Tropical Plant Research
An International Journal
© Society for Tropical Plant Research

ISSN (E): 2349 – 1183
ISSN (P): 2349 – 9265
3(1): 213–220, 2016

Research article

Characterization and validation of teak plus trees ramets of national teak germplasm bank through microsatellites

Shashank Mahesh[1], Vivek Vaishnav[1]*, Pramod Kumar[1], Naseer Mohammad[1] and S. A. Ansari[2]

[1]Genetics and Plant Propagation Division, Tropical Forest Research Institute, Jabalpur, Madhya Pradesh, India
[2]Institute of Forest Productivity, Ranchi, Jharkhand, India

*Corresponding Author: vivekvaishnaw@live.in

[Accepted: 19 April 2016]

Abstract: National Teak Germplasm Bank, Chandrapur (Maharashtra) holds one of the largest collections of teak plus tree ramets collected in the form of bud grafts from 12 natural teak populations of India. These grafts were planted with three ramets of each tree. The objective of the present investigation was to validate 118 ramets of 48 plus trees and also to characterize the germplasm bank using microsatellite markers. 12 microsatellite primers amplified 27 loci with estimates of expected and observed heterozygosity (0.33 and 0.2). Moderate values of resolving power (1.05) and polymorphic information content (0.27) confirmed its stringency for analysis. The genetic structure of the germplasm was determined through the F_{ST} (0.075), Shannon's information index (0.59), Nei's heterozygosity (0.41) and Chi-square tests to confirm its HWE state. 7.54% variation existed among populations and 92.46% within populations. Ramets were validated through UPGMA dendrograph. Majority of the plus trees grouped as per their sampling location. 35 ramets belonging to 14 plus trees (APT-22, MHSC-A1, MHSC-A2, MHSC-A3, ORPUB-6, UP-M, UP-A, AI-K, MHALP-8, ST-26, ST-35, ORANP-3, ORPUB-23 and ORPUB-0) clustered strictly as per their parental origin (ortet). For eight plus trees (APKEA-23, APKEA-24, APKEN-1, APNLP-1, MHSC-J1, MYHV, ORPB-12 and ORPUB-10) with three ramets, two ramets grouped with each other; whereas the third ramet could not be validated.

Keywords: Ortet - HWE - PIC - RP - Gene diversity.

[**Cite as:** Mahesh S, Vaishnav V, Kumar P, Mohammad N & Ansari SA (2016) Characterization and validation of teak plus trees ramets of national teak germplasm bank, Chandrapur, Maharashtra through microsatellites. *Tropical Plant Research* 3(1): 213–220]

INTRODUCTION

Teak (*Tectona grandis* L. f., 2n=36) is one of the premier timber tree species of the world belonging to family Lamiaceae, planted widely in the tropics (Gill *et al.* 1983). It is native to India, Thailand, Myanmar and Laos. Molecular level characterization of the species has confined India and Laos as the centers of the genetic origin (Verhaegan *et al.* 2010). In India, teak improvement program started in 1960s and intended mostly on the selection of phenotypically superior plus trees, establishment of provenance/progeny trials and seed orchards and creation of seed production areas. In teak the traditional breeding methods to find out good genotype is a long process. Therefore, germplasm of the plus trees are considered fit to be used for raising seed orchards. The scientific program related to molecular breeding and tree genomics also needs a structured population of the species.

To fulfill the need, national teak germplasm bank (NTGB) at Chandrapur (Maharashtra) was established in 1978. It is having collection of teak plus tree clones raised from the selected plus trees bud grafts of 12 natural populations of India. 260 plus tree clones with three ramets of each had been planted in row/column design with 8m X 8m spacing (Kumar *et al.* 1998). It is the only collection of its kind in the country. The main object of the germplasm bank is to conserve a broad spectrum of genetic variability to serve as a reservoir for different conservation and management needs.

Received: 30 January 2016 Published online: 30 April 2016

Assessment of genetic variability employing molecular markers has proved to be a keystone to understanding the genomic constitution, categorizing the genes responsible for important traits, the classification and conservation of genetic variation in plant germplasm and developing selective proliferation approaches for breeding and ex-situ conservation. The neutral genetic diversity of teak from the natural area and introduced populations has been studied with molecular markers (Vaishnaw *et al.* 2014). Studies based on highly stringent dominant (RAPD, ISSR and AFLP) and co-dominant marker (SSR) technique state that the teak populations have genetic variability from 57% to 99.6% within population and from 0.04% to 43% between populations (Ansari 2014). Teak in India exhibits high variability not only at gene level (Verhaegan *et al.* 2010, Vaishnaw *et al.* 2014, Ansari 2014, Fofana *et al.* 2009, Fofana *et al.* 2013) but varies greatly in timber characteristics (mainly central and southern Indian teak) also. The germplasm bank must have collection covering varied characteristic of teak and it must represent the entire genetic variability found in natural population as well.

Therefore, present investigation was performed aiming, (1) validation of plus trees ramets, (2) assessment of the genetic variability and (3) molecular level characterization of national teak germplasm bank using microsatellite markers.

MATERIAL AND METHODS

Plant material and DNA extraction

Branch cuttings were collected from total 118 ramets representing 48 plus trees maintained at NTGB, Chandrapur (Maharashtra). Three ramets from each of 22 plus trees and two ramets from each of 26 plus trees were sampled which were the collection from nine states of the country (Table 1). The surface sterilized cuttings were administered 200 ppm IAA + 200 ppm thiamine for 4 hours followed by sealing of the top cut end with paraffin wax. The treated cuttings were planted in polybags filled with potting mixture. After one month of the planting, the cuttings produced leaf sprouts, which were harvested for the extraction of genomic DNA.

Genomic DNA was extracted following the modified Doyle and Doyle method (Narayanan *et al.* 2006). To remove large amounts of polyphenols and polysaccharides, 3% PVP was added to the extraction buffer. An additional washing step with cold ethanol was also included for removal of remaining impurities. Purity and quantity of the extracted genomic DNA were estimated through spectrophotometer and the quality of DNA was visually verified on 1% agarose gel electrophoresis.

Table 1. Teak (*Tectona grandis* L. f.) plus tree clones selected for the characterization of germplasm bank through microsatellite markers.

Locations	**Accessions**	
	With two Ramets	**With three Ramets**
All India	AI, AI-8, AI-C, AI-D, AI-I, AI-K, AI-N	-
Andhra Pradesh	APT-20,	APKEA-23, APKEA-24, APKEN-1, APNLP-1, APNPL-0, APT-22
Maharashtra	MHAL-P2, MHALP-3, MHALP-8,	MHSC-A1, MHSC-A2, MHSC-A3, MHSC-J1
Karnataka	MYHD-1, MYHD-3, ST-26, ST-27, ST-35, ST-44, ST-45	MYHV, ST-43,
Orissa	ORANP-3, ORANP-7, ORPUB-23, ORPUB-0, ORPUB-00	ORANR-4, ORPB-12, ORPUB-10, ORPUB-11, ORPUB-2, ORPUB-6,
Madhya Pradesh	-	PT-3
Tamilnadu	TNT-10,	TNT-8
Uttar Pradesh	UP-G,	UP-M, UP-A,
West Bengal	WB-4	-
TOTAL	26 genotypes X 2 ramets each = **52 trees**	22 genotypes X 3 ramets each = **66 trees**

Microsatellite markers assay

In prior investigation, 35 microsatellite primers were screened in teak genome. Out of them twelve primers could amplify the genome and were selected for final analysis with selected genotypes (Table 2). PCR amplification was carried out in 15 µl of reaction volume containing 15 ng genomic DNA, 1 unit Taq polymerase (Promega GoTaq M3001), 0.2 mM of each dNTPs, 1X Taq polymerase buffer and 0.66 µM each of forward and reverse primers. Amplifi cation cycle consisted of an initial 5 min denaturation at 94^{o}C, 34 cycles for 30 sec at 94^{o}C, 30 sec at 55^{o}C (Ta), 1 min at 72^{o}C, and final extension step for 8 min at 72^{o}C. The PCR

products were mixed with 3µL gel loading dye and were separated through electrophoresis on a 3.5% (Agarose SFR) gel at 100 V in 1X TBE buffer with 0.5 µg/ml ethidium bromide.

Data analysis

Banding profiles generated by the microsatellites compiled into a data matrix based on the molecular weight of amplicons. The informativeness of primers was determined through genetic parameters; expected and observed heterozygosity (He and Ho) and polymorphic information content (PIC) value were calculated by software POWERMARKER Version 3.25 (Liu & Muse 2005). The stringency of the primers to discriminate the genotypes was confirmed through calculation of resolving power (RP) as described by Prevost and Wilkinson (1999).

To evaluate the genetic variability and structure of the germplasm bank, the analysis of molecular variance (AMOVA) and F_{ST} was conducted using software Arlequin Version 3 (Excoffier *et al.* 1980). Shannon's information index (I), Nei's heterozygosity (H) and Hardy-Weinberg equilibrium (HWE) through chi-square test confirmation was determined applying software POPGENE Version 1.32 (Yeh *et al.* 1999). The ramets were validated through their pairing with identical ramet in a neighbor joining dendrograph based on the Nei's genetic distance (1983) method implemented in software POWERMARKER Version 3.25 (Liu & Muse 2005).

RESULTS AND DISCUSSION

Molecular markers are widely used in plant genetics, breeding, biological diversity analysis, and cultivar identification since they can directly manifest genetic differences at the DNA level. SSR motifs are polymorphic, abundant, and randomly distributed in eukaryotic genomes (Tautz 1989). Compared to other biomarkers, such as RAPDs and AFLPs, SSR markers are stable, co-dominant, and low cost. Therefore, they have been widely used in genetic analysis and genomic linkage mapping of tree population. A total of 118 ramets belonging to 48 teak plus trees were sampled (Table 1) from NTGB located at Chandrapur, Maharashtra. However many accessions in the assemblage of NTGB were initially acquired from different states of India and the morphological data of the accessions have been maintained but their original geographic origin and parentage is unknown due to slipshod management of the tags in each bud graft. This study is first and only report for molecular characterization of the National teak germplasm bank.

Informativeness of primers

27 markers were amplified by 12 microsatellite primers and the number of alleles detected among the 27 markers studied ranged from 2 to 7 (Table 2). The expected heterozygosity (also called gene diversity) of the primers ranged from 0.22 (by Tg-7) to 0.47 (Tg-11) with average value of 0.33. The observed heterozygosity of primers was found with average value of 0.2 (Table 2). These values of heterozygosity are comparably low to the SSR primers resulted (0.576) for population of *Quercus suber* (Gomez *et al.* 2001) and closely similar to the values of SSR primers resulted (0.22 to 0.54) for Birch (Hao *et al.* 2015). Low value of observed heterozygosity than expected indicates low variability covered by the microsatellites. With average value of 1.05, RP ranged from 0.2 (by Tg-13) to 5.42 (by Tg-11). The PIC is determined by both, allele numbers and allele frequency distribution and can be used to evaluate the variation of microsatellites (Botstein *et al.* 1980). In this study, the PIC values of the primers ranged from 0.2 (by Tg-7) to 0.36 (by Tg-11) with average 0.27 (Table 2). PIC and RP have been used in several studies (Prevost & Wilkinson 1999, Smith *et al.* 1997, Korkovelos *et al.* 2008) to analyze markers for their informativeness in genotyping, genetic diversity assessment, and discriminatory power. In the current research, markers with higher RP values were more informative in other genetic parameters also. These results are in agreement with Prevost & Wilkinson (1999) who observed a strong linear relationship between resolving power and discriminatory power of a marker. However, these values are dynamic and changeable, depending upon the number and nature of the genetic material involved. The low to moderate values of gene diversity and PIC indicated that the microsatellites applied for the investigation were stringent but covered lower genetic variability of entire germplasm bank. Primer Tg-11 showed highest values not only for gene diversity and heterozygosity but for resolving power and PIC also (Table 2). Therefore, the amplicons of primer Tg-11 can be used further for linkage disequilibrium or association mapping of teak.

Genetic structure of germplasm bank

AMOVA resulted moderate values of genetic variation. 7.54 % variation was found among populations and 92.46% variation within populations of germplasm bank. In earlier reports, teak populations have been

characterized through dominant (RAPD, AFLP and ISSR) and co-dominant (SSR) marker systems (Ansari 2014) and among populations variation was revealed minimum 0.04% (Kumar 2011) to maximum 43% (Shreshta *et al.* 2005). On the other hand, within population variation was found minimum 57% (Shreshta *et al.* 2005) to maximum 99.6% (Kumar 2011). The earlier report based on microsatellites based characterization of teak populations revealed that only 0.17% variation exhibited among six natural teak populations of India and, within population variation was found non-significant (Verhaegan *et al.* 2010). The F_{ST} value of the germplasm bank was found 0.075 which is lower than the estimates (0.15 to 0.34) revealed through dominant marker systems (Ansari, 2014) but it was found higher than the estimate (0.02) revealed by microsatellite systems (Verhaegan *et al.* 2010). Since the germplasm bank has a large coverage of genotypes collected from north, central and south states of India therefore, higher value of F_{ST} was obtained in present investigation compared to earlier report (Verhaegan *et al.* 2010) in which genotypes only from south Indian states were sampled. The Shannon's information index and Nei's heterozygosity was found 0.59 and 0.41 respectively. These values strengthen the fact that the germplasm bank exhibits maximum variability of the natural teak population. Chi-square values for different loci indicate that 89% loci deviated from HWE at significant level ($p<0.05$). Since the germplasm bank had been established by the selection of phenotypically superior trees from several natural populations, it deviated from the equilibrium state. Estimates of parameters depicting genetic variability of the germplasm bank confirm that the bank maintains high genetic diversity comparing to the earlier reported variability through microsatellites and it represents the genetic structure and variability of natural population of teak in India. Of course its mode of establishment through selection of superior trees causes its deviation from the HWE state but the available resources can be managed for breeding and mapping population.

Table 2 Genetic parameters showing informativeness of 12 microsatellite primer sets on teak genotypes.

Primer ID	Forward (F) and Reverse (R) sequences (5'-3')	Amplicon size (bp)	He	Ho	RP	PIC
Tg-2	**F** GCTTTAGTGATTCTCGCCTA	622 -644	0.36	0.25	1.02	0.29
	R CTCAATAATTCCAAACCGAC					
Tg-3	**F** TCTCTCATTCTCTICGGTTC	651-693	0.30	0.11	0.42	0.25
	R TTTCTAGAGGCCCATAATGA					
Tg-7	**F** TTATTGCTCTTTGGGTTTGT	522-559	0.22	0.07	0.27	0.20
	R TATTCTCGCTTCCATGACTT					
Tg-8	**F** AAGAAAGACGACAACCTTG	722-749	0.31	0.21	0.85	0.26
	R GCTTTAGTGATTCTCGCCTA					
Tg-9	**F** CAACTGGAAATCCACAATTT	478-577	0.45	0.28	1.10	0.35
	R GGCCTATATTTCTTTCCTCC					
Tg-11	**F** TCATGCACACATGTAACACA	797-849	0.47	0.68	5.42	0.36
	R ACCGCAAATAATCATAATGG					
Tg-12	**F** GCTATCAAATTTGCTGCTTT	687-760	0.38	0.16	0.64	0.31
	R ACTGATTGCTATAAAGGCCA					
Tg-13	**F** ATCGTATTGCAGCTTTGTCT	708-769	0.25	0.05	0.20	0.22
	R GGAACTCCTTTCTCGTCTTT					
Tg-15	**F** TTATCAACTTCTGCAACCCT	610-739	0.24	0.12	0.69	0.21
	R GAGTATGTCACCTGCCTGTT					
Tg-16	**F** AACCATGACAGAAACGAATC	520-596	0.42	0.23	0.89	0.33
	R GACATTCAGCCTGCTACTTC					
Tg-18	**F** TCTTGATGGTTTGCCTATTT	820-862	0.34	0.15	0.61	0.27
	R TATCTTCATGGTTGCCTTCT					
Tg-21	**F** GCAGTAATGAAAGGATTTC	806-846	0.24	0.11	0.45	0.21
	R ACATTACTTCTCACATGCCC					
		Average	**0.33**	**0.20**	**1.05**	**0.27**

Note: He= expected heterozygosity, Ho= observed heterozygosity, RP= resolving power, PIC= polymorphic information content.

Validation of ramets

The NJ dendrograph revealed that out of 118 ramets belonging to 48 PTs, 35 ramets exactly grouped as per their parental origin/ortet (Fig. 1). 100% ramets from the ortets APT-22, MHSC-A1, MHSC-A2, MHSC-A3, ORPUB-6, UP-M, UP-A, AI-K, MHALP-8, ST-26, ST-35, ORANP-3, ORPUB-23 and ORPUB-0 were validated (Table 3). 66% ramets could be validated from the ortets APKEA-23, APKEA-24, APKEN-1,

APNLP-1, MHSC-J1, MYHV, ORPB-12 and ORPUB-10. Only two out of three ramets could be validated of these ortets (Table 3). Unlike the earlier characterization of these 48 genotypes based on dominant marker

Table 3 Validated ramets of teak assembled in National teak germplasm bank Chandrapur, Maharashtra through microsatellite markers.

S. N.	Accessions/ Ortet	Ramets	Validated Ramets	Validation (%)
1	AI	2	0	0
2	AI-8	2	2	100
3	AI-C	2	0	0
4	AI-D	2	0	0
5	AI-I	2	0	0
6	AI-K	2	0	0
7	AI-N	2	0	0
8	APKEA-23	3	3	100
9	APKEA-24	3	2	66.6
10	APKEN-1	3	2	66.6
11	APNLP-1	3	3	100
12	APNPL-0	3	0	0
13	APT-20	2	0	0
14	APT-22	3	3	100
15	MHAL-P2	2	0	0
16	MHALP-3	2	0	0
17	MHALP-8	2	0	0
18	MHSC-A1	3	3	100
19	MHSC-A2	3	3	100
20	MHSC-A3	3	3	100
21	MHSC-J1	3	2	66.6
22	MYHD-1	2	0	0
23	MYHD-3	2	0	0
24	MYHV	3	0	0
25	ORANP-3	2	2	100
26	ORANP-7	2	0	0
27	ORANR-4	3	0	0
28	ORPB-12	3	2	66.6
29	ORPUB-10	3	2	66.6
30	ORPUB-11	3	0	0
31	ORPUB-2	3	0	0
32	ORPUB-23	2	2	100
33	ORPUB-6	3	3	100
34	ORPUB-0	2	2	100
35	ORPUB-00	2	0	0
36	PT-3	3	0	0
37	ST-26	2	0	0
38	ST-27	2	0	0
39	ST-35	2	0	0
40	ST-43	3	0	0
41	ST-44	2	0	0
42	ST-45	2	0	0
43	TNT-10	2	0	0
44	TNT-8	3	0	0
45	UP-A1	3	3	100
46	UP-G	2	0	0
47	UP-M	3	3	100
48	WB-4	2	2	100
Overall		118	47	39.8

(Narayanan *et al.* 2007), the majority of PTs were grouped together as per their geographical location of sampling (Fig. 1). Few of the ramets could not be validated through applied microsatellites and characterized distinct from the labeled ortet. There could be chances of mislabeling or failure of bud graft vis-à-vis rootstock overtaking the growth. The genetic fidelity of these invalidated ramets needs should be further checked using

multiple marker system supported by the authentic phenotyping of these ramets. However, microsatellites markers efficiently revealed the status of genetic diversity in these collections and also clustered most of geographically related PTs clones together.

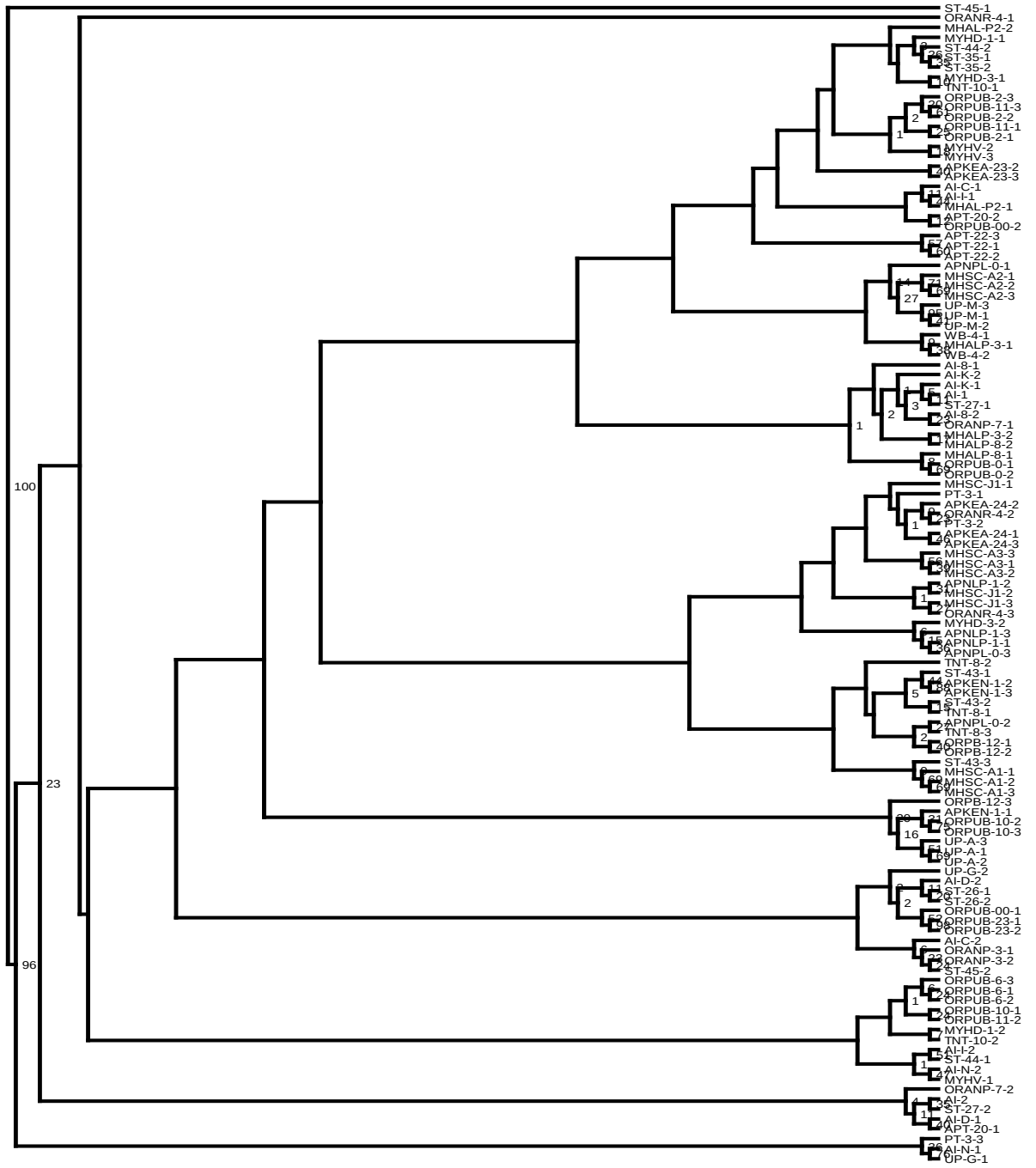

Figure. 1 Neighborjoining (NJ) dendrograph of 118 ramets of plus trees of teak (*Tectona grandis* L. f.) based on Nei's (1983) genetic distance method.

CONCLUSION

Gene diversity is a result of gene evolution in plant species (Guo *et al*. 2012) and becomes a foundation of genetic improvement of species. The results of the present study indicated that the National teak germplasm bank located at Chandrapur, Maharashtra was a good initiative to assemble clones of plus trees collected from all India. Prior information related to its genetic variability and validation of the ramets with their ortets will be helpful to manage mapping and breeding population for next generation breeding program. Our study confirms that the germplasm bank represents the actual variability of teak population that has been reported by earlier findings. Mismatch of the ramets with their ortet can be considered as a limit which can be overcome by the

establishment of bud grafts from same ortet. The investigation recommends for increasing number of selected PTs from natural teak populations to widen the genetic base of National Teak Germplasm Bank, Chandrapur because a large proportion of genetic variation in teak occurs among individuals within populations. It is also recommended to maintain the equal number and size of germplasm representing various geographical locations of teak natural population of the country. Further, this study highlights the effectiveness of microsatellite markers to distinguish the ramets with respect to genetic diversity estimates of assembled germplasm, promoting efficient conservation of genetic resources of teak in India.

ACKNOWLEDGEMENT

We are thankful to Department of Biotechnology, Government of India, New Delhi for funding the project and to the Maharashtra Van Sanshodhan Sansthan (Maharashtra State Forest Department) for permitting access to the National Teak Germplasm Bank, Chandrapur. There is no conflict of interest among authors.

REFERENCES

Ansari SA (2014) *A molecular diversity of teak (Tectona grandis L. f)*. In: Silviculture Conference 2014, held in ICFRE Dehradun, India.

Botstein D, White RL, Skolnick M & Davis RW (1980) Construction of a genetic linkage map in man using restriction fragment length polymorphism. *American Journal of Human Genetics* 32: 314–331.

Excoffier L, Laval G & Schneider S (2005) Arlequin ver. 3.0: An integrated software package for population genetics data analysis. *Evolionary Bioinformatics Online* 1: 47–50.

Fofana IJ, Ofori D, Poitel M & Verhaegen D (2009) Diversity and genetic structure of teak (*Tectona grandis* L. f.) in its natural range using DNA microsatellite markers. *New Forest* 37: 175–195.

Fofana IJ, Silue S, Diarrassouba N, Kadio AA & Sangare A (2013) Comparative analyses of amplified fragment length polymorphism (AFLP) and simple sequence repeat (SSR) in genetic diversity of teak (*Tectona grandis* L. f.). *International Journal of Advance Agriculture Research* 1: 114–123.

Gill BS, Bedi YS & Bir SS (1983) Cytopalynological studies in woody members of family Verbenaceae from north-west and central India. *Journal of Indian Botanical Society* 62: 235–244.

Gomez A, Pintos B, Aguiriano E, Manzanera A & Bueno MA (2001) SSR Markers for *Quercus suber* tree Identification and Embryo Analysis. *Journal of Heredity* 92 (3): 292–295.

Guo Y, Li YL, Huang Y, Jarvis D, Sato K, Kato K, Tsuyuzaki H, Chen L & Long C (2012) Genetic diversity analysis of hulless barley from Shangari-la region revealed by SSR and AFLP markers. *Genetic Resources and Crop Evolution* 59: 1543–1552.

Hao W, Wang S, Huajing L, Zhou B, Wang X & Jiang T (2015) Development of SSR markers and genetic diversity in White Birch (*Betula platyphylla*). *PLoS ONE* 10(4): e0125235 [DOI: 10.1371/journal.pone.0125235]

Korkovelos AE, Mavromatis AG, Huang WG, Hagidimitriou M, Giakoundis A & Goulas CK (2008) Effectiveness of SSR molecular markers in evaluating the phylogenetic relationships among eight Actinidia species. *Scientia Horticulturae* 116: 305–310.

Kumar R (2011) *Studies on genetic diversity analysis of teak (Tectona grandis L.F.) of central India using RAPD marker*. Ph.D. Thesis (Forest Biotechnology), FRI Deemed University, Dehra Dun.

Kumar V, Kotrange HR & Dhotekar UP (1998) Genetic improvement of teak. *Indian Forester* 124: 687–695.

Liu K & Muse SV (2005) Power Marker: Integrated analysis environment for genetic marker data. *Bioinformatics* 21: 2128–2129.

Narayanan C, Dubey S, Wali SA, Shukla N, Kumar R, Mandal AK & Ansari SA (2006) Optimization of DNA extraction for ISSR studies in *Tectona grandis* L. f. - an important forest tree species. *African Journal of Biotechnology* 5(13): 1220–1223.

Narayanan C, Wali SA, Shukla N, Kumar R, Mandal AK & Ansari SA (2007) RAPD and ISSR markers for molecular characterization of teak (*Tectona grandis*) plus trees. *Journal of Tropical Forest Science* 19 (4): 218–225.

Prevost A & Wilkinson MJ (1999) A new system of comparing PCR primers applied to ISSR fingerprinting of potato cultivars. *Theoretical and Applied Genetics* 98: 107–112.

Shreshta MK, Volkaert H, & Straeten DVD (2005) Assessment of genetic diversity in *Tectona grandis* using amplified fragment length polymorphism markers. *Canadian Journal of Forest Research* 35(4): 1017–1022.

Smith JSC, Chin ECL, Shu H, Smith OS, Wall SJ, Senior ML, Mitchell E, Kresovich S & Ziegle J (1997) An evaluation of the utility of SSR loci as molecular markers in maize (*Zea mays* L.): comparisons with data from RFLPs and pedigree. *Theoretical and Applied Genetics* 95: 163–173.

Tautz D (1989) Hypervariabflity of simple sequences as a general source for polymorphic DNA markers. *Nucleic Acid Research* 17: 6463–6471.

Vaishnaw V, Mohammad N, Wali SA, Kumar R, Tripathi SB, Negi MS & Ansari SA (2014) AFLP markers for analysis of genetic diversity and structure of teak (*Tectona grandis*) in India. *Canadian Journal of Forest Research* 45(3): 297-306.

Verhaegan D, Fofana IJ, Logossa ZA & Ofori D (2010) What is the genetic origin of teak (*Tectona grandis* L.) introduced in Africa and Indonesia? *Tree Genetics and Genomics* 6(5): 717–733.

Yeh FC, Yang RC & Boyle TJ (1999) POPGENE, Version 1.3. Microsoft Windows-based freeware for population genetic analysis. Distributed by the author (http://www.ualberta.ca/~fyeh/fyeh/).