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GENETIC DIVERSITY AND GENETIC VARIATION ASSESSMENT AMONG Tectona grandis SEED PRODUCTION AREA POPULATIONS

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Abstract: Tectona grandis L a highly appreciated timber for centuries , Teak is indigeneous to the Indian Peninsula. Random Amplified Polymorphic DNA (RAPD) primers were designed to evaluate the level and pattern of genetic diversity in two populations of Wyanad Seed Production area. 12 RAPD primers yielded 90 amplification products with different sizes, of which 83 (91.20%) were polymorphic. A high species-level genetic diversity was detected with Nei's (H = 0.2491) and Shannon's diversity (I = 0.3009). In contrast, the population-level genetic diversity was relatively lower (PPB = 44.25%, H = 0.1336, I = 0.1851). Coefficient of populations differentiation (GST) was 0.5055, indicating that Intra-population and intra-population variation contributed 50.45% and 48.44% respectively to the total genetic variability. This corresponding ratio of variation was supported by AMOVA analysis, limited gene flow (Nm = 0.5122), habitat disintegration and geographical disconnection possibly capable for the population structure of that particular population. UPGMA cluster analysis classified the two populations into twogroups which signifies no notable relationship between the genetic similarity coefficient and geographic origin but showed distingished consociation with morpho-physiological characters. The results of the study provide species-level and population-level genetic profiles for further exploitation and conservation of genetic diversity of Wyanad SPA.

Index Terms **- Wyanad SPA, Genetic Diversity, Population Structure, RAPD.**

I. INTRODUCTION

Teak (*Tectona grandis* L.) is an indigenous unique timber species of India. Often considered to be one of the most important timbers in the world, Teak has managed to remain in popularity because of its rare combination of mechanical and physical properties. A Seed Production Area is a plus stand, or a young plantation of the required parent seed origin which will be upgraded and opened later after the removal of all unwanted trees, and managed as a seed source. Wayanad is nestled along the southern belt of mountainous plateau in the Western Ghats, 700 to 2100 meters above sea level which holds many teak plantations and high productive plantations converted into seed production area.

The present study was carried out at Seed production sites in Wayanad district, situated in the southernmost Indian state of Kerala, a place well known for its high degree of endemism and richness of flora and fauna. Set high on the Western Ghats with north latitude 110 26' 28" and 110 58' 22", east longitude 750 46' 38" and 760 26' 11" , the altitude ranges from 700 to 2100 m. The average rainfall is 2322 mm per year. Nearly 14% area were under cover of Teak plantations, apart from natural forest. Many teak plantation is present outside the zones of its natural distribution. Nevertheless, in the previous years, due to extended biological or environmental pressure, the teak resources got fragmented, because of that homogeneity or reduction of genetic variability may occurred. The lack of systematic studies of genetic diversity among teak species and ecotypes could seriously restrict genetic improvement by limiting exploitation in teak culture and breeding of many excellent traits. Accordingly, it is essential to properly characterize and assess the genetic diversity of teak SPA resources for protection and breeding utilization. In the past few years, a quantity of molecular markers, such as RAPD (Randomly Amplified Polymorphic DNA) (Nazeer et al., 2012; Changtrgoon et al., 2000), AFLP (Amplified Fragment Length Polymorphism) (Wang et al., 2011), SSR (Simple Sequence Repeat or microsatellites) (Huang et al., 2016) and ISSR (Inter Simple Sequence Repeat) (Dania et al.2019) have been generally employed to examine genetic diversity and structure in teak. While previous reports concentrated on the fingerprinting and genetic variability of teak germplasm, there are few studies on the population genetic diversity of different ecotypes especially the wyanad teak. RAPDs technique have been broadly employed in different economically important forest species to find out genetic diversity and variation (Alcantara and Veasey, 2013). RAPD markers yields better results, easy to handle and less expensive and covers whole genome. Moreover, they can be used as a primary method of choice for screening the genotypes to find duplicates in collections. We

evaluate the population-level genetic diversity of two teak populations throughout their known distribution by RAPD analysis to give some references for conservation measures and germplasm resource development and utilization.

II. MATERIAL AND METHODS

II.1 Plant material

Two Teak Seed Production areas in Wayanad, Kerala were sampled across 10 TSPAs under South Wayanad Division, Kerala Forest Department, has been taken for this study viz., including Changampam and Cheeyambam areas. Interpopulation distance ranged from a minimum of 30.0 km (CG-CY). The number of samples analyzed depended on the usability of trees (65-150 cm at breast height diameter, height 14-23 m) in each sampling location. In each population, 10 - 25 adult Teak trees were randomly sampled in each quadrant at the distance exceeding at least 5 m from each other to reduce the probability of sampling from family clusters. From each individual tree, fresh mature leaves were randomly sampled, stored in plastic bags and taken to the lab for experimental studies.

II.2 DNA Extraction

Genomic DNA was extracted from storage leaves of Teak by using the modified and optimized Cetyl Trimethyl Ammonium Bromide (CTAB) method (Borges et al. 2009). Concentration and purity of DNA was determined by electrophoresis in 0.8% agarose gels and spectrophotometrically (Analytik Jena, Germany). This extraction method yielded an average DNA content of 400 µg g -1 of leaf tissue, and purified DNA revealed A260/A280=1.80 ± 0.11, revealing the high purity of DNA samples. Purified DNA was diluted to approximately 20 ng·µL−1 prior to polymerase chain reaction (PCR) amplification, and preserved in the refrigerator at −20˚C.

II.3 RAPD amplification

DNA from each Teak genotype was screened with 12 RAPD primers (Tables 1). For RAPD, the PCR reaction (50 µl) consisted of: 1X reaction buffer (20 mM Tris-HCl pH 8.4, 50 mM KCl), 0.2 mM dNTPs, 2 mM MgCl2 , 10 pM primer, 1.0 unit of Taq DNA polymerase, and 25-50 ng genomic DNA. PCR products were separated on a 1.5% agarosegel. The gel was stained with ethidium bromide (0.5 µg/mL) and visualized under UV light and the image captured using BioRad Gel Doc XR+ imaging system, India. A total of 30 RAPD primers were screened, of which 12primers (IDTDna primers) that gave consistent results and higher number of polymorphic bands were selected. A 100 bp DNA ladder (Bangalore Genei, India) was used for molecular weight estimation of PCR products. Only the bands between 100 and 1500 bp which were clear and unambiguous were recorded.

II.4 Data Analysis

Polymorphic bands were scored visually as either present “1” or absent “0” to form a binary matrix. On the supposition of Hardy-Weinberg equilibrium, POPGENE versions 1.31 (Yeh et al, 1994) were employed to compute the following basic statistics. To assess genetic diversity at species level and population level, the parameters calculated included; percentages of polymorphic bands (PPB%), mean number of alleles (Na), mean effective number of alleles (Ne), Nei’s gene diversity index (H) and Shannon-diversity index (I). To evaluate gene differentiation between populations, the parameters calculated included Nei’s coefficient of population differentiation (GST) with the formula: ST G = − (Ht Hs) Ht (Nei, 1973), and Gene flow among populations (Nm) which was calculated using the formula of S (1) 2 Nm G G = − ST T . Genetic relationships among populations were estimated by Nei’s unbiased genetic distances (D) and genetic identities (I) using NTSYSpc-2.0 (Roulf, 1998) and a cluster analysis was performed using UPGMA (the Unweighted Pair Group Method with Arithmetic mean) (Nei, M. (1978). In addition, population differentiation coefficients within and among the populations were computed from AMOVA 1.55 (analysis of molecular variance) (Excoffier et al., 1992).

III. RESULTS AND DISCUSSION

III.I RAPD Analysis

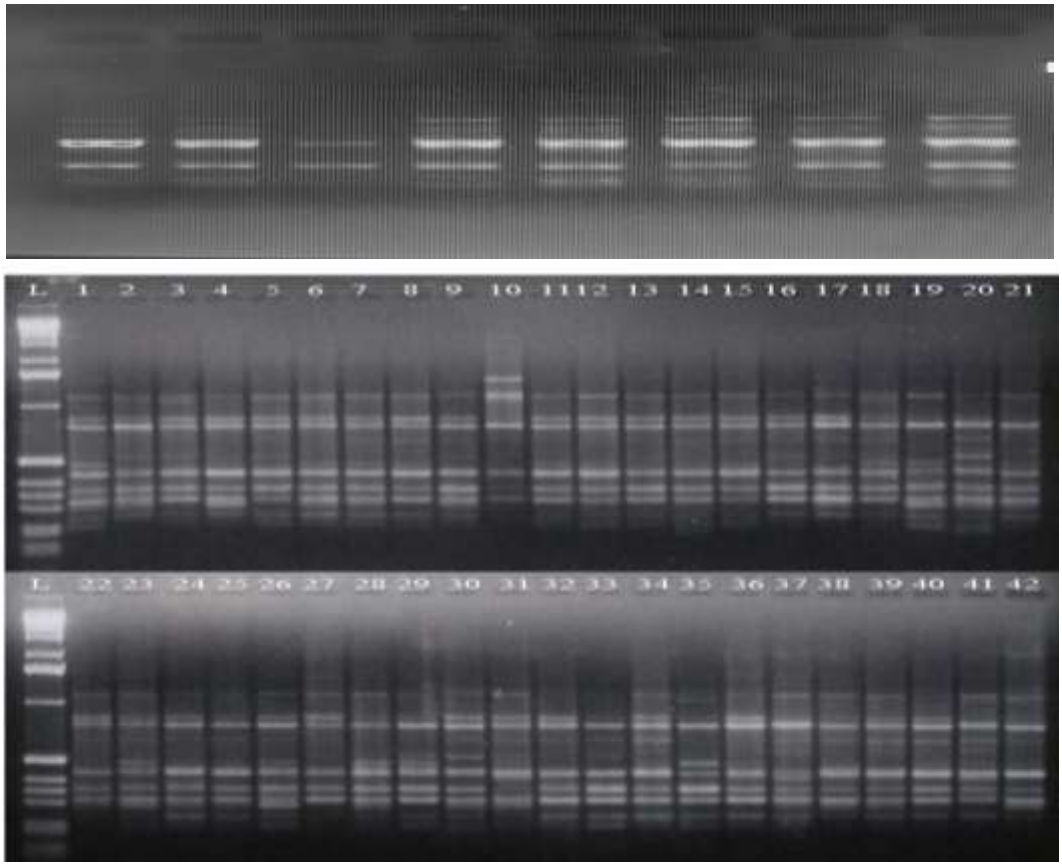
In our results 90 bands in sum was bring out from 12 screened primers for the 202 individual samples from the two populations, of which 84 (92.13%) were polymorphic bands. The size of the amplified band ranged from 300 bp to 1200 bp. The percentage of polymorphic band (PPB) of the 12 RAPD primers differed from 68.65% (P6) to 100% (P8, P9, P10 and P12), with an average of 91.41%. The number of bands presented by each primer also differed from 6 (P6) to 11 (P10) with an average of 10.10 (Table 1, Fig.1).

Table. 1. Polymorphic bands generated by RAPDS in Two populations of TSPAs

Primer	Sequences	Tmb ºC	Number of Band	Number of Polymorphic band	PPB%
P1	CAGGCCCTTC	33.4	9	9	100
P2	AGTCAGCCAC	31.8	10	10	100
P3	AATCGGGCTG	34.7	12	11	81
P4	AGGGGTCTTG	34.1	10	10	99
P5	GGTCCCTGAC	32.5	15	10	100
P6	GAAACGGGTG	32.0	6	6	80
P7	CTACTGCGCT	35.0	4	3	99
P8	ACGGACGTCA	32.1	10	10	100
P9	TGACGCATGG	33.4	12	9	100
P10	CTGAGGTCTC	32.7	11	9	100
P11	GGAGAGACTC	32.6	9	9	100
P12	CCTCTGACTG	39.5	10	10	100

Sequence 5' - 3'; PCR reaction annealling temperature for tabulated primers (˚C) ; PPB Percentage of polymorphic band.

Fig.1 Variation pattern generated by the RAPD primer P-5- GGTCCCTGAC **- between CG and CY population**



At the species level, the effective number of alleles (Ne), Nei's genetic diversity (H) and the Shannon information index (I) were 1.4041, 0.2673, 0.4112 respectively. At the population level, PPB ranged from 19.67% for the CG population to 47.24% for the CY population, with an average of 42.25%, and the average Ne, H, and I were 1.1214, 0.1236, and 0.1772, respectively. The results showed that the genetic diversity of Wyanad TSPAs from the Cheeyambam CY population (Ne = 1.3601, H = 0.1726, I = 0.2656) was the richest in two estimates among the two populations. The STRUCTURE results estimated the fixation index (Fst) for each of the populations and suggested that there was no significant divergence within both of the two subpopulations.

III.2 Genetic Variation and Populations Genetic Structure

In accordance with the GST value (GST = 0.5055), found in this study a significant amount of genetic differentiation is observed among and within two populations of Wayanad Teak TASPs. The end results of AMOVA, points that 50.45% of the genetic variation was subdivided inter-populations and 48.44% intra-populations. The difference might be due to the sampling locations, sampling size, and the different marker systems used. Similar studies have shown that there are inconsistencies in genetic differentiation value found between populations when using different genetic markers in other species populations (Maguire et al., 2002). High genetic differentiation in Wayanad TSPAs is attributed to the following points: Firstly, because of low gene flow of the TSPAs teak habitat (Nm = 0.5122), the present population structure was shaped by genetic drift mainly. In order to adapt to the diverse habitats of the region in the evolutionary process, variants to some extent occurred, which may have been preserved and fixed gradually due to limited gene flow, thereby, genetic differentiation among populations occurred. Similar type of conclusion was found in walnuts genetic diversity studies in a group of genotype using RAPD markers (Wani et al. 2014)

The GST value and AMOVA analysis results obtained in this study indicated that Wayanad has a significant genetic differentiation among populations, which suggests that the species has a strong environmental adaptability. Put briefly, high genetic variation among populations is due to genetic drift and geographical isolation of the populations. Based on the genetic similarity, two populations of Wayanad TSPAs were grouped into 3 clusters, but genetic differentiation did not completely cluster according to geographical distances. Our results co insides with moderate genetic diversity aspect as like many researchers findings in teak (Alcântara and Veasey 2013; Hansen et al. 2014; Sreekanth et al. 2014; Vaishnaw et al. 2014; Hirao et al. 2016, Chaudhari et al., 2018).

Table: 2 The STRUCTURE results 202 *Tectona grandis* accessions for the fixation index (Fst), average distances (expected heterozygosity/*He*) and number of genotypes assigned to each subpopulation.

Population	Inferred clusters	Mean Fst	Exp. Hete	No. of Genotypes
Popu1	0.455	0.1622	0.2638	102
Popu2	0.522	0.2002	0.2500	100

IV. CONSERVATION IMPLICATIONS

To know about the genetic diversity and genetic variation is indispensable for the protection of germplasm resources. Our study provides an insight into genetic diversity and genetic differentiation at population levels of Wayanad Teak seed production area, and indicates a moderate genetic diversity caused by variation in geographical level. The high level of polymorphism possibly reflects the outcrossing character of teak because almost similar results have been obtained in many fruits and nut tree species like pistachio, walnut or olive. Accordingly, so as to secure existing diversity, preservation areas covering large populations as well as many small populations should be established.

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