PLANT SCIENCE TODAY
ISSN 2348-1900 (online)
Vol x(x): xx–xx
https://doi.org/10.14719/pst.5745

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RESEARCH ARTICLE

Unveiling genetic diversity in teak (*Tectona grandis* L. f.) accessions through ISSR profiling

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OPEN ACCESS

ARTICLE HISTORY

Received: 12 October 2024
Accepted: 20 December 2024

Available online
Version 1.0 : 18 February 2025

Check for updates

Additional information

Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

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Ashwath M N, Santhoshkumar A V, Shankar C Elizabeth M, Mathew D, Shiran K. Unveiling genetic diversity in teak (*Tectona grandis* L. f.) accessions through ISSR profiling. Plant Science Today (Early Access). https://doi.org/10.14719/pst.5745

Abstract

The present study focuses on understanding the genetic diversity among the selected teak accessions using Inter simple sequence repeat (ISSR) markers. Genomic DNA from ten selected teak accessions was extracted using a modified Cetyltrimethylammonium Bromide (CTAB) protocol, ensuring high quality and yield. ISSR primers were employed to amplify DNA fragments and resulting bands were visualized. Banding patterns were analyzed to determine the presence of alleles and polymorphism. The study revealed significant genetic diversity among teak accessions, with 212 alleles identified, of which 199 were polymorphic. Cluster analysis grouped accessions into three distinct clusters based on genetic relatedness, highlighting the influence of environmental factors and geographic location on teaks' genetic variability. Cluster I comprised two accessions, A1 (Top slip-6) and A2 (Top slip-10) (Jaccards' coefficient=0.361). Notably, A6 (Nellikutha-1) and A4 (Nedumkayam-2) show a close genetic relationship, sharing a common ancestry with A10 (Karulai) and finally, all three are linked with A7 (Nedumkayam-1), forming cluster II. Cluster III included two pairs of closely related accessions: A3(TC4)-A5(TC11) and A8(Nellikutha-2) - A9 (Shankaram-thode-1). Neis' genetic identity and distance indices provided further insights into the degree of genetic similarity and differentiation among accessions.

Keywords

diversity; evolution; population; molecular-markers; teak

Introduction

Genetic diversity within a species encompasses the range of genetic variation among the individuals. Teak (*Tectona grandis* L. f.) is a tropical timber with a considerable genetic variation due to its presence over vast geographical regions. In such situations, molecular diversity is more significant in intra-population than inter-population (1). This diversity results from evolutionary processes and selection, underpinning a species' capacity to adapt to varying ecological conditions (2). Restricted gene flow, illegal logging, anthropogenic activities and unknown or secondary seed sources often cause genetic deterioration in teak. Understanding its genetic diversity is essential for maintaining the quality and quantity of timber resources and ensuring its continued survival and resilience in the face of emerging climatic challenges (3). Application of ISSR (Inter Simple Sequence Repeat) markers enables the identification and characterization of distinct genetic

profiles among teak populations, shedding light on their genetic relatedness and evolutionary history. This knowledge is the foundation for informed conservation and breeding efforts in forest trees (4,5). ISSR primers target abundant sequences throughout the plant genome and exhibit rapid evolution. As a result, ISSR markers can unveil a significantly higher number of polymorphic fragments. These characteristics make ISSR markers invaluable in genetic diversity studies and population genetics analyses (6).

The teak populations face the risk of genetic deterioration due to uncontrolled logging and land degradation and early breeding efforts for increased yield with improved wood quality (7). Teak breeding is needed to improve the wood supply without erosion of genetic diversity (8). Understanding genetic diversity and composition is becoming one of the prime objectives in many breeding programs due to maintaining the differentiated population in breeding to reduce inbreeding and allow the mixing of gene pools (9,10,11). To combat drift and have a powerful evolutionary force, artificial movements of individuals in every generation of breeding are needed (12). The successful management and conservation of species are based on the extant knowledge of genetic variation with its natural distribution and introduced populations (5). The diversity is often compromised when plus tree selections are made from a limited geographical area. Amidst this backdrop, the current study explores molecular diversity using ISSR markers in teak to explore the diversity present among plus teak trees selected earlier for the vigour of growth and form.

Materials and Methods

The molecular diversity of selected teak accessions (Table 1) was assessed using Inter Simple Sequence Repeats (ISSR) primers (Table 2). Healthy, matured and fresh leaves were collected from vegetatively propagated plantlets (of plus trees selected from Kerala and Tamil Nadu under the AICRP program on Agroforestry and stored at -20˚C for later use (13).

Table 1. List of teak accessions selected for assessment of molecular diversity

Accession No.	Accession name
A1	Top slip-6
A2	Top slip-10
A3	TC4
A4	Nedumkayam-2
A5	TC11
A6	Nellikutha-1
A7	Nedumkayam-1
A8	Nellikutha-2
A9	Shankaramthode-1
A10	Karulai

Table 2. List of ISSR markers used for molecular diversity analysis

Sl. No.	Primer	Sequence
1		
2	UBC 807	AGA GAG AGA GAG AGA GT
3	UBC 808	AGA GAG AGA GAG AGA GC
4	UBC 809	AGA GAG AGA GAG AGA GG
5	UBC 810	GAG AGA GAG AGA GAG AT
6	UBC 811	GAG AGA GAG AGA GAG AC
7	UBC 818	CAC ACA CAC ACA CAC AG
8	UBC 826	ACA CAC ACA CAC ACA CC
9	UBC 827	ACA CAC ACA CAC ACA CG
10	UBC 848	CAC ACA CAC ACA CAC ARG
11	UBC 858	TGT GTG TGT GTG TGT GRT
12	UBC 866	CTC CTC CTC CTC CTC CTC
13	UBC 874	CCT CCT CCT CCT
14	UBC 876	GAT AGA TAG ACA GAC A
15	UBC 878	GGA TGG ATG GAT GGA T
16	UBC 880	GGA GAG GAG AGG AGA
17	UBC 884	HBH AGA GAG AGA GAG AG
18	UBC 886	VDV CTC TCT CTC TCT CT
19	UBC 887	DVD TCT CTC TCT CTC TC
20	UBC 888	BDB CAC ACA CAC ACA CA
21	UBC 889	DBD ACA CAC ACA CAC AC

Plant genomic DNA isolation

Genomic DNA was extracted using the CTAB protocol with some modifications (14). Around 200-300 mg of the leaf sample was weighed and ground along with 1.50-2.00 mL of pre-warmed extraction buffer (3 % CTAB) and 50 mg of polyvinylpyrrolidone (PVP) was added. The green viscous liquid was then transferred to 2 mL Eppendorf tubes containing 25 µL of β-mercaptoethanol and mixed by gentle inversion. Tubes were placed in a floating rack and kept in a water bath (60 to 75 min) preheated to 65 °C (gentle inversion every 10 minutes). Warmed tubes were taken out, cooled at room temperature and centrifuged at 10000 rpm for 10 min at 4 °C and the clear (greenish tinge) supernatants were transferred to fresh Eppendorf tubes. An equal volume of Phenol: Chloroform: Isoamyl alcohol (25:24:1) was added, emulsified by gentle inversion for 5 minutes to get a turbid (light yellowish green) solution and centrifuged at 10,000 rpm @ 4 °C for 15 min.

The top aqueous layer was carefully transferred to fresh tubes without any disturbance to the organic interphase layer and an equal volume of Chloroform: Isoamyl alcohol (24:1) was added and mixed by gentle inversion for 5 minutes, followed by centrifugation at 10000 rpm @ 4 °C for 10 minutes. The top aqueous phase was carefully transferred to 1.5 mL centrifuge tubes without any disturbance to the thin interphase layer and mixed by gentle inversion after adding an equal volume of ice-cold isopropanol. Tubes were cooled to -20 °C for two to three hours to encourage DNA precipitation, followed by centrifugation at 12000 rpm for 15 min (4 °C) to pellet the DNA as a translucent film.

The supernatant was discarded without losing DNA pellets at the bottom of the centrifuge tubes. DNA pellets were washed twice with 50 µL of 70 % ethanol to remove any traces of salts by centrifuging at 4500 rpm for 5 mins at 4 °C. After decanting off the ethanol and air drying, DNA pellets were dissolved in 100 µL TE buffer (1M) by gentle inversions. The DNA samples were stored at -20 °C freezer till further analysis. The quality and quantity of the DNA were estimated using a NanoDrop Spectrophotometer (Jenway - Genova Nano). Since the absorption maxima for nucleic acid and proteins are at 260 and 280 nm, respectively, absorbance was recorded at both wavelengths and the purity of the sample was estimated using the OD260/OD280 ratio.

ISSR amplification

The selected 21 ISSR primers were used to amplify and understand the polymorphism among the accessions, as shown in Table 2 (15, 16). Thermal cycling was carried out with a 5 µL reaction mixture. The reaction mixture was prepared by mixing 1 µL of genomic DNA sample (50 ngµL^{-1}), 1.5 µL of readymade master mix (Takara), 1.5 µL molecular grade nuclease-free water and primer (5 µM) - 1 µL. The tubes were placed in the thermal cycler for 20 cycles of PCR. The PCR program was followed as given in Table 3. The PCR products were electrophoresed on 2.5 percent agarose gel at 70 volts, using a 100 bp DNA Ladder (HiGenome). The gel profile was visualized under a UV transilluminator (Genetix GXFC-15-FCX), documented using a Gel Doc image analyzer (GeNei TM- UVITEC Fire Reader, Merck, UK+computer system) and saved for further analysis (Plate 1.).

Table 3. Details of the PCR program

Steps	Temperature (˚C)	Time
Stage 01 (Hotstart)	94	3 min
Stage 02		(20 cycle)
Denaturation	94	30 sec
Annealing (Ta)	45- 50	30 sec
Extension	72	1 min
Stage 03		
Final extension	72	10 min
Hold	4	∞

Statistical analysis

The GelAnalyser software was used to generate a banding profile to determine the size and readability of the bands. Additionally, manual scoring was conducted, where alleles were assessed for their presence (1) or absence (0) based on the results obtained from ISSR assays. Characteristics related to the primer amplification pattern, including the total number of alleles (NA), monomorphic alleles (Am), polymorphic alleles (Ap) and the percentage of polymorphic alleles (PP), were derived through analyzing of scored alleles.

The constructed matrix data were used to estimate polymorphisms and genetic relatedness of teak accessions using the DARwin (Dissimilarity analysis and representation for Windows version 6.0.021) software. Pairwise genetic similarity using Jaccards' coefficient was calculated by considering both monomorphic and polymorphic bands (17). The dendrogram was constructed following the UPGMA (Unweighted pair-group method with arithmetic mean) (18). PopGene32 (Population genetics) software program was employed to compute Neis' coefficient among accessions (19,20). The correlation analysis was run using the software KAU-GRAPES (General r-shiny-based analysis platform empowered by statistics).

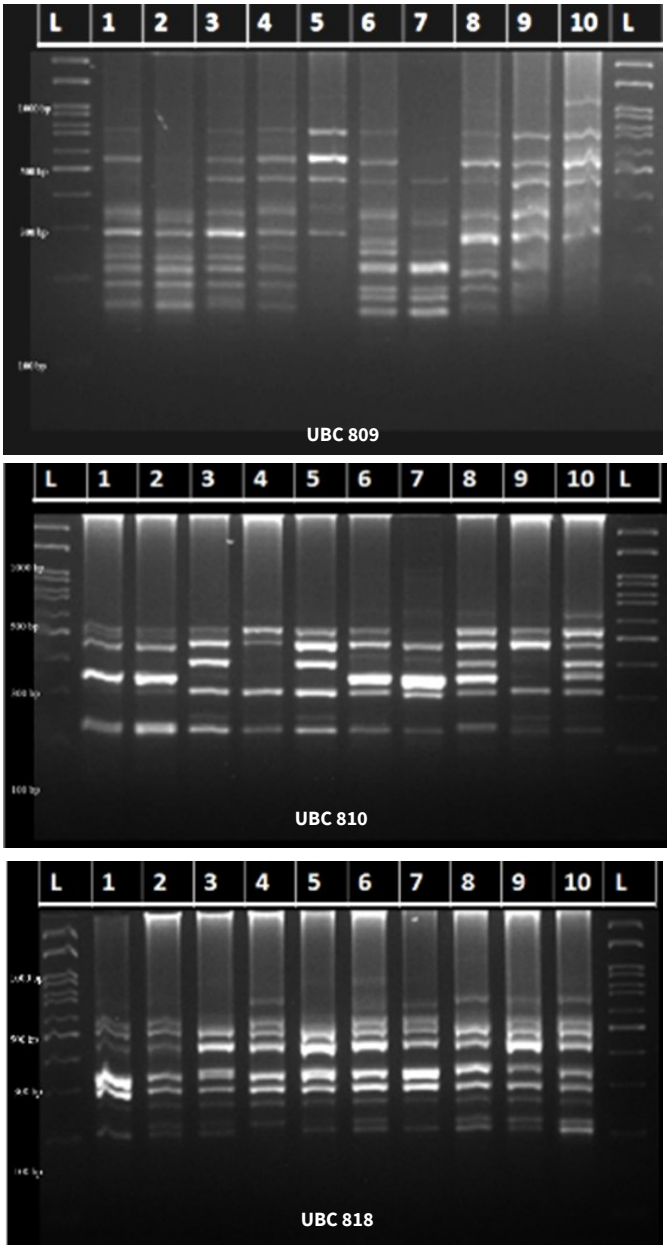

Plate 1. ISSR fingerprints of 10 accessions generated by different primers (UBC 809, 810, 818).

Results

Quality and quantity of genomic DNA

The extracted DNA yield ranged from 166.7 to 269.4 ng/µL. The quality of DNA ranged from 1.86 to 2.08 (Table 4).

Primer-wise analysis of banding patterns

Table 4. Quality and Quantity of DNA extracted from each accession

Accession No.	Quality (260/280)	Quantity (ng/µl)
A1	1.86	265.7
A2	1.90	162.3
A3	2.03	173.3
A4	1.81	170.3
A5	1.89	269.4
A6	1.86	174.3
A7	1.91	259.2
A8	2.08	166.7
A9	1.95	246.5
A10	1.92	181.3

Comprehensive information about the Amplicon Size Range (bp), Number of Alleles (NA), Monomorphic Alleles (Am), Polymorphic Alleles (Ap) and Percentage of Polymorphism (PP) of each primer are tabulated in Table 5. The size of the amplified bands varied between 102 and 2044 base pairs, resulting in 212 alleles. Among these alleles, 199 displayed polymorphism, showcasing genetic diversity. At the same time, 13 remained monomorphic, indicating genetic uniformity within specific markers (Table 5 & Fig. 1). Notably, UBC 886 exhibited the highest number of alleles (18). In contrast, UBC 858 and UBC 866 produced the lowest with only five alleles. The other primers generated different numbers of alleles, including 16 for UBC 809, 14 for UBC 807, UBC 808, UBC 818, UBC 827 and UBC 884, 12 for UBC 887, 10 for UBC 826, UBC 876, UBC 880 and UBC 888, 9 for UBC 811, 8 for UBC 810, UBC 848 and UBC 878, 7 for UBC 889 and 6 for UBC 874. Out of the primers used, only nine primers showed monomorphic alleles namely, UBC 808 (1), UBC 810 (2), UBC 811 (1), UBC 818 (1), UBC 826 (1), UBC 827 (1), UBC 848 (3), UBC 858 (1) and UBC 880 (2), resulting to varying percentage polymorphism of 92.86, 75.00, 88.89, 92.86, 90.00, 92.86, 62.50, 80.00 and 80.00 respectively. The remaining alleles displayed whole polymorphism, with a 100 percent polymorphism rate. The graphical representation of data in the matrix set shows the presence (red) and absence (blue) of alleles at a particular base pair (Fig. 1).

Table 5. Primer-wise analysis of banding patterns by ISSR markers

Primer	Amplicon Size Range (bp)	NA	Am	Ap	PP
UBC_807	231 - 1108	14	0	14	100.00
UBC_808	190 - 945	14	1	13	92.86
UBC_809	170 - 1259	16	0	16	100.00
UBC_810	230 - 620	8	2	6	75.00
UBC_811	195 - 663	9	1	8	88.89
UBC_818	209 - 758	14	1	13	92.86
UBC_826	193 - 957	10	1	9	90.00
UBC_827	223 - 920	14	1	13	92.86
UBC_848	193 - 484	8	3	5	62.50
UBC_858	285 - 682	5	1	4	80.00
UBC_866	397 - 1298	5	0	5	100.00
UBC_874	370 - 2044	6	0	6	100.00
UBC_876	212 - 660	10	0	10	100.00
UBC_878	350 - 1410	8	0	8	100.00
UBC_880	163 - 603	10	2	8	80.00
UBC_884	210 - 970	14	0	14	100.00
UBC_886	192 - 779	18	0	18	100.00
UBC_887	232 - 726	12	0	12	100.00
UBC_888	254 - 490	10	0	10	100.00
UBC_889	102 - 421	7	0	7	100.00
Total/ Average	102 -2044	212	13	199	92.75

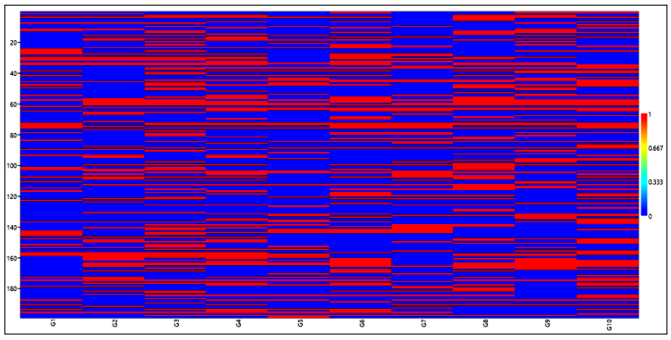

Fig. 1. Allelic data matrix showing presence and absence of bands at different bands produced by all primers (y-axis) among ten teak accessions (x-axis).

Jaccards' similarity coefficients and cluster analysis

The estimates of Jaccards' similarity coefficients among the teak accessions reveal the degree of genetic similarity or dissimilarity. Table 6 of coefficients show the similarity or dissimilarity between pairs of accessions, with higher values indicating more significant similarity. The similarity coefficient between A1 and A2 was 0.361, representing the degree of similarity between A1 and A2. Further, accession six is closely related to A4, A10, A8 and A7 with coefficient values of 0.504, 0.476, 0.465 and 0.440, respectively. Meanwhile, A9 was closely related to A8, with a coefficient of 0.452. Similarly, A3 showed a close relatedness with A6 (0.437). Cluster analysis utilizing the coefficients obtained from 1000 bootstrap repetitions grouped the ten accessions into three distinct clusters, as depicted in Fig. 2. Cluster I comprised two accessions, A1 and A2. Notably, accessions 6 and 4 show a close genetic relationship, sharing a common ancestry with A10 and finally, all three are linked with A7, forming cluster II. Cluster III included two closely related accessions: A3 and A5 and A8 and A9.

Neis' diversity indices and correlation

Neis' genetic identity and distance are measures used to assess genetic relationships and diversity between accessions (Table 7). Genetic identity (I) measures the proportion of genetic similarity between two accessions and quantifies the alleles that are identical between the two accessions. A higher genetic identity indicates more significant genetic similarity between individuals, with values ranging from 0 (entirely dissimilar) to 1 (identical). A1 and A2 have a genetic identity of 0.675, indicating a relatively high level of genetic similarity. Genetic distance (D) measures the genetic dissimilarity between two accessions. A smaller genetic distance indicates more significant genetic similarity, while a larger one suggests more signifi-

Table 6. Jaccard's similarity coefficient matrix among the teak accessions

	A1	A2	A3	A4	A5	A6	A7	A8	A9
A2	0.361								
A3	0.330	0.412							
A4	0.344	0.320	0.424						
A5	0.296	0.295	0.426	0.446					
A6	0.325	0.365	0.437	0.504	0.410				
A7	0.271	0.283	0.353	0.380	0.361	0.440			
A8	0.312	0.331	0.402	0.431	0.372	0.465	0.379		
A9	0.341	0.286	0.424	0.345	0.394	0.378	0.281	0.452	
A10	0.284	0.313	0.337	0.431	0.373	0.476	0.414	0.388	0.453

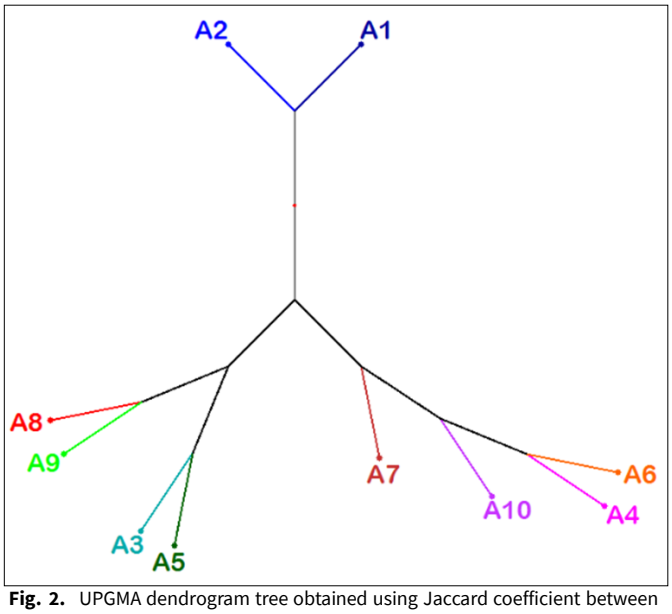

Fig. 2. UPGMA dendrogram tree obtained using Jaccard coefficient between the ten teak accessions.

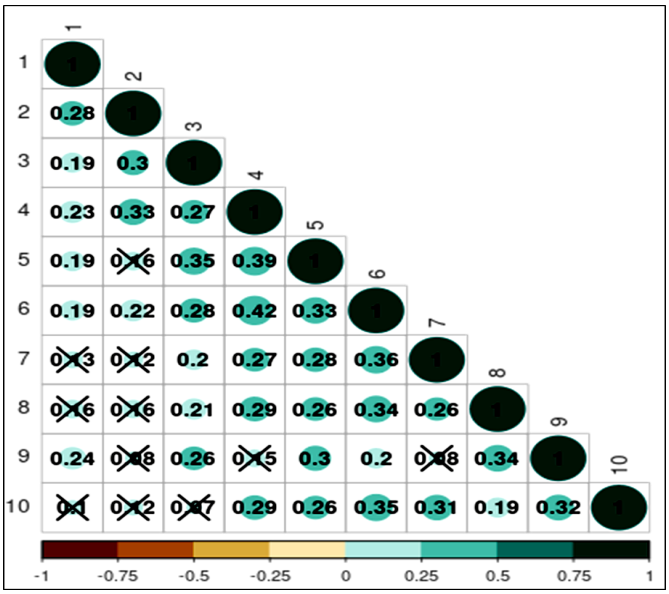

Fig. 3. Correlation matrix showing the relationship between teak accessions using ISSR banding profile.

Table 7. Nei's genetic identity (above diagonal) and genetic distance (below diagonal) among the teak accessions

	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10
A1	***	0.675	0.599	0.632	0.642	0.609	0.609	0.594	0.637	0.561
A2	0.394	***	0.651	0.675	0.618	0.623	0.594	0.590	0.566	0.566
A3	0.512	0.429	***	0.637	0.675	0.642	0.604	0.609	0.642	0.538
A4	0.459	0.394	0.451	***	0.708	0.712	0.646	0.651	0.590	0.646
A5	0.444	0.481	0.394	0.346	***	0.675	0.675	0.642	0.675	0.637
A6	0.497	0.474	0.444	0.339	0.394	***	0.689	0.675	0.613	0.679
A7	0.497	0.520	0.505	0.437	0.394	0.373	***	0.637	0.566	0.660
A8	0.520	0.528	0.497	0.429	0.444	0.394	0.451	***	0.675	0.599
A9	0.451	0.569	0.444	0.528	0.394	0.489	0.569	0.394	***	0.670
A10	0.578	0.569	0.620	0.437	0.451	0.387	0.415	0.512	0.401	***

cant genetic differentiation. Among the accessions, A1 and A2 have a genetic distance of 0.394, indicating they are genetically more similar to other pairs with higher genetic distances. Accessions A4 and A9 have a relatively high genetic distance of 0.528, indicating they are genetically dissimilar. Accessions A7 and A10 have a genetic distance of 0.415, indicating a moderate level of genetic dissimilarity.

Supporting the results of genetic distance and diversity, the correlation coefficients ($p < 0.001$) between ten accessions are shown in Fig. 3. A1 had a higher correlation coefficient of 0.284 with A2 than other accessions. Further, A2 had higher coefficient values of 0.326, 0.298, 0.225 and 0.163, with A4, A3, A6 and A4 showing related connections. The dendrogram shows that A8–A9 had correlation values of 0.342 and A6–A4 had 0.415. A3 and A5 shared a close relation, showing a correlation coefficient of 0.351.

Discussion

Understanding genetic diversity is becoming one of the prime objectives in any breeding program to maintain the differentiated population, reduce inbreeding and allow the mixing of gene pools (9). Using dominant, multiallelic markers like ISSR is the most efficient approach for revealing genetic diversity at a genomic or molecular level (15).

Young or growing teak leaves contain high concentrations of polyphenols and matured leaves are loaded with polysaccharides and carbohydrates, accounting for low yield and quality of genomic DNA (21,23). Adding extra PVP to the sample forms a hydrogen bond, which facilitates the separation of DNA from polyphenolic components. Treatments involving phenol, chloroform and isoamyl alcohol were employed to minimize protein contamination (14,24). A wash with chloroform and isoamyl alcohol (24:1) effectively eliminated polysaccharides and reduced protein contamination. Reports also suggest that exposure of genomic DNA in a buffer to normal temperature during different steps involved in washing may cause DNA to degrade or break down, resulting in lower yield (25). The extracted DNA yield quantities ranged from 166.7 to 269.4ng/µL, with OD260/280 values falling within the acceptable range of 1.86 to 2.08.

The individual marker-wise assessment provided valuable insights into genetic diversity among accessions. Among the alleles reproduced, 13 were monomorphic, indicating the genetic uniformity of a particular allele. Monomorphic bands indicate the homogeneous situation among the accessions at a given allelic position. In other words, there is no genetic variation at that specific locus. These regions are under strong evolutionary constraints or genetic heritage among the populations. On the other hand, polymorphic alleles represent heterogeneity among the individuals representing genetic variants at the specific locus. The primers UBC 807, UBC 809, UBC 866, UBC 874, UBC 876, UBC 878, UBC 884, UBC 886, UBC 887, UBC 888 and UBC 889 showed 100 per cent polymorphism under the investigation.

The Jaccards' similarity coefficients among the teak accessions ranged from 0.271 (A1, Top slip-6 and A7, Nedumkayam-1) to 0.504 (A4, Nedumkayam-2 and A6, Nellikutha-1). The coefficient values indicate the degree of similarity, with higher values indicating more similarity, facilitating the identification of clusters. Based on these coefficients, accessions were grouped into three groups (Fig. 2). The cluster I consisted of two accessions, A1 (Top slip-6) and A2 (Top slip-10), with a coefficient value of 0.361. Accessions 6, 4, 10 and 7 were grouped into a single cluster, indicating a shared ancestry primarily with accession 6, with coefficient values of 0.504, 0.476 and 0.440 for accessions 4, 10 and 7, respectively. Cluster III included two closely related accessions: A3 and A5 (0.426) and A8 and A9 (0.452). Neis' genetic identity reveals a similarity between the two accessions, whereas genetic distance measures the genetic dissimilarity between two accessions. Genetic identity and distance ranged between 0.566 and 0.716 and 0.339 and 0.578, respectively. The correlation among accessions also supports the results of genetic diversity and cluster analysis by showing relatedness among selected trees (Fig. 3). Accession A1 (Top slip–6) had a statistically significant positive correlation with A2 (Top slip–10) ($r = 0.284$, $p < 0.001$), indicating a close relation compared to any other accessions. Some correlations are not statistically significant, suggesting insufficient evidence to conclude a meaningful relationship between those particular accessions as found by genetic diversity and distance.

Similarly, A Jaccard coefficient value ranges from 0.31–0.85 and 0.27–0.88 in RAPD and ISSR markers with 93.2 and 95.9 per cent polymorphic loci, respectively, for teak populations (22). Among teak populations in southern Gujarat, the highest Jaccard similarity coefficient observed was 0.72 and the highest Nei coefficient was 0.84 (16). On the other hand, the lowest values recorded were a Jaccard similarity coefficient of 0.42 and a Nei coefficient of 0.59. These findings indicate varying genetic similarity and diversity within teak populations in the southern Gujarat region. The average Neis' gene diversity value of 0.32 was observed in Indian teak populations from RAPD analyses (26). The genetic distinctions among accessions could arise from adaptations to varying environmental conditions (27).

The Neis' coefficients ranging from 0.32 to 0.40 for 29 teak populations sampled from central and peninsular India were noticed (28). These coefficients enabled the categorizing of the entire set of populations into three distinct clusters by effectively delineating the moist teak populations from those in dry regions and the semi-moist teak populations, highlighting the genetic differentiation among these ecological categories. Moreover, a species' genetic variability can be shaped by several factors, including geographic distribution, reproductive strategies, dispersal methods and position within ecological successional stages (29). The 48-plus trees selected from 11 natural populations did not form distinct groups based on their geographical distribution (22). This observation implies the possibility of either a shared genetic foundation among these accessions or regular occurrences of natural or human-mediated gene flow across various teak populations, from which these plus tree selections were made.

Most studies represented higher variation in natural populations spread over southern and central parts of India, attributing it to the diverse climatic conditions that influenced trees' adaptation to the location. Molecular diversity studies showed the migration path of teak from southern India to Myanmar, Thailand and Laos, where the natural distribution area is less and the diversity is at its lowest, which may be seen as a diminishing genetic diversity moving eastward. Reduced diversity may hamper growth and productivity by altering inherent characteristics like biotic and abiotic stress tolerance. Introducing wild and distinctive clones helps conserve them and improve breeding strategies.

Conclusion

The research highlights the vital significance of compre-

hending genetic diversity among teak populations, especially considering its importance as a tropical timber species and its various challenges because of limited gene flow, illicit logging and human activity. By applying ISSR markers, this research has provided valuable insights into the molecular diversity among selected teak accessions. Extracting high-quality genomic DNA using a modified CTAB protocol tailored to the unique requirements of teak leaves ensured reliable results for ensuing molecular analyses. Numerous polymorphic alleles were found in the ISSR amplification, indicating a high degree of genetic variation among the accessions under investigation. Teak accessions could be clustered by analyzing Jaccards' similarity coefficients, revealing genetic distinctiveness and relatedness patterns. These results were corroborated by Neis' genetic identity and distance indices, which showed the degree of genetic differentiation among the accessions and their shared genetic linkage. The studys' findings are consistent with earlier investigations into the genetic diversity of teak, highlighting the influence of environmental conditions, geographic location and human activities on the genetic variability of the species. Comprehending these dynamics is crucial for well-informed conservation and breeding initiatives that seek to preserve and improve the resilience and productivity of teak populations.

Acknowledgements

The authors would like to express their sincere and profound gratitude to the College of Forestry, Kerala Agricultural University, for the support and facilities provided for the study. We also thank the Ministry of Tribals, GoI, for the grants given to research scholar (Award No—202122-NFST-KAR-00498). AICRP on Agroforestry, College of Forestry, KAU, Thrissur, is duly acknowledged for providing mother plant materials.

Authors' contributions

AMN conducted the experiment, recorded the data, and performed the statistical analysis. SAV, ME, and DM provided guidance by developing the research concept, facilitating the laboratory setup, and approving the final manuscript. SC carried out the lab experiments and worked on data visualization. AMN and SC drafted the manuscript, while SK contributed to summarizing and revising it.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

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