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REVIEW

Review on the application of molecular approaches for the genetic analysis of teak (*Tectona grandis* L.f.) populations

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Abstract: **Teak (*Tectona grandis* L.f.), a prominent tropical hardwood tree species, yields one of the finest timbers in the world. Because of its capability to provide high quality timbers, the demand of teak wood has been on the rise for centuries. However, the natural populations of teak have been declined drastically because mainly of over-exploitation and deforestation. Geographically, Bangladesh is a very potential region for the growth of teak plants. Although variations in ecologically and economically important morphological traits of teak have been investigated worldwide for many decades, molecular data regarding genetic variation between and among populations have received relatively less attention. Among all the available molecular markers, some notable markers are- Random Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP), Inter Simple Sequence Repeats (ISSR) and Simple Sequence Repeats (SSRs), although each of them has their own merits and demerits. Different parameters and components of genetic diversity study have been estimated by using different techniques and analytical tools. These components of genetic diversity help us to give a precise picture on the proper utilization of plant genetic resources and also help in initiating the effective timber plant management techniques.**

Keywords: Genetic diversity, Molecular markers, Statistical analysis, Assessment of variations, Teak timbers.

Teak (*Tectona grandis* L.f.), a member of the plant family Lamiaceae, yields one of the finest timbers in the world. Teak timbers have been widely used predominantly since around the 7th century and due to its versatile uses, it has become a popular timber across the globe (1,2). Although the natural occurrence of the species is limited to only in the South and South-East Asian countries like India, Laos, Myanmsar and Thailand, currently the species has been grown in more than 60 countries in the world (3). This species is providing not only the provisioning services it is also supporting biodiversity in the terrestrial ecosystems. Therefore, proper conservation and management of this species is of utmost importance from both economic and ecological aspects.

Morphological traits of teak have been investigated worldwide in the context of timber production. However, morphological parameter may not always be accurate to bring out the overall picture of variations between or among the plant populations (4). Moreover, morphological studies can be susceptible to phenotypic plasticity. Therefore, molecular approach is getting popularity to explore the genetic variation at population and individual levels of the organisms. In the recent years, the use of molecular techniques has been significantly increased (4), because of its relevance in proper management and conservation. Variations within or between the populations of teak can be directly estimated from the study of genetic diversity. The analysis of genetic diversity is mainly based on the applications of various types of DNA-based markers. This chapter discusses the use of molecular approaches for better understanding about the population genetics of teak for proper management and conservation.

Biology of Teak

Teak is deciduous in nature that shows most of the leaf fall during winter. From the early Spring, new leaves of this plant start to grow. Leaves of this plant are very large in size (generally 20-70 cm long), opposite, pointed at the end, broadly elliptic in shape, green in color, mostly hairy, yellowish beneath and rough above (5). The entire canopy

of teak trees is covered by white-colored flowers during the rainy season. Flowers are small in size, fragrant and usually, borne on a large terminal panicle which is of 1-3 feet tall. According to some reports, teak plant can grow up to 500 years (6). The shape of fruit of teak is quadrangular (about 3 cm in diameter in size); bark of the tree is very thin and the outer layer is sometimes hairy. Dark brown colored fruits of teak can be found hanging on the tree during the winter. For spreading of the seeds, teak plant is highly dependent on the air. Propagations of teak are caused by seed germination and stump cuttings (5).

Teak grows in a variety of habitats and climates, from desert regions with barely 500 millimeters of annual rainfall to extremely wet locations with up to 5000 millimeters of yearly rainfall (7). Teak flowers have both male and female reproductive organs. Flowers are loosely protandrous and they are arranged such that the anthers of teak flowers mature before the stigma, and the pollen is discharged within a few hours after the flower opens.

Importance of Teak

Teak is mostly used for day-to-day commodities and furniture. A number of features such as moderate weight, suitable strength, easy workability, finishing qualities and appealing colour and texture of teak wood have made this plant very demandable. Durability and resistance against fungal attacks are two of the main qualities that have made teak a popular timber plant (8). Teak woods are used in making poles, beams, roofs, columns, doors, windows, frames, floorings, planking, paneling, staircases, luxurious furniture and many more. Moreover, cabinet making, wagon, railway carriages and marine construction can also be made from the best quality of teak woods (8).

Teak wood also has some resistance properties to some harmful chemicals (9). It also possesses some medicinal properties as well. Different plant parts of this species are being largely used in ethnomedicinal treatment for various diseases. Powdered wood can be used to produce wood tar which is used as vermifuge and it can also be used in relieving headaches (10). Teak leaves possess about 6-7% of tannin and other secondary metabolites (10). Moreover, leaves of this plant are used for the production of various types of oily substances.

Beside its economic benefits, teak is also a very potential tree species from the point of ecological aspects. This plant can mitigate the microclimate and water-shedding. Reduction of soil erosion is another profound importance of this plant (11). Teak is a fine sustainable timber yielding plant because of its high carbon sequestration, carbon storage and rooting system of the tree (11). Teak plantations or natural teak population can supply larger forest-like canopy and also enhance the biodiversity to a great extent. By following appropriate agroforestry practices, teak plantations can have a lower economic cost (12).

Teak plants are now under the threats of continuous and frequent logging all over the world. Due to over-exploitation and degradation of ecosystems, diversity of this tree is in great danger rendering potential risks of extinction. Understanding the population genetics of this species is important for taking appropriate measures for the conservation of this species.

Population Genetics of Teak

Teak is mostly a out-crossing species and the flowers of this plant are usually insect pollinated. However, wind pollination can also be occurred occasionally (13). This type of pollination suggests that teak has a larger genetic diversity. To investigate the population genetic structure and genetic variation on natural populations and distinct landraces of teak, numerous molecular markers were used. Reduced population size, habitat fragmentation, and natural population isolation result in decreased levels of genetic diversity, which is crucial for tree species to react to environmental changes such as climate, pests, insects and diseases. Seeds are the vital part for teak populations, because the seeds from the earlier teak plantations are the basis from which plantation of teak scales up and it leads to the formation of various landraces (14). Most of the breeding and domestication activities of teak plants around the world were based on mainly local landraces, as a result the actual genetic origin is not clearly known yet. The majority of teak genetic diversity research has focused on a small number of chosen populations or trees, rather than covering the whole teak distribution range.

Application of Molecular Approaches to Study Population Genetics of Teak

Studies on population structure and genetic diversity by using DNA-based molecular markers have been carried out in some parts of the world. Molecular markers are mainly used to investigate the differences within the sequence of nucleic acid between different individuals. A molecular marker is able to distinguish polymorphisms, even though these polymorphisms may not produce any phenotypic variations (5). Molecular markers can provide a true representation of the genetic makeup.

Polymerase Chain Reaction (PCR)-based and restriction-hybridization-based techniques are the two types of basic molecular marker techniques. The variation in DNA samples or polymorphism can be identified using PCR and restriction-hybridization techniques followed by gel electrophoresis. The principal PCR-based molecular markers include allozymes, random amplified

polymorphic DNA (RAPD), amplified fragment length polymorphisms (AFLPs), inter simple sequence repeats (ISSRs), and simple sequence repeats (SSRs). Further, molecular markers can also be categorized as co-dominant (e.g. allozymes, RFLP, SSR and SNPs) and dominant marker (e.g. RAPD and AFLP) while the former can identify both dominant and recessive alleles and the later one can identify dominant alleles only.

Genetic Diversity Analysis Using Molecular Markers

Various molecular markers have been utilized to assess genetic diversity of teak although in limited extents. Among the various markers used in such studies, some notable ones include Allozymes (15), RAPD (16,17), ISSR (18-20), AFLP (21,22) and SSR (23-26). Allozyme was employed to investigate genetic diversity in teak populations from Laos, Thailand, Indonesia and Western India (15). This study showed a comparative data on the genetic variation present in the teak populations distributed in the sites located in geographically distant places. Microsatellite markers (SSRs) are often utilized in the study of teak genetic variation. Alcântara et al. (2013) evaluated 60 teak genotypes from various Brazilian provenances using nine microsatellite primers (26). They also studied genetic diversity within 13 teak clones collected from Malaysia, Honduras, Malaysia, Indonesia, India, Solomon Islands and Ivory Coast by using microsatellite primers. Microsatellite markers or SSR markers are popular and reliable in studying genetic diversity. By using ISSR marker, Prasetyo et al. (2020) showed the subdivision of teak populations which indicated the relevance of using molecular marker in the conservation of forest plants (20).

Teak Genetic Diversity Study in the Indian Subcontinent

Several conservation efforts have been implemented in the last two decades to reduce the loss of genetic resources of teak plants in countries where wild teak populations may be found. In India, the Maharashtra Forest Department established a National Teak Germplasm Bank (NTGB) at Chandrapur for the protection of teak genetic resources, which is currently the country's biggest field gene bank (27).

Although, India is the major center of origin of teak, a few numbers of the study has been conducted over the years to investigate genetic diversity of this species. Using an AFLP molecular marker, Sreekanth (2014) evaluated genetic and morphological diversity in nine wild teak populations containing 180 genotypes of teak from the Western Ghats of South India (28). According to genetic distances derived from AFLP data, only the petiole and tree height were strongly connected. A correlation test indicated a substantial positive association between geographic and genetic distance matrices supporting the Isolation by Distance hypothesis.

Using five ISSR primers, Narayanan et al. (2012) calculated the molecular diversity and genetic structure of 29 Indian teak populations from Rajasthan, Kerala, Tamil Nadu, Andhra Pradesh, Madhya Pradesh, Karnataka, Maharashtra and Orissa (19). In this study, AMOVA test suggested high genetic diversity (almost 91%) within populations. UPGMA (Unweighted Pair Group of cluster Analysis) dendrogram revealed several clusters and it distinguished the moist teak populations from comparatively drier teak populations from Southern part of India. The study further suggested enforcing in-situ conservation method within natural populations for germplasm conservation of teak plants of South India.

Vaishnaw et al. (2014) analyzed the genetic diversity and structure of 96 teak genotypes from 10 natural populations in India using five AFLP primer combinations (21). AMOVA research also revealed that within-location genetic diversity varies more than between-location genetic diversity. Analysis of Principal Coordinate Analysis (PCoA) and Neighbor Joining Tree indicated two separate centers of teak diversity (Peninsular India and Central India).

Statistical Tools Used in the Measurement of Genetic Diversity of Teak

Franco et al. (2001) described the requirements that are necessary in measuring genetic variations within or between the populations in research such as using the multiple data sets comprising the morphological, physiological or DNA-based molecular collections (29). Furthermore, another factor that is necessary to study genetic diversity is the choice of statistical tools in estimating genetic distance and the level of clustering of the genetically related individuals. These tools help in finding out the spectrum of expected genetic variations.

A large number of genetic distance measurement techniques have been formulated for analyzing molecular DNA data for the study of genetic diversity of many plants. Narayanan et al. used Popgene, a software program (Version 1.31)(19,30) to assess Nei's genetic distance (31), gene diversity and Gst (32), Shannon's information index (33) and gene flow (Nm) among populations in the study of genetic structure and molecular diversity of teak by using 5 ISSR markers in India. They also used a computer software NTSYS- pc (Version 2.20e) to construct UPGMA dendrogram (34) and Principle Coordinate Analysis (PCoA). Huang et al. (2016) used GenAlEx v6 in estimating genetic distance and also for the analysis of molecular variance (AMOVA)

(35). They also used the software NTSYS to construct an UPGMA dendrogram and Principle Coordinate Analysis (PCoA). Bayesian cluster analysis were used to describe the genetic structure of sampled provenances using the program Structure (v. 2.3.1).

Table 1. Merits and demerits of some most commonly used molecular markers for genetic diversity study.

Sl. No.	Marker	Type of marker	Merits	Demerits	Ref.
1	Allo-zymes	Co-dominant	➢ Comparatively easy and fast to analyze. ➢ Not so expensive.	➢ Only a few markers available. ➢ Lower polymorphism. ➢ Can only detect coding genes.	(15).
2	RFLP	Co-dominant	➢ Highly reproducible. ➢ Markers are well distributed throughout the genome. ➢ No prior sequence information needed.	➢ High quality and large amount of DNA is required. ➢ Polymorphism level is low. ➢ Laborious and expensive.	(36)
3	SSR	Co-dominant	➢ Small amount of DNA is enough. ➢ Highly polymorphic and reproducible. ➢ Easy to interpret in genotyping.	➢ Development cost is high, ➢ Primer development is time consuming.	(37)
4	ISSR	Dominant	➢ Highly polymorphic, ➢ No prior sequence information is needed.	➢ Non-reproducible. ➢ Detection system is complicated.	(19)
5	RAPD	Dominant	➢ Only a small amount of DNA in enough. ➢ Quick and simple process. ➢ No prior DNA sequence information is required.	➢ Non-reproducible. ➢ Interpretation in terms of allele and loci levels is not possible.	(17)
6	AFLP	Dominant	➢ Highly polymorphic and reproducible. ➢ No prior sequence information is required. ➢ Large amount of amplicons are generated.	➢ Laborious and Complex process. ➢ High quality of DNA is required.	(22)

Alcantara and Veasey (2013) applied nine microsatellites to assess 60 genotypes of teak sampled from India, Brazil, Malaysia, Honduras, Indonesia and Solomon Islands and Ivory Coast (26). They employed the Bayesian technique using computer software Structure (v. 2.1) to differentiate between the genotypes they sampled (38). They ran AMOVA to determine the diversity inside and between the groups generated in the Structure analysis using the program Arlequin (39). The GDA software program was used to assess genetic diversity characteristics such as number of alleles per locus (A), observed heterozygosity (Ho), anticipated heterozygosity or gene diversity (He), and fixation index (F) (40). FSTAT software was used to determine allele frequencies and molecular diversity parameters from Nei (1973) such as total genetic diversity (HT'), genetic diversity between groups (DST'), the proportion of genetic diversity between genotype groups (GST'), and genetic diversity within groups (HS) (32).

Molecular Approaches Used in the Study Genetic Diversity of Teaks

Assessment of teak genetic diversity utilizing different molecular DNA markers from different studies suggested an overall variation among phenotypic and genotypic data. Sreekanth et al. (2014) used the AFLP marker to explore the pattern of morphological and genetic diversity among teak populations in Southern India (28). They found a low correlation between phenotypic and genotypic data. The multivariate analysis of morphological data suggested that the neighboring teak populations did not show any tendency to group together. The PCA analysis also

supported the depiction. Though they have found low correlation between most of the morphological and genetic data, positive correlations between genetic distance and a few morphological properties such as petiole shape and height of the tree were found from AFLP data analysis. Positive correlations between overall morphological and genetic data were not found using the Mantel test. In addition, the Mantel test demonstrated a significant correlation between geographic distance and genetic distance, which was verified by PCoA analysis and the UPGMA dendrogram.

Table 2. Some studies on genetic diversity of teak plants across the globe.

Sl. No.	Markers	Parameters	Country	Ref.
1	Allozymes	➢ Nine quantitative characters and 10 allozyme loci were used to detect genetic differentiation between teak populations Laos, Thailand, Western India and Indonesia. ➢ Multivariate analysis revealed that the populations of Laos showed less variation than other regions. ➢ Fixation Index was only 4% and no clear geographical pattern was found. ➢ Another study was conducted between two Tanzanian and two Nicaraguan landraces using Allozyme data. ➢ Comparatively enhanced genetic variation was found in the Tanzanian landraces than the Nicaraguan landraces. ➢ Population bottleneck might be the reason for the reduced variation in the Nicaraguan landraces.	Laos, Thailand, western India, Indonesia, Nicaragua and Tanzania.	(15)
2	RAPD	➢ Ten RAPD primers were used where 73% polymorphism was observed. ➢ Total genetic diversity within the species (HSP) was found 0.3. ➢ About 73% of variation was found within the populations.	India	(17)
3	AFLP	➢ Two chloroplast SSRs and sixty nine AFLP markers were applied to estimate 4 Tectona grandis and 3 T. hamilitoniana populations. ➢ AFLP analysis revealed higher genetic diversity in teak populations. ➢ AMOVA revealed significant genetic differentiation between the two species (38.4%, $p<0.05$). ➢ Significant pairwise genetic differentiation (FST) was detected between most populations.	Myanmar	(23)
4	SSR	➢ Sixty teak genotypes from various Brazilian provenances using nine microsatellite primers. They also studied genetic diversity within 13 teak clones collected from Malaysia, Honduras, Malaysia, Indonesia, India, Solomon Islands and Ivory Coast. ➢ There was moderate genetic diversity, with an average of 4.1 alleles per location and a heterozygosity of 0.329. ➢ Using Bayesian Structure analysis, two sets of genotypes were discovered. ➢ The AMOVA for the two groups revealed that there was a	Brazil	(26)

		lot of variability among the groups (73%, P<0.001).		
5	ISSR	➢ Three main regions (East, West and Central) of Java Island were selected to detect the population structure and genetic diversity of teak using multiplexed ISSR. ➢ AMOVA results concerning native provenance populations showed the most of the variation (44%) resulted from among populations. ➢ According to genotyping sequencing, the FST value, or population differentiation index among local regional populations was very high (0.446). ➢ The PCoA analysis revealed that the studied populations can be classified into two distinct categories.	Indonesia	(20)

Chaudhari et al. (2018) conducted genetic diversity analysis of 10 teak populations of South Gujarat with the help of 20 RAPD primers (16). The first three coordinates were found to account for 20.53, 14.90, and 13.31 percent of total variation, respectively. According to PCoA analysis, the total cumulative variance accounted for by the three locations was 48.73 percent which also supported the results from their UPGMA dendrogram analysis. Overall, they discovered a lot of variance across the 10 teak populations in South Gujarat.

Prasetyo et al. (2020) used multiplexed ISSR genotyping by sequencing (MIG-seq) to determine the origin of commercial teak plantations and assess the genetic differentiations of Indonesian teak from three main regions (East, West, and Central) of Java Island, and contrasted the estimated population structure and genetic diversity with that of natural teak forests in Myanmar, India, Laos, and Thailand (20). According to genetic analysis, the AMOVA results for native origin populations revealed that the majority of variation (44%) came from across populations, followed by within individual plants (31%) and within populations (25%) variants. Furthermore, genotyping sequencing indicated that the FST value (population differentiation index) across native regional populations was quite high (0.446), whereas pairwise FST values ranged from 0.004 to 0.736 and for all populations, they were statistically significant (P<0.05). The findings of the PCoA analysis for all of the populations revealed that the analyzed teak populations may be separated into two different groups, which was supported by the Neighbor joining tree or dendrogram analysis.

Win et al. (2015) investigated on the genetic diversity of 10 teak populations (including 4 natural and 6 plantation populations) in Myanmar by 15 selected microsatellite markers (41). According to their findings, Myanmar teak has much more genetic diversity than the teak populations from Thailand and Laos. In compared to other teak native regions like as Thailand, Laos, and India, Myanmar's natural forests area is bigger, resulting in increased genetic variety and a moderate amount of genetic differentiation. Furthermore, Principal component analysis of this study showed not much variation of genetic components between natural teak populations and plantations of teak in Myanmar.

Lyngdoh et al. (2013) compared the genetic parameters between 8 unimproved and 7 improved teak populations in Karnataka state of India by employing the DNA based ISSR markers (42). AMOVA data suggested almost 39% and 32% of genetic variance among the unimproved and improved teak populations respectively and almost 61% and 68% of variance within the populations of unimproved and improved teak, respectively.

According to these studies, it was found that the maximum teak populations still maintain a high level of genetic variation and possess higher level of polymorphism. AMOVA analysis from these studies suggested that the most of the genetic variations were found within the populations rather than among the populations. These studies mostly showed the importance of conservation actions to protect the genetic resources of teak population in the sites where genetic diversity is dwindling.

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CONCLUSION

Molecular markers are considered as the tools that are employed to evaluate genetic diversity and construct genetic maps. To analyse the genetic diversity of teak populations, the use of molecular markers (SSRs, RAPDs, ISSRs, AFLPs, and others) has been widely done.

Although molecular methodologies have been used to explore teak genetic diversity in other regions of the world, similar research has yet to be done in Bangladesh, where teak populations are abundant. Information regarding the study of genetic diversity of natural population is relevant for better management and conservation of forest plant populations. Since, continuous and frequent logging of timber of teak trees is a very common phenomenon all over the world, the natural distributions of teak populations are jeopardised. As a result, studying the genetic diversity of teak using molecular analysis methods is a highly practical way to go before undertaking any of the proper management practices. Selective logging of natural teak forest land has been considered as the ideal solution and one of the practical forest management solutions to the deforestation of natural teak populations. Proper logging can be beneficial in paving the way of growth of new plants to protect natural ecosystems from degradation (23). Furthermore, the evaluation of the phylogenetic relationships among the teak populations and investigation on the history of origin of teak plants are also possible from the molecular analysis study. However, use of appropriate number of replicates and several markers simultaneously can generate reliable data.

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CONFLICT OF INTEREST

This article has no conflict of interest.

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