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Research Article

COMPARISON OF MORPHOLOGICAL TRAITS AND GENETIC POLYMORPHISM OF *TECTONA GRANDIS* L.F. FROM SELECTED LOCALITIES OF KERALA, INDIA

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ABSTRACT

Teak belongs to Verbenaceae show wide geographic distribution in South East Asia. The natural populations develop heritable phenotypic adaptations to local environmental factors in order to survive in different ecological conditions. Phenotypic variation was the result of interaction between genetic and environmental factors. In this juncture, present study aims to analyze morphometric and genetic polymorphism among the teak selected from six different localities of Kerala, India. Morphometric analysis resulted variations among the accessions. A dendrogram is constructed showed highland accessions in to a small clad whereas, midland and lowland accessions in to the larger cluster. Subsequently, RAPD is used for polymorphism detections and is possible for identifying large number of loci and ascribes unambiguous taxonomic and genetic relationships among different taxa. DNA was extracted from fresh young leaves from six regions belongs to high land, mid land and low land of Kerala, India. RAPD analysis were carried out with 20 decamer primers from Operon Technology. DNA was amplified using cycler gradient Eppendorf with 35 cycles. RAPD products were separated on 1.5 % agarose gel and detected by staining with ethidium bromide. There were 374 bands generated in 18 random primers. The number of monomorphic bands, polymorphic bands and the percentage of polymorphism were 21 bands, 353 bands and 94.38 % respectively. The high number and percentage of polymorphic bands revealed genomic DNA variation. This variation is in accordance with phenotypic variation detected in this experiment. Dendrogram clustered the clones into two major clades, the first clad further bifurcated into two having taxa from Munnar, Palakkad and Idukki. The second clad have taxa from Kochi, Kollam and Thiruvananthapuram, India

Keywords: Genetic polymorphism, *Tectona grandis,* RAPD, morphological traits, primers.

INTRODUCTION

Teak (*Tectona grandis*), an entomophilous out crossing woody plant, is naturally distributed in the moist and dry monsoon forests of India, Myanmar, Laos and Thailand. It is widely planted outside of its natural distribution in Tropical Asia as well as in Africa and Latin America mainly due to the outstanding durability and high commercial value of its timber. In India, the teak were selected from natural forests on the basis of their superior phenotypes, namely, (1) vigour (height and girth), (2) straight and cylindrical woody stem devoid of basal fluting or buttressing, (3) narrow or compact crown with light branching, (4) reasonable amount of seed production, and (5) free from pest and disease. The selected teaks serve as source material for production of improved planting stock by clonal propagation for large scale plantation programmes or are used as parents in breeding programme for superior traits[1]. In the National Teak Germplasm Bank in Chandrapur, India, a large number of *T. grandis* has been established and maintained for their sustainable use in tree improvement programme. However, *T. grandis* do not possess distinct morphological characters and hence need to be characterized at molecular level for maintenance of genetic identity of their clones and thus preventing errors in identification visually[2]. DNA markers precisely characterize cultivars, provenances or genotypes and measure their genetic relationships. The markers are highly heritable, environmentally stable and exhibit sufficient polymorphism to discriminate closely related genotypes[3]. RAPD (random amplified polymorphic DNA) markers are rapid and economical and are extensively used for diversity analysis, mapping and genotype identification of plant species including forest trees[4]. RAPD markers through PCR (polymerase chain reaction) amplification of genomic DNA with random decamer primers are fast, simple and most effective tool for identification[5,6]. They make precise distinction among clones, varieties and cultivars of many forest trees[7,8]. The present study reports the use of RAPD markers for assessment of DNA polymorphism among *T. grandis* collected from six different localities from Kerala, India. Results will be useful for better identification and their sustainable use as superior diverse parents in future breeding.

MATERIALS AND METHODS

Plant Material

Teak plants are collected from six different localities of Kerala viz., Trivandrum, Kollam, Kochi, Idukki, Palakkad and Munnar, India. All of these plants were collected from their wild habitat.

Morphological Analysis

31 morphological features such as girth, height, leaf size, stomata, palisade ratio, epidermal cell, number of flowers in a bunch, pedicel length, petiole length, sepal, petal, stamen, carpel, fruit and seed characters are evaluated for discriminating the accessions.

DNA Extraction

The DNA genome was extracted from fresh young leaves using Doyle and Doyle's[9] method with slight modification in CTAB extraction buffer. 0.1 g fresh young leaves were grinded using mortar and pestle with 1 ml of extraction buffer. The extraction buffer consists of 2 % CTAB, 0.1 M Tris-HCl, 3.5 M NaCl, 20 mM Na_2EDTA, 2 % PVP and 2 % β-3-mercaptoethanol.

DNA amplification using RAPD

RAPD analysis were carried out using 20 decamer primers from Kit A of Operon Technology. RAPD reaction was prepared with mixture of 5 µl PCR master mix 2x solutions from Intron Biotechnology, 2 µl OPA primers (10 µmol), 2 µl deionized H_2O and 1 µl DNA (25-50 ng). DNA was amplified using Master Cycler Gradient Eppendorf. The RAPD program started with 5 min of 95°C incubation, followed by 35 cycles of 1 min at 95°C denaturing, 1 min at 36°C annealing, and 2 min at 72°C extension. The reaction finished with 5 min at 72°C incubation and stopped at 4°C. RAPD products were separated on 1.5 % agarose gels and were detected by staining with ethidium bromide.

Data Analysis

Bands of equal molecular weight and mobility generated by similar primer were considered to be identical locus. Genetic and morphological similarities between teaks from the six different localities were measured using similarity index and were used to construct a dendogram using UPGMA (Unweighed pair group method with arithmetic average). All the statistical analysis were carried out using the NTSYSpc 2.1 version (Exeter Software, Setauket, N. Y.) software package.

Table 1: DNA polymorphism scored by the 18 OPA primers in teak accessions from Kerala, India

Primers	Total Bands	No. Monomorphics bands	No. Polymorphics bands	Polymorphism (%)
OPA3	12	0	12	100
OPA4	19	0	19	100
OPA5	26	3	23	88.46
OPA6	19	5	15	78.94
OPA7	23	3	20	86.95
OPA8	18	0	18	100
OPA9	25	2	23	92
OPA10	24	0	24	100
OPA11	17	1	16	94.11
OAP12	23	0	23	100
OPA13	22	0	22	100
OPA14	16	1	15	93.75
OAP15	18	3	15	83.33
OPA16	25	2	23	92
OPA17	20	2	18	90
OPA18	20	1	19	95
OAP19	28	3	25	89.28
OPA20	19	3	16	84
Total bands	374	21	353	94.38

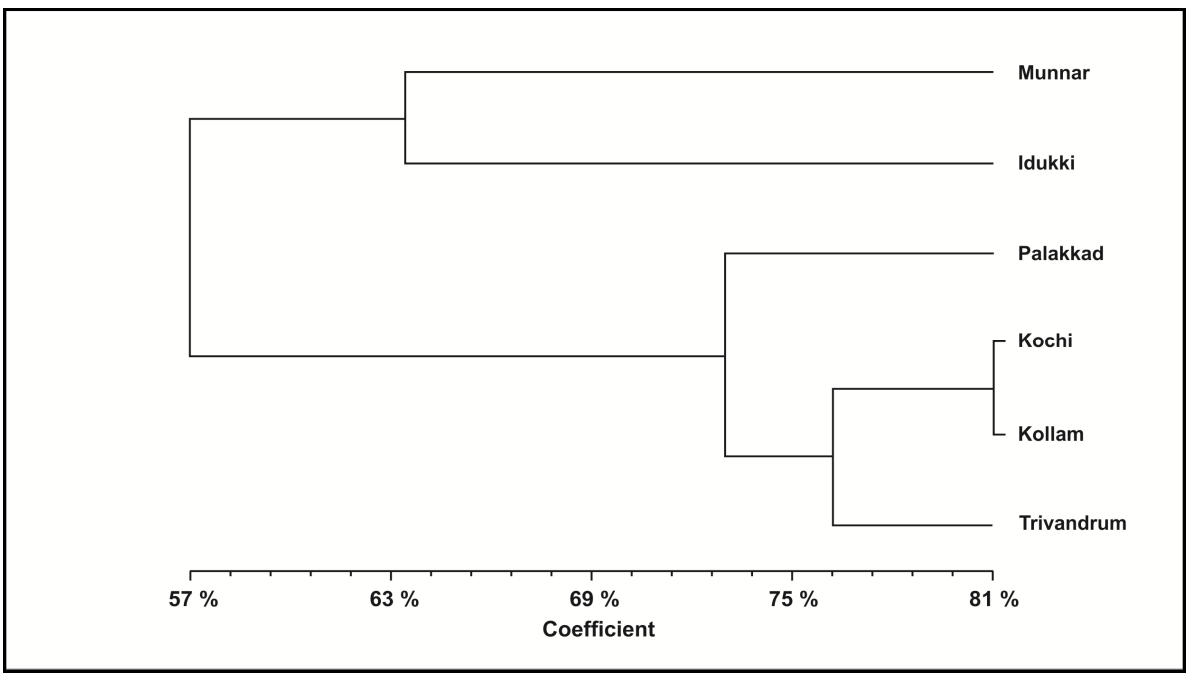

Figure 1: Phylogenetic tree based on UPGMA analysis using morphological characters

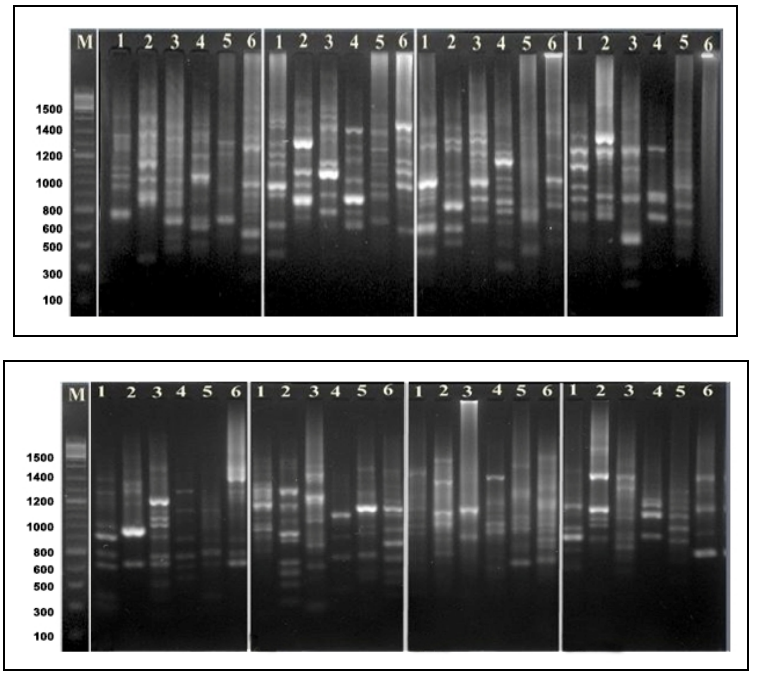

Figure 2 and 3: RAPD banding profile among six teak accessions of Kerala using 20 primers

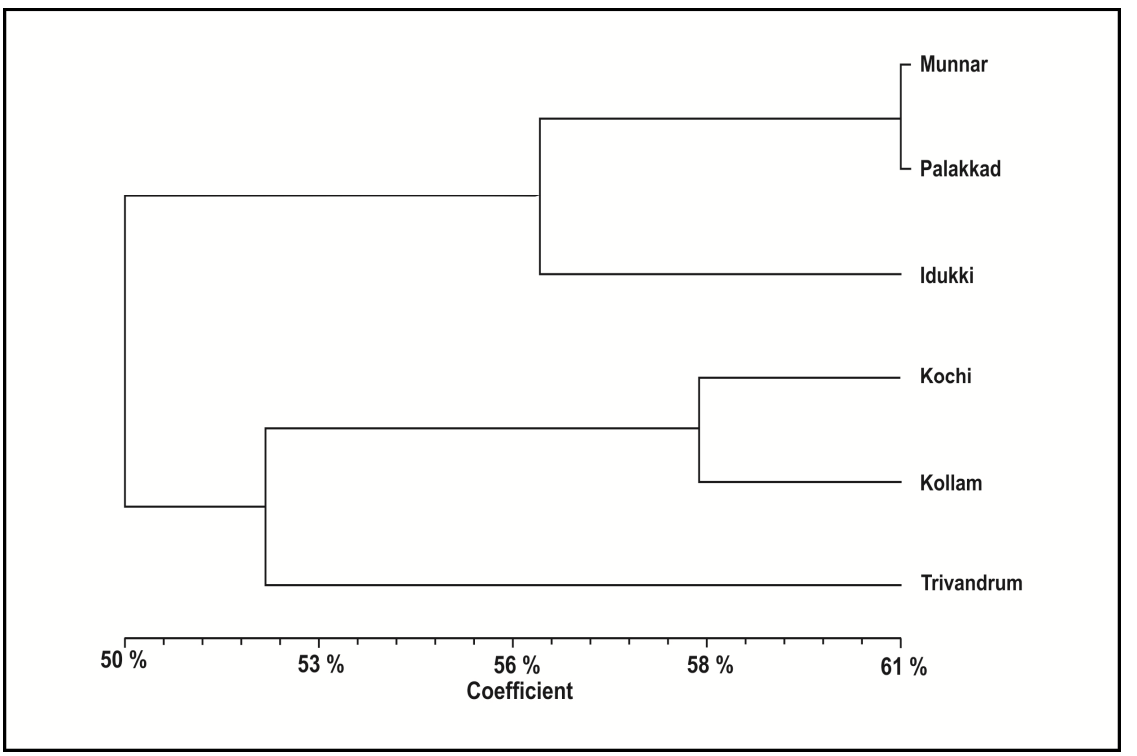

Figure 4: Phylogenetic tree based on UPGMA analysis of the RAPD data

RESULTS AND DISCUSSION

Phylogeny of teak based on morphological features

As many as 31 characters were used for the morphological analysis (data not shown). All of the characters were conferred into character states. Character states data were converted to binary digits that formed the source of data for phylogenetic analysis. The

phylogenetic tree based on UPGMA analysis of the morphological data comprising 31 character states indicated a similarity between all the 6 accessions at the 57 % level (Figure 1). Nevertheless, it was interesting to note that the accessions are clustered in to two major clades suggesting two different morphology bearing teak groups. The first branch consisted of Munnar and Idukki with 63 % degree of similarity. The second branch gets bifurcated in to two sub clades. One of the sub-clad culminated in to Palakkad and the other sub-clad further branched in third order two branches. The first branch of the third order leaded to Kochi, Kollam and free third order branch ended in to Trivandrum, India. These four accessions had 73.5 % degree of similarity. The accessions in the sub-branches had the same characters and are called synapomorphy characters. Morphological data had limitations; however the morphological analysis was often useful for field analysis. The morphological analysis also showed relevance in plant phylogeny.

DNA polymorphism

The number of DNA bands among the accessions showed remarkable variations depending on the primers employed. Out of the 18 primers used in this experiment only OPA3, OPA4, OPA5, OPA6, OPA7, OPA8, OPA9, OPA10, OPA11, OPA12, OPA13, OPA14, OPA15, OPA16, OPA17, OPA18, OPA19 and OPA20 which were capable of amplifying the DNA genome. The total numbers of bands noticed in the RAPD analysis are 374. The number of scorable markers produced per primer ranged from 12 to 28 and size of the products ranged from 100 bp to 2000 bp. The total number of polymorphic markers and the percentage of polymorphism were 353 and 94.38 respectively (Table 1). RAPD marker profiles produced by the primers are shown in the Figure 2 and 3. The primer OPA 19 generated the maximum number of bands i.e., 28 bands while OPA 3 yielded the minimum 12 bands. Monomorphic bands are comparatively minimum among the accessions i.e., 0 - 5. Further, OPA 16 may be used to identify the Trivandrum teak accession which generated a unique band with 400 bp size. OPA12 produced 500, 550 bp while, OPA13 - 1450 bp for Kollam accessions. Similarly, OPA11 – 1100 bp, OPA12 – 1400 bp and OPA18 – 500 bp for Kochi accession; OPA13 – 1400 bp and OPA17 – 200 bp for Idukki accession and OPA15 with 550 bp for Munnar teaks. Cluster analysis was performed based on the Jaccard's similarity coefficient matrices, calculated from RAPD markers to generate dendrogram of teak accessions. The dendrogram separated teak accessions into two clusters. First cluster included Munnar, Palakkad and Idukki teaks. The second cluster was further divided into two sub-clusters. Among the two sub-clusters, first sub-cluster bifurcated with Kochi and Kollam teaks and the free branch ended in to Trivandrum accession (Figure 4). The phylogenetic tree based on UPGMA analysis of the RAPD data indicated a similarity between all the 6 accessions at the 50 % level. Accessions of Munnar, Palakkad and Idukki displayed 56 % degree of similarity. The second cluster consisted of Kochi, Kollam and Trivandrum, India with 52.5 % degree of similarity. DNA bands generated from RAPD had high genetic diversity and supported with high percentage of polymorphism (94.38 %) (Table 1). Dendrogram that was generated from molecular data had marginally different from morphological data. Dendrogram from the molecular analysis showed that Trivandrum accession was included along Kollam and Kochi clade. The molecular data suggested that there were two sub-zones in Teak one represent that of high land and the other the low land teaks. Members of teak varied with morphological characters are correlated with genetic diversity. Differences among the accessions may be due to their environmental adaptation. Morphological analysis has limitations especially due to high subjectivity in selection of characters. Molecular markers can be used for phylogenetic analysis at any taxonomic grades including intraspecific level. Molecular markers have high objectivity. Based on the morphological variation, the teak was divided into two zones. The first sub-zone, namely northern and the second zone, namely Southern and has four accessions. Phylogeny inferences with UPGMA indicated that the two sub-zones formed a well supported paraphyletic clade. Genetic diversity analysis using RAPD markers in ten teak populations of Western Ghats and Central India regions showed low genetic distance within the populations of same geographic regions. Greatest diversity was observed for Western Ghats population compared to the teak populations of Central India. Thus Nicodemus *et al.*[10] proposed zones from where these accessions were taken as separate genecological zones for teak. DNA polymorphism among the 48 assorted teak accessions of National Teak Germplasm Bank, Chandrapur, by RAPD and ISSR markers exhibited very high genetic diversity. ISSR primers produced 95.9 % polymorphism compared to RAPD primers (93.2 %)[3]. 100 % polymorphism was observed among the 29 populations of teak using 5 UBC, ISSR primers. ISSR proved as an effective tool for genetic diversity analysis among teak accessions/populations among the central and peninsular India[11]. Watanabe *et al.*[6] studied the genetic diversity in teak plus trees using 120 RAPD decamer primers in which 24 primers produced reproducible bands. The discrimination study could be useful in the clone management of *Tectona grandis.* AFLP was employed by Vivek Vaishnaw *et al.* [12] for the diversity analysis of 10 accessions of *Tectona grandis* from India. The clonal variations in teak of Kerala and Tamil Nadu, India using RAPD profiling resulted in the highest variation between the clones of Tamil Nadu and Kerala, India which is useful in long term breeding programmes[13]. AFLP markers were employed for the identification of origin of African teak which showed approximately 95 % of teak came from North India[14]. Fifteen microsatellite markers were used to analyse the genetic diversity of 166 teak distributed in the natural area of teak by Fofana *et al*[15].The dendrogram grouped the accessions into four clusters. First two clusters comprising India which confirmed the main center of origin of genetic diversity in *Tectona grandis.* Third and fourth with the Thailand and Laos in which Thailand stood apart from Laos in distinct genetic diversity. Five genotypes of Apocynaceae members from different agro-ecological regions and along with their *in vitro* grown callus were analyzed for genetic diversity. Genetic variation was observed in the *in vitro* and *in vivo* samples of each sample which may be developed during the course of ecological adaptations[16].

CONCLUSION

Both molecular and morphological marker systems generated enough polymorphisms for the distinction of teak trees and some of them even at individual levels. Accessions of trees included in this investigation were highly diverse according to their territorial distribution, thus, suggesting either a common genetic base or frequent natural or anthropogenic gene flow among various populations of teak from where the tree selections were made. The putative tree specific RAPD may be converted into much stringent SCAR markers for the preparation of precise passport database of entire teak. The genetic diversity of trees may be effectively employed for making choices for selection of parental material for breeding programme aimed at improving productivity and wood quality of teak.

REFERENCES

1. Mandal AK, Rambabu N. Quantitative genetic aspects of teak improvement (Eds.) Genetics and Silvi culture of Teak. International Book Distributors, Dehra Dun; 2001. p. 135−145.
2. Wilhelmina TGV, Mcnicol RJ. The use of RAPD markers for the identification of Sitka spruce (*Pice sitchensis*) clones. Heredity 1995; 75: 126−132. http://dx.doi.org/10.1038/hdy.1995.115
3. Narayanan C, Mandal AK, Ansari SA. Molecular markers and their applications in forest trees. Biotechnology Current Perspectives and Potential Applications; 2007. p. 31−56.
4. Karp A, Issar PG, Ingram DS. Molecular Tools for Screening Biodiversity, Chapman and Hall, London; 1998. http://dx.doi.org/10.1007/978-94-009-0019-6
5. Takata K, Shiraishi S. Discrimination of Japanese cedar (*Cryptomeria japonica* D. Don) cutting cultivars in Kyushu region with RAPD markers. Bulletin of Kyushu University of Forestry 1996; 75: 1−14.
6. Watanabe A, Widyatmoko A, Rimbawanto A, Shiraishi S. Discrimination of teak (*Tectona grandis*) plus trees using selected random amplified polymorphic DNA (RAPD) markers. J. Trop. For. Sci 2004; 16(1): 17−24.
7. Goto S, Ieiri R, Miyahara F. RAPD analysis of cutting cultivars and plus tree clones of sugi (*Cryptomeria japonica* D. Don) in Fukuoka Prefecture. J. Jap. For. Soc 1999; 81: 187−193.
8. Tessier C, David J, This P, Boursiquot JM, Charrier A. Optimization of the choice of molecular markers for varietal identification in *Vitis vinifera*. Theoretical and Applied Genetics 1999; 98: 171−177. http://dx.doi.org/10.1007/s001220051054
9. Doyle JJ, Doyle JL. Isolation of plant DNA from fresh tissue. Focus 1990; 12: 13−15.
10. Nicodemus A, Nagarajan C, Narayanan C, Varghese M, Subramanian K. RAPD variation in Indian teak plantations and its implications for breeding and conservation. In: Proceedings of International conference on quality timber products of teak from sustainable forest management. Kerala Forest Research Institute, Peechi, India; 2003. p. 189-201.
11. Ansari SA, Narayanan C, Wali SA, Kumar R, Shukla N, Rahangdale SK. ISSR markers for analysis of molecular diversity and genetic structure of Indian teak (*Tectona grandis* L. f.) populations. Ann. For. Res 2012; 55(1): 11-23.
12. Vivek V, Naseer M, Syed AW, Randhir K, Shashi BT, Madan SN, Shamim AA. AFLP markers for analysis of genetic diversity and structure of teak (*Tectona grandis*) in India. Can. J. For. Res 2014; 10: 296-305.
13. Srinivasan R, Ganapathy SG, Gopi M, Karthikeyan M, Baskaran R, Mathivanan K, Govindasamy C. Research article RAPD profiling assessment of genetic diversity on *Tectona grandis*. Int. J. Curr. Res 2011; 3(11): 041-044.
14. Fofana IJ, Diarrassouba N, Silue S, Koffi KK, Adou K. Origin of African Teak (*Tectona grandis*) provenances in côte d'ivoire using AFLP markers. Elite Res. J. Biotech. Microbiol 2013; 1(1): 1-8.
15. Inza JF, Daniel O, Mireille P, Daniel V. Diversity and genetic structure of teak (*Tectona grandis* L. f) in its natural range using DNA microsatellite Markers. New Forests 2009; 37: 175-195. http://dx.doi.org/10.1007/s11056-008-9116-5
16. Garima Z, Amla B. *In vitro* and *in vivo* RAPD analysis in Apocynaceae family: population growing in different regions of Rajasthan, India. J Pharm Sci Innov 2014; 3(3): 230-239. http://dx.doi.org/10.7897/2277-4572.033145

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