did not express polymorphism in any clones. (Fig 1 and Table 2). The results of genetic similarity index for all pair wise combination of the teak sources were computed using binary data. The lowest similarity coefficient value of 0.53 was found between the sources of Kerala and Tamil Nadu. The highest similarity coefficient value of 0.93 was observed between TNT 1 and TNT 20. The pair wise combination expressed the lowest genetic distance of 0.07. From the phenotic tree two major clusters could be visualized at a coefficient level of 0.53. Cluster A was the largest with 8 sources and Cluster B had 7 sources. The major cluster B resolved into 2 sub clusters at a coefficient level of 0.57 viz., B1 and B2. All the sources from Thunakadavu were grouped in sub cluster B2 whereas sub cluster B1 consists of only 1 source from Bhadrachalam (Fig. 2). At a coefficient level of 0.61, the major cluster, A resolved into two sub clusters viz., A1 and A2. Among the two sub clusters, A1 consists of 3 sources and A2 consists of 5 sources. A1 is further resolved into sub clusters viz., A11 and A12 at a coefficient level of 0.855. Among the two sub clusters, A11 consists of 2 sources and A12 consists of only 1 source. All the sources from Nilambur were grouped into sub cluster A1 and all the sources from Karulai and Sungam were grouped in A21 and A22 respectively (data not shown).

DISCUSSION

In general, RAPD technique have successfully utilized to detect polymorphism in many genera and species of Forest trees (Mohapatra and Singhal, 2000). The results of the present studies showed the genetic variability between the same species with different clones this could be due do the reproductive strategies such as selfing and vegetative propagation (Waller *et al.,* 1987). Similarly, (Chalmers *et al.,* 1992) use RAPD markers to partition the genetic variation in *Gliricidia sepium* and *G. maculate* using 11 primers and obtained 60% of genetic variation between the population and 40% within population. Further, the results of clustering co-efficient analysis showed the variations between the clones and this might be due to the different geographical locations of the clones. Similar results had been reported by (Chalmers *et al.,* 1992) the using RAPD markers in *Gliricidia spp* and estimated levels of genetic variation between 0.4 and 0.7. Several tropical trees had also reported to exhibit low levels of genomic variations within and between populations. Despite being sampled from geographically distinct locations, species that had wide distribution might show very low genomic variation (Lakshmi *et al.,* 1997) and species introduced from a small genetic base could have more genomic variation (Varghese *et al.,* 1997). In conclusion the RAPD genetic variations studies showed the major genetic variations between Kerela and Tamilnadu clones. The highest genetic diversity showing individual can be taken for further breeding programme to develop genetically superior clones. Even though, the studies related with the advanced markers *viz*., SSR, ISSR, STS are highly warranted.

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