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Agronomy

Nilambur- Genotypically A Unique Teak Population in India

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Abstract

Gene diversity and population structure were analyzed in 14 natural teak (*Tectona grandis* L.f.) populations in India belonging to Kerala, Madhya Pradesh, Gujarat and Orissa using microsatellite markers. The data collected through microsatellite marker studies from 550 trees of the natural teak populations in different geographic areas indicate that it has different genetic structure forming separate genetic clusters. Populations from Madhya Pradesh and Gujarat form the first cluster while Orissa is in the second cluster and Kerala populations except Nilambur fall in the third cluster. Phenotypically Nilambur teak stands are quite distinct among Kerala populations were reported earlier but this is the first report proving the uniqueness of Nilambur teak through DNA analysis. The results of this study using molecular markers also support and confirm that gene diversity within teak populations of teak are in higher percentage than the gene diversity among populations.

Highlights

- The program Structure Version 2.2 implements a model-based clustering method for inferring population structure using genotype data of microsatellite markers
- Genotypic variation showed that the microsatellite markers could differentiate Nilambur teak, a unique teak population in India.
- Population structure and patterns of diversity in teak have been studied using molecular markers showed that there is significant gene diversity within the teak populations.

Keywords: Genetic diversity, genetic conservation, teak diversity, DNA markers, microsatellite markers

Teak is an important timber species naturally occurring in forests of India, Myanmar, Northern Thailand and Laos. India is considered to be the only known center for genetic diversity and variability of teak, having its natural distribution zone confined pre dominantly to peninsular region below 24°N latitude (Satish Kumar *et al.* 2014). Although detailed studies on the distribution of genetic variability in teak are limited, considerable variation in a quantitatively inherited trait in *T. grandis* has been reported in provenances from natural populations in India, Thailand, and Laos. Molecular characterization is one of the tools to identify the hidden genetic diversity (Datta *et al.* 2014). Population structure and patterns of diversity of teak have been studied using molecular markers like isozymes, RAPD, AFLP and Microsatellites (SSR). In recent years, of these different types of molecular markers microsatellites have been utilized most extensively, because they can be readily amplified by PCR and the large amount of allelic variation at each locus (Gous *et al.* 2013). Genetic diversity of teak in Thailand was revealed by RAPD (Changtragoon and Szmidt 2000). A couple of studies have been carried out for estimating the genetic diversity of Indian teak in comparison with other countries teak populations through DNA marker studies (Shrestha *et al.* 2005; Fofana *et al.* 2008). Genetic diversity of nine natural populations of the Western Ghats in Southern India by AFLP markers were also carried out (Sreekanth *et al.* 2012).

The teak genetic resources have been altered due to uncontrolled logging and mixing of germplasm (Shrestha *et al.* 2005). Anthropogenic destruction of tropical forests has dramatically increased in recent decades, and the

threat to tropical ecosystems is well articulated (Lowe *et al.* 2005). In the light of anthropogenic disturbances and uncontrolled mixing of germplasm with out analyzing the long term effects, there is a crucial need for conservation of genetic resources. The genetic diversity of teak from India, Thailand and Indonesia has been estimated by isozyme variation of provenances showing that the Indian teak provenances were clearly differentiated from the Thailand and Indonesian provenances (Kertadikara and Prat 1995). Among the Indian populations, the genetic diversity analysis using RAPD markers showed that the Western Ghats and Central Indian regions fall into two distinct clusters which could be designated as separate breeding zones (Nicodemus *et al.* 2005). AFLP marker studies followed by cluster analysis and principal coordinate analysis indicated that Indian teak populations (Konni from Kerala, Allapalli from Maharashtra, Mount Stuart from Tamil Nadu) clearly separated from those in Thailand and Indonesia (Shrestha *et al.* 2005). Microsatellite marker studies showed four main centers of genetic variability in teak forming two clusters in India, one cluster from South Indian provenances comprising Nilambur (Kerala), Masale valley, Virnoli and Bairluty (Karnataka), the second cluster from the North Indian provenance, Purunakote (Orissa), the third cluster mainly consisting of populations from Thailand and South Laos and the fourth cluster from Central Laos (Fofana *et al.* 2008).

However, in many trial regions, the superiority of the Nilambur provenance in terms of productivity and tree form was reported. The Nilambur provenance is generally well accepted among other seed sources from Kerala due to the fast growth, tree form including less tapering and high extractive contents (Bhat and Indira 1997). The genetic diversity of North and South Indian teak samples from natural populations and plantations were analyzed by using RAPD markers and found that the UPGMA dendrogram grouped the Western Ghats and Central Indian populations into two distinct clusters (Nicodemus *et al.* 2005). Parthiban *et al.* (2003) did a genetic diversity study of 28 Indian teak seed samples by RAPD markers and observe state-wise grouping into separate sub clusters. The teak provenances of the Western Ghats origin is reported genetically superior (Fofana *et al.* 2008). Through the present study using Microsatellite markers, we have investigated how south western populations genetically vary within and among populations and how they differ from other north Indian, north western and north eastern populations. The DNA required for these studies were extracted from 550 trees of the natural teak populations from different states of India. The information available from the studies is expected to contribute for the sustainable management of teak genetic resources.

Materials and Methods

Natural teak forests were identified from states of Kerala, Madhya Pradesh, Orissa and Gujarat representing the Southwest, North, Northeast and Northwest of India, respectively. In Kerala, populations were selected in four natural teak growing areas viz. Konni, Thrissur, Nilambur and Wayanad Forest divisions. Natural teak growing areas of Khurda division from Orissa, Jabalpur division from Madhya Pradesh and Valsad division from Gujarat were also selected for the study. Hence, from

Table 1.

Sl. No.	Population/ Location State	Forest Range	Latitude and Longitude
1	Kaduvappara-Konni **(Kerala)**	Naduvathamuzhy	9°09'N and 77° 00' E
2	Kattathi-Konni **(Kerala)**	Naduvathamuzhy	9° 10' N and 76° 57' E
3	Vazhani - Peechi **(Kerala)**	Machad	10°63'N and 76° 32'E.
4	Thamaravellachal - Peechi **(Kerala)**	Peechi	10°50'N and 76° 37 E
5	Poochappara- Nilambur (Kerala)	Karulai	11° 38' N and 76° 26' E
6	Padukka- Nilambur **(Kerala)**	Karulai	11° 20' N and 76° 21' E
7	Tholpetti- Wynad **(Kerala)**	Tholpetty	11° 57' N and 76° 05' E
8	Bavali-Wynad **(Kerala)**	Tholpetty	11° 51' N and 76° 05' E
9	Sara-Valsad **(Gujarat)**	Valsad North	20° 47' N and 73° 24' E
10	Mahwas-Valsad **(Gujarat)**	Valsad North	20° 46' N and 73° 25' E
11	Ghotgharakpur- Jabalpur **(Madhya Pradesh)**	Burgi	22° 51' N and 79° 53' E
12	Disharad-Jabalpur **(Madhya Pradesh)**	Burgi	22° 55' N and 79° 55' E
13	Balunda-Khurda **(Orissa)**	Balugaon	19° 52' N and 85° 05' E
14	Ranjin- Khurda **(Orissa)**	Balugaon	19° 53' N and 85° 07' E

these seven geographic areas, pair populations (two populations each, one protected/undisturbed and the other disturbed) were sampled leading to a total of 14 populations (Table 1). The criteria for the classification of protected/undisturbed and unprotected/disturbed had been classified as per the data collected about natural regeneration, records about the occurrence of forest fires, number of stumps, and distance to human inhabitation from sample collection site. From each population, 40-50 adult trees were randomly selected and juvenile leaves were collected for DNA extraction.

Genomic DNA was extracted using modified CTAB method (Doyle and Doyle 1987). The quality and quantity of DNA was checked on agarose gel electrophoresis. Ultra Lum Total lab software (Rita and Animesh 2006) was used to quantify the DNA to obtain 30ng DNA uniformly in each sample. Hypervariable micro satellite markers were used for the studies (Gous *et al.* 2013), they are co-dominant markers and considered as neutral. Hence, they are suitable for genetic diversity estimation, paternity analysis and gene pollen flow studies. Microsatellite markers designed for teak by Dr. Hugo Volkaert, Kasetsart University, Thailand, out of which, four micro satellite markers namely AC01, AC28, AG04 and AG14 (Genbank/EMBL/DDBJ accessions AJ511746, AJ511764, AJ539416 and AJ539417 respectively) were used for genetic diversity studies (Sabna *et al.* 2011).

DNA extracted from all the selected trees from fourteen populations in the seven Forest Divisions in four states of India as mentioned earlier were amplified using the four microsatellite markers. The method of DNA amplification, polyacrylamide gel elctrophoresis and staining were done as detailed in Sabna *et al.* (2011). Clear bands seen on the gel were scored for allelic polymorphism based on the position of bands on the gel and the heterozygosity/ homozygosity was noted. The number of alleles, allelic richness, gene diversity, inbreeding and genetic differentiation were estimated using FSTAT version 2.9.3.2 (Goudet, 2002). The gene diversity was estimated using the observed heterozygosity (Ho), expected heterozygosity (He) and allelic richness (Goudet 2002). The geographical patterns were drawn by the program STRUCTURE (Pritchard *et al.* 2000) which are among the most common data formats in population genetics software (Excoffier and Heckel, 2006). Structure Version 2.2, using the data on allele frequency in different populations. It implements a model-based clustering method for inferring population structure using genotype data consisting of unlinked markers. The method was introduced by Pritchard *et al.* (2000) and extended in sequels by Falush, *et al.* (2003, 2007).

Results and Discussion

According to Nicodemus *et al.* (2005), the Western Ghats and Central Indian regions may be designated as separate breeding zones since these populations are genetically distant and grouped into two distinct clusters using RAPD markers. Fofana *et al.* (2008) show two distinct clusters for Indian teak by microsatellite markers, one comprising populations from Western Ghats and the second group from North eastern Orissa.

From the present study, it is observed that all the microsatellite markers exhibited very high polymorphism with a total of 30 alleles and AC01, AC28, AG04 and AG14 have shown 8, 7, 7, and 8 alleles respectively. Hence, they are suitable for assessment of genetic diversity and other related parameters. Among these populations, the observed heterozygosity (Ho) 0.584 was found to be more at Nilambur (Table 2) than the expected heterozygosity (He) 0.547. The lowest expected heterozygosity 0.521 was observed in the undisturbed population at Wayanad (Table 2). With regard to the undisturbed populations, the maximum observed heterozygosity was seen in the Nilambur population. The minimum value in the undisturbed population was observed in Jabalpur (0.326).

Table 2. Expected heterozygosity (He) Observed heterozygosity (Ho)

Genetic parameter	(He)		(Ho)	
Populations	1a	2b	1a	2b
Thrissur	0.604	0.591	0.572	0.552
Konni	0.637	0.645	0.571	0.559
Nilambur	0.521	0.537	0.521	0.485
Wayanad	0.547	0.593	0.584	0.484
Khurda	0.718	0.625	0.522	0.519
Jabalpur	0.643	0.477	0.326	0.254
Valsad	0.654	0.570	0.348	0.337

Legend: a: *Undisturbed population b: Disturbed population*

Genetic markers are normally considered not to be influenced by natural selection (Suangtho *et al.* 1999). Important genetic differentiation following divergent natural selection in a few generations may therefore not be detected by the markers (Karhu *et al.* 1996). This is supported by the fact that several studies of forest trees, including teak, have shown larger differentiation between adaptive traits than between biochemical markers (Kjaer, *et al.* 1996). Kertadikara and Prat (1995)

concluded that rapid differentiation is not probable, particularly in the case of isozyme markers. Earlier studies on phenotypic variations in Indian teak revealed that 70% trees from Nilambur have sessile leaves while less than 16% trees have sessile leaves from all other provenances of Kerala (Arienkavu, Konni, Thrissur, Parambikulam and Wynad) (Indira, Bhat and Thulasidas 2010). It is also recorded that Nilambur teak (Malabar teak) has good growth (with wider rings), greater log dimensions and pleasing colour and hence, has a wider reputation in the world especially for ship building (Bhat and Priya 2004).

Allelic richness is estimated by measuring the number of alleles in different population with sample size of 40-50 trees. From this study, it is clear that allelic richness was highest (5.91 and 5.74) in Thrissur (Table 2) and the least in the disturbed population (4.10) at Wayanad. The results show that the estimated gene diversity is higher in populations at Khurda in Orissa (0721 and 0.629) followed by Konni Division in Kerala (Table 3). In the present study the disturbed population at Jabalpur exhibited lowest gene diversity (0.48). The total gene diversity obtained from the study is 0.765 and from this, the genetic diversity within population found to be 0.599 or 78.3%. From the current study it is also clear that, with respect to gene diversity, variation of disturbed over undisturbed populations was more in populations in other states compared to Kerala. Within Kerala populations, Nilambur had shown higher variation. Genetic differentiation (Fst) (Table 3) between paired population were significantly different except Trichur was found low (0.0028) and non-significant.

Table 3. Allelic richness and gene diversity

Genetic parameter	Allelic richness		Gene diversity		Fst
Populations	1a	2b	1a	2b	
Thrissur	5.91	5.74	0.608	0.596	0.0028 ns
Konni	4.31	5.06	0.639	0.647	0.0537*
Nilambur	4.52	4.53	0.594	0.532	0.0672*
Wayanad	5.08	4.10	0.526	0.538	0.0955*
Khurda	5.46	5.48	0.721	0.629	0.0832*
Jabalpur	4.62	4.95	0.645	0.480	0.1895*
Valsad	4.97	5.09	0.657	0.575	0.1938*

a: *Undisturbed population* **b:** *Disturbed population* significant at 5 % level*

The present study on genotypic variation showed that the microsatellite markers could differentiate Nilambur teak from the rest of the Kerala populations which are geographically on the southern and northern side of Nilambur. Microsatellite markers help to analyses the recent changes occurring in the genome over short time span and they are more sensitive indicators of fine scale genetic structure (Butcher *et al.* 1999). The separation of the Nilambur populations could be due to the recent changes occurred following natural selection and adaptation (Fofana *et al.* 2008). However another study in the same lab using nuclear gene marker (a nuclear gene coding for one of the cytoplasmic isoforms and catalyses in teak) studies, which show long term changes, could not differentiate Nilambur populations from the rest of Kerala provenances (Indira *et al.* 2010).

In the present study, the two populations from Balugaon Range, Orissa distinctly separate from the teak populations in the nearby state like Madhya Pradesh. Shrestha *et al.* (2005) also observe the Berbera population from Orissa near the north-eastern coast of India as a distinct cluster separating from the other cluster comprising Allapalli (Maharashtra), Konni (Kerala) and Mount Stuart (Tamil Nadu). They also note it as an exception, as Berbera from Balugaon Range seems to have associations with both the Indian and the Thai–Indonesian populations. Samples for the present study also were collected from Balugaon Range.

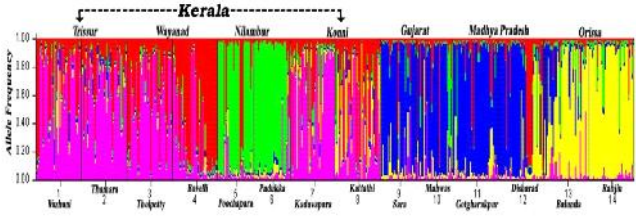

Fig. 1. Bar diagram showing geographical patterns in allele frequency

The program Structure Version 2.2 implements a model-based clustering method for inferring population structure using genotype data consisting of unlinked markers (Pritchard *et al.* 2000). The structure analysis show geographical patterns and population subdivision patterns based on the allele frequencies. In the present study the analysis showed four clusters comprising the fourteen populations (Figure 1). One cluster comprised the north and north western populations, i.e. Madhya Pradesh and Gujarat. The north eastern population from Orissa formed a second cluster. The populations from south western India were split into two clusters. The first cluster was Nilambur Division (with two populations) separated from the rest of Kerala. Other populations from Kerala namely Thrissur, Wayanad and Konni formed the second cluster and shows similar genetic pattern. But in each of the locations, there are no apparent differences between the paired populations.

Population structure and patterns of diversity in teak have been studied using molecular markers like isozyme, RAPD and AFLP in recent years (Changtragoon, and Szmidt 2000, Shrestha, *et al*. 2005, Balasundaran *et al*. 2010). Significant assessment of genetic diversity was obtained both at the morphological and molecular level in *Aloe barbadensis* (Pushpa and Sanghamitra 2015) and they clearly mentioned that, the molecular markers technique is more precise than the morphological traits, this results supports the present result in cluster analysis. All the earlier studies on genetic diversity in teak using DNA markers, though with few populations and samples, showed that there is significant gene diversity within the teak populations. The study with ten populations of teak from Western Ghats and Central Indian regions showed that 78 percent of variation exist within the population and rest between populations (Nicodemus *et al.* 2005). Changtragoon and Szmidt (2000) also reported 79 percent gene diversity within populations after analyzing 16 teak populations in Thailand using RAPD markers. The present study also showed that 78% of total gene diversity is within populations and matched with earlier studies.

Conclusion

Genetically broad as well as gene-ecologically distinct populations should be maintained as a basis for present and future conservation practices. Populations with low genetic variability have a reduced potential to adapt to environmental changes. Thus, genetic variation is important for the long-term survival of a species (Babik *et al.* 2009). The remaining natural teak populations are to be protected efficiently since, purity of gene-ecologically distinct populations is not being taken care at present. State level mixing of teak seeds from seed production areas of different geographical areas is practiced for developing new plantations which will reduce the extent of such distinct populations. Nilambur is one among them to be conserved and it is genotypically different from other provenances of Kerala. Many more teak populations in the Western Ghats region are to be analysed for establishing an effective network of conservation populations.

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