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ISSR markers for analysis of molecular diversity and genetic structure of Indian teak (*Tectona grandis* L.f.) populations

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Abstract. Inter simple sequence repeats (ISSR) constitute a powerful dominant DNA molecular marker system used for diversity analysis, which is indispensable for making estimates of genetic base and demarcation of populations for undertaking conservation and improvement program of forest tree species. Twenty nine populations of teak (*Tectona grandis* L.f.) were collected from central and peninsular India for analysis of genetic diversity and structure. Genomic DNA from ten randomly selected individuals of each population was extracted and amplified using five ISSR primers (UBC-801, 834, 880, 899 and 900). The primers showed 100% polymorphism. UBC-900 recorded the highest Nei's genetic diversity (0.32 to 0.40) and UBC-899 had the highest Shannon's Information Index (0.49 to 0.59). AMOVA revealed a very high intra-population genetic diversity (91%), in comparison to inter-population genetic diversity among states (6.17%) and within states (2.77%) which were also indirectly confirmed by large standard deviations associated with genetic diversity estimates for individual population, as well as poor bootstrapping values for most of the cluster nodes. However, UPGMA dendrogram revealed several clusters, with populations from central India being present almost in each cluster, making groups with populations of adjoining states and distant states. Nevertheless, the cluster analysis distinguished the drier teak populations of central India from the moist teak populations of south India, which was also confirmed by Principle Coordinate Analysis. The findings advocates the need not only for enhancing selection intensity for large number of plus trees, but also for laying out more number of *in situ* conservation plots within natural populations of each cluster for germplasm conservation of teak aimed at improving the teak productivity and quality in future.
Keywords *Tectona grandis*, teak, genetic diversity, population differentiation.

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Introduction

The availability of information on the genetic variation within populations and the differentiation between populations plays a significant role in the formulation of appropriate management strategies for conservation of genetic resources (Milligan et al. 1994). ISSR (Inter-Simple Sequence Repeats) are dominant markers and detect polymorphisms in microsatellite and inter-microsatellite loci and do not require prior information of DNA sequences (Zietkiewicz et al. 1994). These markers have been widely used to assess genetic diversity and population structure and require comparatively small amount of DNA (Wolfe et al. 1998, Esselman et al. 1999). Each ISSR primer is composed of not only 2-3 repeats complementary to microsatellite region of the genome, but also 1-3 additional arbitrary nucleotides at the 5' or 3' end. The latter serve as anchors against reverting strand slippage during amplification (Gupta et al. 1994). Further, the anchoring nucleotides facilitate attachment of primers to specific inter microsatellite regions, resulting in diverse banding patterns from identical repeat sequences with varying anchoring nucleotide(s) (Wolfe et al. 1998a, b). In contrast to other molecular markers, the target sequences for ISSR primers are abundant throughout the eukaryotic genome and evolve rapidly. Consequently, ISSR markers help in revealing a much higher number of polymorphic fragments than RAPD (Random Amplified Polymorphic DNA) markers. In addition, the ISSR reaction is more specific than RAPD reaction (Williams et al. 1990, Fang & Roose 1997, Wolfe et al. 1998a, b).

Teak (*Tectona grandis* L. f.), an important source of tropical timber, grows naturally in forests of India, Myanmar, northern Thailand, Laos and Indonesia. The natural teak forests in India are the largest in the world, occupying an area of 8.9 million ha in central and peninsular region of the country (Tewari 1992). It may be reiterated that teak of central India, comprising the states of Maharashtra and Madhya Pradesh, is pure/mixed dry forests, possesses beautiful decorative grains and is commercially exploited more than moist teak of peninsular and coastal region (Kumaravelu 1993). The superior quality, the durability and the decorative value of teak wood have led to the establishment of teak plantations world-wide, outside of its natural distribution, and in particular, in tropical Asia, Africa and Latin America (Meniaud 1930, Muniswami 1977, Keogh 1979, Dupuy 1990).

However, the uncontrolled logging and unrestricted movement of planting stock has eroded the genetic resources of natural teak in India, ushering in the launch of teak improvement programme. As a result, the phenotypically superior teak plus trees on the basis of their growth, straight bole and resistance to insect pests and diseases, have been selected in great numbers from discrete populations existing in different geo-climatic regions of the country (Mandal & Rambabu 2001). Thus, the teak germplasm of the country exists both as plus trees and natural populations. Our group has already analyzed the molecular diversity and identity of the Indian teak plus trees using RAPD and ISSR markers (Narayanan et al. 2007). The genetic diversity of very few populations/ provenances of teak from India, Thailand and Indonesia has been assessed by RAPD (Changtragoon & Szmidt 2000, Nicodemus et al. 2005), AFLP (Shrestha et al. 2005) and microsatellite markers (Verhaegen et al. 2005, Fofana et al. 2009). The above works were mostly carried out on *ex situ* raised provenance trials (Shrestha et al. 2005) or on second generation plantations from *ex situ* conserved germplasm (Verhaegen et al. 2005, Fofana et al. 2009). Further, the studies considered only a few unrelated populations rather than those of the entire natural area. As a result, the information on genetic diversity and structure of the original teak populations from India remained fragmentary and preliminary. Therefore, the ISSR assay was chosen

for estimating genetic diversity and structure analysis of teak populations belonging to the entire natural range of the species in India.

Materials and methods

Population sampling, plant material and DNA extraction

Twenty nine populations were collected from different teak growing areas in the states of Andhra Pradesh, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Orissa, Rajasthan and Tamil Nadu (Table 1, Figure 1). Open pollinated bulked drupe fruits of one kg weight containing ≈ 2,000 fruits were collected from fifty trees of each population and stored at room temperature (30 ± 2°C) until further use. The half of the bulked fruits of each population was separately sown in nursery beds to raise seedlings. There was about 20% seedling emergence (Ansari & Singh 2003). Fully expanded young leaves of ten randomly selected seedlings per population were used for the extraction of genomic DNA, following a slightly modified method of Doyle & Doyle (1990) as the percentage of PVP in the extraction buffer was raised to 3% for effective removal of excess polyphenols. An additional washing step using 90% cold ethanol was included before DNA pellet was dried and dissolved in Tris-EDTA buffer. Quantity and quality of extracted DNA was determined in 1% agarose gel buffered with TBE.

ISSR assay

The ISSR amplification assay developed by Zietkiewicz et al. (1994) was employed for genomic DNA amplification. Five ISSR primers (Table 2) previously used by our group for teak plus tree analysis (Narayanan et al. 2007) were employed in the present study. The reaction mixture for ISSR amplification assay had a total volume of 10 µl, which contained 20 ng genomic DNA, 1x Taq polymerase buffer, 0.1 mM of each dNTPs, 2.5 mM $MgCl_2$, 1 unit *Taq* polymerase, and 0.8 µM primer. The assay also incorporated a sample without genomic DNA, as a negative control to rule out the possibility for self amplification of the primers or the contamination of genomic DNA. The amplification was carried out on a PalmCycler (Corbett Research Inc., Australia), with an initial 3 min denaturation at 94°C, followed by 35 cycles of 30 s at 94°C, 30 s at 50°C and 1 min at 72°C, and a final extension step for 10 min at 72°C. The amplification products of each sample, along with a 1 kb DNA ladder, were size fractionated on 2% agarose gel (in 0.5 x TBE) for 3h at 200 mA, and stained with 0.5 µg ml^{-1} ethidium bromide. The fractionated amplified genomic DNA bands were visualized on UV transilluminator and photographed with a Kodak® digital camera (Kodak, USA).

Data analysis

Only consistently amplified DNA bands from three repetitions of each ISSR assay were scored as present (1) or absent (0) and compiled into a data matrix. Both monomorphic and polymorphic bands were used for genetic characterization of populations. POPGENE (Version 1.31, Yeh et al. 1999) software program was employed to compute Nei's coefficient (Nei 1972), Shannon's information index (Shannon & Weaver 1949), gene diversity and Gst (Nei 1973) and gene flow (Nm) among populations (Slatkin & Barton 1989). Values of standard deviations and Duncan's multiple range tests for comparison of Nei's coefficient and Shannon's information index of each population were incorporated in order to get information about significant differences between and within populations. To ensure better explanation of our results, we also calculated the coefficients of Lynch and Milligan (1994) using AFLP-SURV (Version 1.0, Vekemans et al. 2002) software for making the distinction between homozygosity and heterozygosity.

Table 1 Details of teak populations used in the ISSR analysis

State	Population (Code)	Geographical location		Teak forest type
		Latitude (N)	Longitude (E)	
Andhra Pradesh	Kawal (AP1)	19°12'30"	78°57'30"	Dry
	Kinnersani (AP2)	17°46'43"	80°33'4"	Dry
	Shivaram (AP3)	19°37'	78°30'	Dry
	Bhadhrachalam (S) (AP4)	17°40'	80°56'	Moist
Karnataka	Chordi (KN1)	16°37'	75°38'	Dry
	Tuppur (KN2)	16°37'	76°51'	Moist
	Veerampalli (KN3)	15°12'	74°28'	Moist
Kerala	Kulathupzha (KE1)	9-10°	76-77°	Moist
	Nilambur (KE2)	11°28'3"	76°25'	Very moist
	Olavakkode (KE3)	10°25'46"	76°45'36"	Moist
	Tholpetty (KE4)	11°39'	76°16'	Moist
Madhya Pradesh	Balaghat (MP1)	21°48'	80°15'	Moist
	Betul (MP2)	22°6'49"	77°53'	Dry
	Hosangabad (MP3)	22°46'	77°45'	Semi-moist
	Seoni (MP4)	22°6'	79°35'	Dry
Maharashtra	Akkalkuwa (MS1)	21°33'20"	74°1'4"	Dry
	Allapalli (MS2)	19°25'36"	80°3'39"	Moist
	Amba (MS3)	19°13'60"	75°28'60"	Very dry
	Behapada (MS4)	18°42'	72°45'	Dry
	Chanda (Central) (MS5)	19°56'46"	79°17'48"	Dry
	Chanda (West) (MS6)	19°59'1"	79°21'12"	Dry
	Deozari (MS7)	21°15'	75°17'60"	Dry
	Karjana (MS8)	21°18"	77°54"	Very dry
	Surewani (MS9)	21°9'	79°9'	Dry
	Yavatmal (MS10)	20°24'36"	78°7'47"	Dry
	Zari (MS11)	21°15'	75°17'60"	Dry
Orissa	Puri (OR)	19°48'	85°52'	Semi-moist
Rajasthan	Dungarpur (RJ)	23°50'	73°50'	Very dry
Tamil Nadu	Varagalayar (TN)	11°00'	77°00'	Moist

Table 2 Characteristics of ISSR markers used for the analysis of teak populations

Primer code	Nucleotide sequence (5'-3')
UBC-801	ATA TAT ATATATATATT
UBC-834	AGA GAGAGAGAGAGAGYT
UBC-880	GGA GAG GAG AGG AGA
UBC-899	CAT GGT GTT GGT CAT TGT TCCA
UBC-900	ACT TCC CCA CAG GTTAACACA

Lynch and Milligan (1994) considered presence of a band as dominant homozygosity and absence of a band as recessive homozygosity, and the estimation of heterozygosity was made indirectly. Different hierarchical analyses of molecular variance (AMOVA) were carried out to determine genetic structure of teak populations using the Arlequin (ver. 3.0, Excoffier et al. 2005) software. To assess the genetic relationships among populations based on Nei's genetic distance coefficients, NT-SYS-pc (Version 2.20e, Rohlf 2000) software

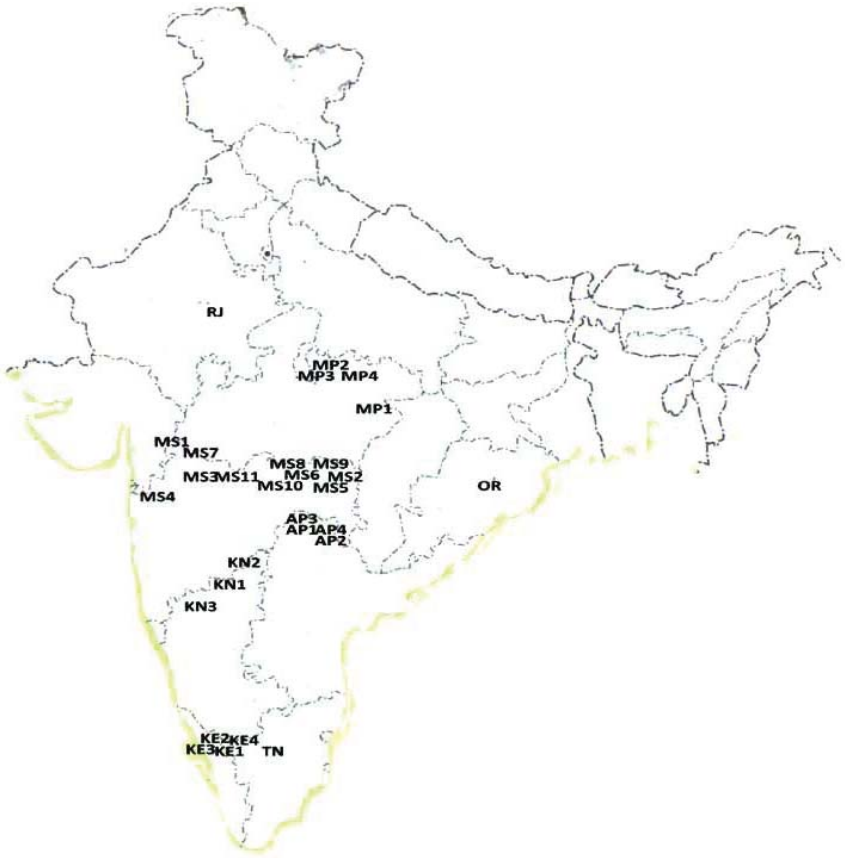

Figure 1 Map of India showing boundaries of states and the abbreviated names of teak populations denoting their locations

was used to construct UPGMA (Unweighted Pair Group Method of cluster Analysis) - dendrogram (Sneath & Sokal 1973) and perform Principle Coordinate Analysis (PCA). The robustness of each UPGMA node was evaluated by bootstrapping Nei's genetic distance coefficient data over loci for 1,000 replicates using the Seqboot and Consensus tree of PHYLIP 3.69 (Felsenstein 2005).

Results

Genetic diversity

The five ISSR markers showed 100% polymorphism, amplifying 43 polymorphic bands across 29 teak populations, corresponding to an average of 8.6 bands per primer. The higher values for Nei's gene diversity and Shannon's information index were in primer UBC 899 and the lower in primer UBC 900 (Table 3, Fig. 2**).**

Percentage of polymorphic loci (PPL), expected heterozygosity, Nei's gene diversity and Shannon's information index had the highest values in the populations of Andhra Pradesh. On the other hand, the PPL was the lowest in the Tamil Nadu population, the expected heterozygosity in Orissa population, Nei's gene diversity in Madhya Pradesh populations and Shannon's information index in Maharashtra

Table 3 Genetic diversity of 29 teak populations detected by five ISSR primers

S. No.	Primer code	nPBa(PPLb)	Nei's genetic diversity (H)	Shannon's Information Index (I)
1	UBC-801	8.0 (100)	0.36	0.55
2	UBC-899	9.0 (100)	0.40	0.59
3	UBC-834	9.0 (100)	0.37	0.55
4	UBC-900	8.0 (100)	0.32	0.49
5	UBC-880	9.0 (100)	0.34	0.51
	Mean	8.6	0.36	0.54

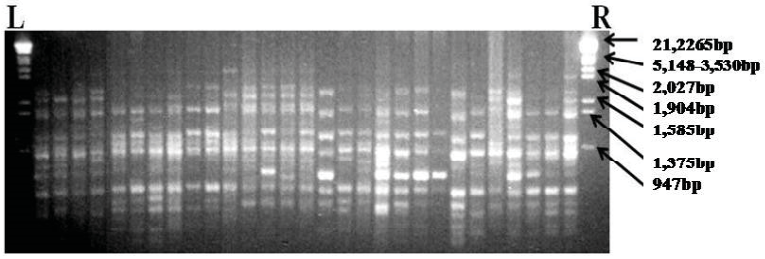

Figure 2 Gel electrophoresis patterns of amplified loci among teak (*Tectona grandis*) populations using ISSR primer UBC-801

Note: Left (L) and right (R) lanes exhibit molecular ladder fragments (λ DNA/ Eco R I/ Hind III double digest) with molecular weights (bp); Lanes 2-30 from 'L to R' sequentially represent 29 teak populations - Kawal (AP1), Kinnersani (AP2), Shivaram (AP3), Bhadhrachalam (S) (AP4), Chordi (KN1), Tuppur (KN2), Veerampalli (KN3), Kulathupzha (KE1), Nilambur (KE2), Olavakkode (KE3), Thol petty (KE4), Balaghat (MP1), Betul (MP2), Hosangabad (MP3), Seoni (MP4), Akkalkuwa (MS1), Allapalli (MS2), Amba (MS3), Behapada (MS4), Chanda (Central) (MS5), Chanda (West) (MS6), Deozari (MS7), Karjana (MS8), Surewani (MS9), Yavatmal (MS10), Zari (MS11), Puri (OR), Dungarpur (RJ) and Varagalayar (TN).

populations. At individual population level, PPL was the highest in Tuppur (Karnataka) and Surewani (Maharashtra) populations but was the lowest in Betul (Madhya Pradesh) population. The expected heterozygosity had the higher values in Shivaram and South Bhadrachalam populations of Andhra Pradesh, and the lower values in Deozari and Zari populations of Maharashtra. Similarly, Nei's gene diversity and Shannon's information index were maximum in South Bhadrachalam population, while the lowest values were in Allapalli population of Maharashtra (Table 4).

Total gene diversity calculated based on the method of Lynch and Milligan was slightly higher than Nei's gene diversity but remarkably less than Shannon's Information Index. The intra-population gene diversity proportion was 97% according to Lynch and Milligan and 84.5% according to Nei. Similarly, AMOVA also allocated a major proportion (91%) of gene diversity to intra-population in comparison to a low proportion (9%) of gene diversity allocated to inter- population differentiation, which was further partitioned among inter- and intra-state populations (Table 5).

Population genetic structure

The coefficient of genetic differentiation among populations (*Gst*) was 0.1533. The level of gene flow (*Nm*), which was computed based on *Gst*, was estimated to be 2.76. Mean genetic distance between populations ranged from 0.004 to 0.174 with an average of 0.062. The UPGMA dendrogram revealed three clus-

Table 4 Genetic variation among teak populations using ISSR markers

State	Population	PPL*	H_j	H ± SD	I ± SD
Andhra Pradesh	Kawal	81.4	0.39	0.30 ± 0.19[a]	0.44 ± 0.26[a]
	Kinnersani	83.7	0.45	0.35 ± 0.18[a]	0.51 ± 0.24[a]
	Shivaram	83.7	0.47	0.37 ± 0.18[a]	0.53 ± 0.24[a]
	Bhadrachalam (S)	86.0	0.47	0.38 ± 0.17[a]	0.54 ± 0.24[a]
	Mean	**83.7**	**0.45**	**0.35**	**0.50**
Karnataka	Chordi	72.1	0.40	0.29 ± 0.21[a]	0.42 ± 0.29[a]
	Tuppur	90.7	0.39	0.33 ± 0.16[a]	0.49 ± 0.21[a]
	Veerampalli	83.7	0.46	0.34 ± 0.18[a]	0.50 ± 0.25[a]
	Mean	**82.2**	**0.41**	**0.32**	**0.47**
Kerala	Kulathupuzha	86.0	0.43	0.36 ± 0.17[a]	0.52 ± 0.24[a]
	Nilambur	76.7	0.43	0.32 ± 0.19[a]	0.46 ± 0.27[a]
	Olavakkode	86.0	0.39	0.33 ± 0.18[a]	0.49 ± 0.24[a]
	Tholpetty	79.1	0.44	0.33 ± 0.19[a]	0.48 ± 0.26[a]
	Mean	**81.9**	**0.42**	**0.33**	**0.49**
Madhya Pradesh	Balaghat	67.4	0.40	0.30 ± 0.21[a]	0.44 ± 0.30[a]
	Betul	62.8	0.39	0.27 ± 0.22[a]	0.38 ± 0.31[a]
	Hosangabad	79.1	0.41	0.29 ± 0.19[a]	0.41 ± 0.26[a]
	Seoni	79.1	0.43	0.32 ± 0.19[a]	0.47 ± 0.27[a]
	Mean	**72.1**	**0.41**	**0.29**	**0.43**
Maharashtra	Akkalkuwa	74.4	0.43	0.31 ± 0.20[a]	0.45 ± 0.28[a]
	Allapalli	69.8	0.34	0.24 ± 0.20[c]	0.36 ± 0.28[b]
	Amba	83.7	0.39	0.31 ± 0.17[a]	0.46 ± 0.24[a]
	Behapada	76.7	0.35	0.26 ± 0.18[a]	0.40 ± 0.26[a]
	Chanda (Central)	74.4	0.37	0.27 ± 0.20[a]	0.40 ± 0.28[a]
	Chanda(West)	74.4	0.37	0.27 ± 0.19[a]	0.40 ± 0.27[a]
	Deozari	74.4	0.33	0.26 ± 0.19[b]	0.39 ± 0.27[a]
	Karjana	81.4	0.37	0.27 ± 0.18[a]	0.41 ± 0.25[a]
	Surewani	90.7	0.40	0.33 ± 0.16[a]	0.49 ± 0.21[a]
	Yavatmal	72.1	0.40	0.29 ± 0.20[a]	0.42 ± 0.28[a]
	Zari	74.4	0.33	0.25 ± 0.18[c]	0.37 ± 0.26[b]
	Mean	**76.9**	**0.37**	**0.28**	**0.41**
Orissa	Puri	88.4	0.38	0.31 ± 0.18[a]	0.46 ± 0.23[a]
Rajasthan	Dungarpur	74.4	0.42	0.31 ± 0.20[a]	0.45 ± 0.28[a]
Tamil Nadu	Varagalayar	86.0	0.40	0.35 ± 0.18[a]	0.50 ± 0.24[a]
	Mean	**80.3**	**0.40**	**0.32**	**0.45**

Note: * Percentage of polymorphic loci (total no. of loci = 43), H_j - expected heterozygosity (AFLPSURV), H - Nei's gene diversity (POPGENE) and I - shannon's Information Index (POPGENE). Numerical values in columns bearing various superscript letters are significantly different at $P < 0.01$ (Duncan's multiple range test).

ters, separating moist teak of Karnataka and Kerala from dry teak of other states. The flanking two clusters included mostly dry teak of various states occasionally intermingled only with their moist or semi-moist teak, except one population each of Orissa and Tamil Nadu and Karnataka (Tuppur). Nevertheless, Maharashtra populations were dispersed in both flanking clusters, making groups with populations of adjoining states (Andhra Pradesh, Karnataka, Madhya Pradesh and Orissa) and distant states (Rajasthan and Tamil Nadu). The moist populations of Karnataka grouped with dry teak of Maharashtra, on the one hand, and very moist teak of Kerala, on the other. But populations of Kerala made a discrete cluster, distinguishing very moist teak from dry teak. However, the bootstrapping of clusters indicated ≥ 40% robustness only for three nodes as Tuppur/Puri > Deozari/Zari > Amba/ Surewani/Behapada

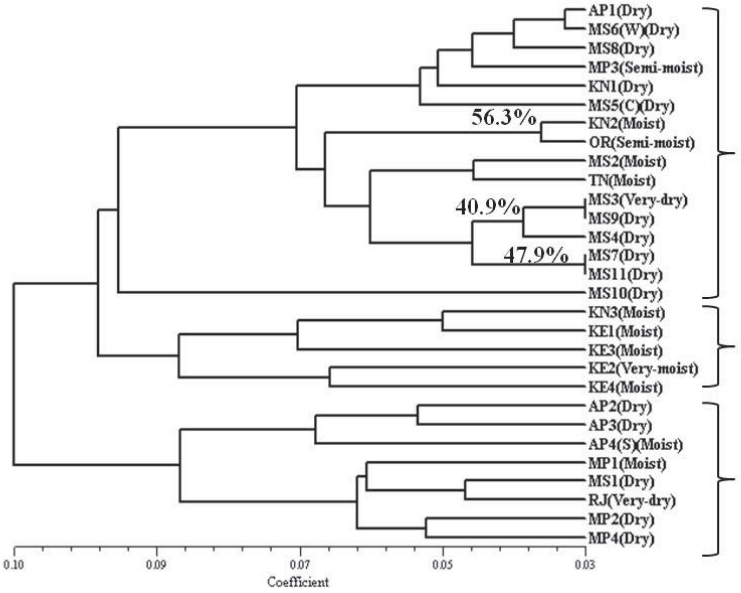

Figure 3 Dendrogram generated using UPGMA method showing relationships between 29 populations of *T. grandis* using ISSR marker data. The robustness of each node was evaluated by bootstrapping data over loci for >1,000 replications. The bootstrap values > 40% are mentioned on the nodes of the cluster analysis, for bootstrap values < 40% being non-significant denote instability of nodes

Table 5 Population genetic structure of teak using AMOVA of ISSR data

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation
Among states	7	163.409	0.45113 Va[1]	6.17
Among populations within states	21	182.218	0.20227 Vb[2]	2.77
Within Populations	261	1736.800	6.65441 Vc[3]	91.06
Total	289	2082.428	7.30780	

Note: Significance tests (1023 permutations), [1]P - value = 0.00000 ± 0.00000; [2]P - value = 0.00098 ± 0.00098; [3]P - value = 0.00000 ± 0.00000.

(Figure 3). The PCA using genetic distance values of teak populations mostly supported the UPGMA cluster analysis and also provided resolution of teak populations according to their geographical locations. Further, both dry and moist teak populations from the same state displayed tendency to remain together in different quadrates on PCA graph. However, irrespective of the geographical boundaries, the dry teak populations occupied positions above the diagonal and moist teak populations below the diagonal on PCA graph (Figure 4).

Discussion

The present study incorporates as diverse populations as possible from the entire range of natural habitats representing from dry to very moist as well as pure and mixed forests of Indian teak. Table 1 presents classification of teak populations on the basis of rainfall (Kumaravelu 1993). The central and peninsular region of the country displays almost similar topography with 300-600 m elevation. However, the region exhibits west to east slope as

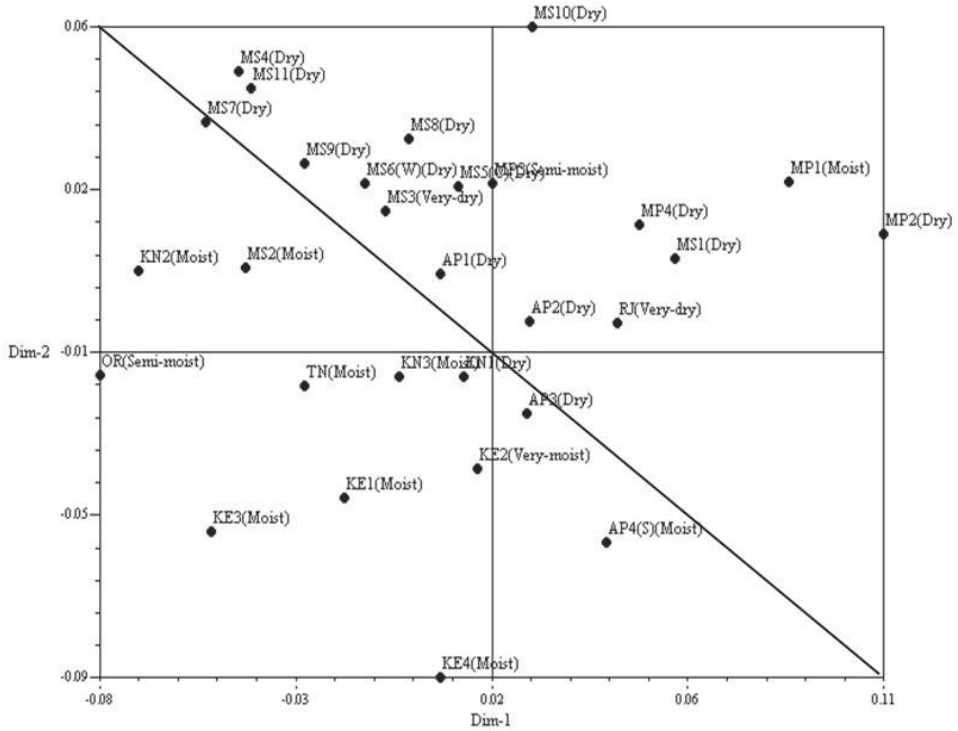

Figure 4 PCA of ISSR data of 29 teak populations

the entire western coast and some part of Karnataka and Maharshtra have elevation of 600-1200 m. The region along the western coast, including Karnataka and Kerala receives more annual rains than the eastern coast. Further, the central region with exception of few pockets by and large, gets less rain and harbours dry teak.

Genetic diversity analysis

Five ISSR primers producing variable values for Nei's genetic diversity and Shannon's information index (Table 3) presumably reflect the differential abundance of their complementary sequences on genomic DNA across teak populations. The fact that the primers UBC 899 and 900 exhibit the highest and lowest values for both parameters of genetic diversity estimates, respectively can be interpreted as UBC 900 having homo-tetramer nucleotide sequence, i.e. CCCC (Table 2). The genomic abundance of complementary sequences to homo-tetramer nucleotide sequence is expected to be less than that of homo-dimer or homo-trimer nucleotide sequences. Toth et al. (2000) have published a survey report on eukaryotic genomes, which indicates less abundance of tetra nucleotide sequences in embryophyta. Mahalakshmi et al. (2002) have also reported higher abundance of di-nucleotide sequence repeats than tetra-nucleotide sequence repeats in *Medicago* spp.

The percentage of polymorphic loci for teak populations obtained in this study was variable, ranging from 62.8% in Betul (Madhya Pradesh) to 90.7% in Chordi (Karnataka) and Surewani (Maharashtra)(Table 4). These values were comparable to the estimates obtained for teak using RAPD (27.4 - 92.2% in 15 Thailand populations, Changtragoon and Szmidt 2000; 73% in 10 Indian teak populations, Nicodemus et al. 2003) and AFLP (82% in nine

populations from India, Indonesia, Thailand, Shrestha et al. 2005). Similarly, Nei's gene diversity average value of 0.32 for Indian teak populations (Table 4) is comparable to that obtained for populations of Indian and Thailand teak through RAPD analysis (Changtragoon & Szmidt 2000, Nicodemus 2003). The expected heterozygosity values in our work are lower than those reported by Fofana et al. (2009). Nevertheless, the deficit in heterozygotes, i.e. difference between expected and observed heterozygosity in all the populations of Indian teak (Tables 4), corroborates the findings of Fofana et al. (2009) and possibly reflects the indiscriminate removal from a population of superior heterozygous trees for commercial purpose on a large scale.

Analysis of molecular variance (Table 5) assigns a very large proportion of genetic variation within teak populations (91%). The large values of standard deviations for Nei's gene diversity and Shannon's information index of each population (Table 4), and the poor bootstrapping values for cluster node stability (Figure 3) also indicate large variations within populations and poor differentiation across populations, respectively. The findings of the present study corroborate that long-lived, out-crossing trees with wide and continuous range retain most of their genetic variation within their populations (Hamrick & Godt 1989, Hamrick et al. 1992, Nybom & Bartish 2000, Nybom 2004). The high intra- population variability could have arisen by high levels of gene flow for a short distance within the populations. Teak is widely accepted as an entomophilous tree, and the insects generally transfer pollen for a short distance mainly on a single tree, producing inbred seeds with poor germination (Bryndum & Hedgegart 1969, Hedgegart 1973, Mathew et al. 1987, Indira & Mohandas 2002, Tangmitcharoen et al. 2009). The obtained inbred individuals or trees exhibit poor growth and survival. Kerdtadikaria & Prat (1995) have reported that the abundance of homozygosity observed at initial seedling level was not found at the reproductive stage in teak. They presume that gene diversity and high heterozygosity in teak populations are maintained by early exclusion of selfed material (no embryo development, low germination), and by progressive selection against homozygous genotypes during stand life, resulting in suppression of consanguineous trees and promoting heterozygous genotypes attaining reproductive stage. As a result, seedlings from heterozygous seeds dominate the population at maturity, thereby tremendously increasing the intra-population gene diversity.

The low gene diversity across various teak populations observed by us, as well as in previous investigations, may be the result of a common gene pool as well as the influence of human activities such as uncontrolled logging and unrestricted movement of planting stock. The first human activity removes teak genetic diversity and the second activity introduces common alleles across populations. The observed moderate values for Gst and Nm are consistent with the argument. Nm value > 1 shows substantial movement of gametes across teak populations satisfying the minimum number of migrants per generation needed to avoid differentiation by genetic drift (Slatkin 1987).

Previously, we have demonstrated that 48 teak plus trees selected from populations of 11 Indian states analyzed by ISSR and RAPD markers did not strictly distinguish themselves according to their territorial distribution, suggesting either a common genetic base or adequate exchange of genetic material among various populations from where the plus tree selections were made (Narayanan et al. 2007). Nevertheless, the moderate gene diversity among populations adequately distinguish dry teak populations of central region from moist teak populations of Karnataka and Kerala as revealed by UPGMA dendrogram (Figure 3) and PCA (Figure 4). Using AFLP marker analysis of teak, Shrestha et al. (2005) have demonstrated distinction between Allapalli popula-

tion (Maharashtra) of drier north-west and two populations from the moist south. Similarly, Fofana et al. (2009) have separated north (i.e. central) Indian populations from south Indian populations on the basis of SSR marker analysis of teak.

Implications for genetic improvement and conservation

The knowledge on genetic diversity and population structure of teak obtained from the present study would help in future plans for conservation and sustainable use of teak genetic resources of the country. The ISSR markers revealed that the majority of variation existed within populations rather than among populations, emphasizing conservation of many individuals within the populations from a wide geographic range in order to capture maximum genetic diversity, i.e. *in situ* conservation stands as suggested for teak in Thailand (Graudal et al. 1997, Suangtho et al. 1999). In this context, the "Preservation Plots", already earmarked within natural forests can be assessed for genetic composition and diversity for their effective conservation (Rodgers 1991). Further, it would be essential to increase the area of the existing preservation plots to include diverse trees or to lay out several new preservation plots within the natural populations representing very dry, dry, semi-moist, moist and very moist teaks of Central and Peninsular regions of India to conserve maximum intra-population genetic variability.

Conclusion

Although teak cultivation in India dates back to several hundreds of years, region-wise or population-wise breeding and conservation strategies have not been implemented. Teak genetic improvement in India mainly focuses on selection of few phenotypically superior (plus) trees from a large number of natural populations or plantations. Our study advocates the need for revision of the strategy towards selecting large number of teak plus trees from a population, due to the existence of the large amount of intra-population variation. Selection of more plus teak trees would help to capture genetic variability of traits of economic interests, which may be utilized for future genetic improvement of timber productivity and quality. In addition, the number of *in situ* conservation plots should also be enhanced for maintenance of broad genetic base in the natural habitat.

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