RESEARCH NOTE

Changes in genetic diversity parameters in unimproved and improved populations of teak (*Tectona grandis* L.f.) in Karnataka state, India

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[Lyngdoh N., Joshi G., Ravikanth G., Vasudeva R. and Uma Shaanker R. 2013 Change in genetic parameters in unimproved and improved populations of teak (*Tectona grandis* L.f.) in India. *J. Genet.* **92**, 141–145]

Introduction

Teak (*Tectona grandis* L. f.; family Verbanaceae) is an important plantation tree species in the tropics and in India one of the first species to be prioritized for improvement. Improvement efforts for the last 50 years have essentially concentrated on augmenting quality seed production by establishing seed production areas (SPA) and clonal seed orchards (CSO). Presently, these two form the main sources of quality planting material for teak throughout the country. However, there is no information on the genetic quality of such sources nor information on the progeny used in plantation programmes. Reports of studies based on coniferous and tropical species provide conflicting results on the impact of domestication on the genetic diversity of populations (Chaisurisri and El Kassaby 1994; Rajora 1999; Moran *et al.* 2000; Godt *et al.* 2001; Icgen *et al.* 2006). Also the impact of domestication on the genetic diversity of progeny populations is poorly understood (Stoehr and El-Kassaby 1997; Schmitdtling and Hiplins 1998). Such studies become pertinent not only for gauging the impact of selection on reforestation stock, but also for effective genetic conservation of existing breeding populations.

We therefore address two issues in the present study: (i) the change in genetic diversity with increasing levels of improvement, and (ii) the impact of the above change on genetic diversity of progeny populations.

Material and methods

Sampling details

Three broad teak-growing regions along the Western Ghats, within the state of Karnataka, India, were chosen for this study (table 1). From each region (except central) one control or unimproved stand (UIS), one seed production area (SPA) and one clonal seed orchard (CSO) were selected. These populations correspond to three levels of improvement arranged in ascending order. Within each region, the distance between SPA and respective UIS was not more than 1 km. From UISs and SPAs, 30 trees separated by a minimum distance of 70 m were selected. Leaf samples and fruits from each tree (for raising seedlings) were collected during the month of March 2006. From the two CSO populations located at the northern and southern regions (both near their respective UIS and SPA), fruits from 2–4 ramets of each clone planted in the orchard were mixed together. However, since these two orchards had 49 similar clones, leaf material for DNA analysis of the parent population was collected from a teak clonal bank maintained by the forest department (Lyngdoh *et al.* 2007).

The leaf samples collected from the populations were used in DNA analysis for assessing the genetic diversity of the parent population. Therefore the parent generation consisted of seven populations, namely three UISs, three SPAs and the 49 clones in a clonal bank representing the orchard parent population.

For assessing the genetic diversity of the progeny populations, seedlings were raised from fruits collected from the

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Keywords. ISSR; progeny diversity; parent diversity; tree improvement.

Table 1. Details of collection sites in Karnataka state, India.

Site	Population	Range	Division	Age (years)	Lat. (°N)	Long. (°E)	Alt. (m)
Verrampali (northern)	UIS	Virnoli	Haliyal	55	15° 27′	74° 79′	599
	SPA	Virnoli	Haliyal	55	15° 27′	74° 79′	599
	CSO	Dandeli	Haliyal	22	15° 28′	74° 56′	573
Kunehusur (central)	UIS	Chordi	Sagar	50	14° 17′	75° 39′	711
	SPA	Chordi	Sagar	50	14° 17′	75° 39′	711
Tithimathi (southern)	UIS	Titimathi	Hunsur	77	12° 30′	76° 03′	933
	SPA	Titimathi	Hunsur	77	12° 30′	76° 03′	933
	CSO	Titimathi	Hunsur	28	12° 57′	76° 04′	866

UIS, unimproved population; SPA, seed production area; CSO, clonal seed orchard.

eight progeny populations. One hundred fruits collected from each mother tree were bulked making sure that each mother tree was represented in the total collection. Leaf samples from a minimum of 30 seedlings were then collected from each population (three UIS, three SPA and two CSO) two months after sowing and DNA was extracted.

DNA isolation and PCR protocol

DNA was extracted from a total of 482 samples (from seven parent and eight progeny populations) by the CTAB (cetyl trimethylammonium bromide) extraction method (Doyle and Doyle 1987). DNA was quantified based on the spectrophotometric measurement of UV absorbance at optical density (OD) 260 nm and was diluted to working concentration (20 ng). Genetic analysis was carried out employing DNA-based inter simple sequence repeat (ISSR) molecular markers. PCR amplification was carried out in a 15-μL reaction mixture containing template DNA (20 ng), primer (0.3 μM), *Taq* polymerase (0.5 units), 10× assay buffer and dNTPs (1 mM). A total of 20 ISSR primers were screened, of which 10 primers (UBC 807, UBC 808, UBC 809, UBC 811, UBC 818, UBC 826, UBC 827, UBC 866, UBC 868, UBC 898) that gave consistent results and higher number of polymorphic bands were selected. PCR products were separated on a 1.5% agarose gel. The gel was stained with ethidium bromide (0.5 μg/mL) and visualized under UV light and the image captured using Herolab Gel Documentation Unit (Herolab, Hamburg, Germany).

Scoring and data analysis

Binary coding was used to score the gels (Wendell and Weeden 1989). Presence of a PCR-amplified product was scored as 1 and its absence as 0. Based on the absence or presence of amplified products, per cent polymorphism and Shannon information index were estimated using software POPGENE v. 1.32 (Yeh and Boyle 1997). To partition the total genetic variation into between-population and within-population variation, analysis of molecular variance was conducted using MicroSoft-Excel-based GenAlEx v. 6 software (Peakall and Smouse 2006).

Results and discussion

Changes in the genetic structure of populations are an integral part of every step of tree improvement such as establishment of SPAs and setting up of CSOs. The selective phenotype-based removal of individual trees from populations, as practised in the formation of SPAs, can potentially alter the genetic structure of residual populations and the levels of genetic diversity. CSO is an assemblage of clonally propagated specific genotypes with elite features identified from a large area. Though such management practices have been conjectured to influence the genetic structure, there are only a few studies that provide empirical data adopting molecular markers in teak, which is a principal timber species of the tropics (Lyngdoh *et al.* 2012). We hypothesized that there would be a gradual decrease in the total genetic diversity of tree populations with the increasing levels of genetic improvement. We tested this both at the level of standing populations and at progeny level adopting ISSR markers.

The 10 ISSR primers generated a total 75 fragments for the 15 populations of teak (seven parent and eight progeny). The number of fragments per primer ranged between five and nine. Only in Tithimathi site (southern region) unimproved stands showed higher levels of genetic diversity than SPA with respect to polymorphic loci (52.00 vs 49.33 % respectively) and Shannon index (0.250 vs 0.238 respectively). However, unimproved stands of Verrampali (northern region) and of Kunehusur site (central region), recorded lower values with respect to both per cent polymorphic loci and Shannon index (56.00%, 0.272; 50.67%, 0.256 respectively) compared to their respective improved populations i.e. SPAs (61.33%, 0.293; 56.00%, 0.280 respectively). We further compared the genetic parameters of CSO with those of unimproved populations and SPAs computed after pooling over all the three sites. This was done since 49 clones deployed in the CSOs were originally sourced from different sites of Karnataka state. In general, there was a gradual reduction in the values of genetic parameters with level of improvement. The highest values were found in the unimproved populations, and CSOs showed the least values; however, per cent polymorphic loci between unimproved populations and SPAs did not differ (table 2).

Table 2. Genetic diversity parameters in parent and progeny populations of unimproved stands, and seed production area and clonal seed orchard populations of teak at three sites of Karnataka.

	Unimproved stands		Seed production areas		Clonal seed orchards	
	% *P*	*I*	% *P*	*I*	% *P*	*I*
Parent						
Verrampali	56.00	0.272 ± 0.271	61.33	0.293 ± 0.273	–	–
Kunehusur	50.67	0.256 ± 0.276	56.00	0.280 ± 0.283	–	–
Tithimathi	52.00	0.250 ± 0.278	49.33	0.238 ± 0.274	–	–
Pooled	70.67	0.375 ± 0.275	70.67	0.359 ± 0.280	64.00	0.295 ± 0.265
Progeny						
Verrampali	69.33	0.319 ± 0.255	62.67	0.292 ± 0.270	61.33	0.303 ± 0.278
Kunehusur	64.00	0.304 ± 0.265	43.33	0.240 ± 0.276	–	–
Tithimathi	62.67	0.254 ± 0.250	52.00	0.224 ± 0.253	62.67	0.310 ± 0.283
Pooled	82.67	0.372 ± 0.247	78.67	0.337 ± 0.259	76.00	0.383 ± 0.277

P, polymorphism; *I*, Shannon information index.

From the above observations it is clear that the data did not completely support the hypothesis. Such conflicting results have been reported on the influence of population improvement on level of genetic diversity. For instance, Neale (1985) and Neale and Adams (1985) have reported that shelterwood harvesting had little impact on the genetic structure of Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco) stands. They attributed this resiliency to the high levels of genetic diversity inherent to Douglas fir and the fact that the species occurs in almost pure, high-density stands. Hawley *et al.* (2005) reported that after three treatment cycles of diameter-limit cutting in eastern Hemlock, residual trees in the diameter-limit cut treatment were significantly higher in genetic diversity measures than trees in the unaltered control. Observed heterozygosity among trees of these diameter-limit cut population was >25% higher than in trees in the control stand; more than twice as many loci were polymorphic in the stand that received repeated diameter-limit cuts than in the control. Even greater differences were also found in which diameter-limit cut treatment trees had 43% higher levels of expected heterozygosity than control. Interestingly the diameter-limit cut populations showed a lower frequency of rare alleles than the uncut control populations. Adopting isozyme studies in *Pinus strobus*, Buchert *et al.* (1997) have shown that observed heterozygosity in the residual population after harvesting increased by 12%. They have attributed this to the unintentional retention of individuals with heterozygosity in the residual population. Leberg (1992) suggested that random genetic drift could account for such patterns in retained populations. Rajora *et al.* (2000) also showed in *P. strobus* that partial removal of populations aimed at promoting natural regeneration resulted in increases in genetic diversity; however, there was a loss of rare alleles. Although no such estimate was made in our study an important observation is that selective thinning has resulted in lower between-population variation among SPA stands compared to unimproved stands implying greater uniformity among them (common alleles retained) following selective retention of phenotypically superior trees in all SPAs (AMOVA; table 3).

The largest changes in genetic structure of forest tree populations occur through natural regeneration of the trees with transmission of genes from the parent generation to the progenies. This process is influenced by factors such as levels of genetic diversity of parental population, outcrossing rates, and deviations from random mating among the constituent individuals. Unfortunately, our understanding of the relationship between level of genetic diversity in parental populations and their offspring among tropical forest tree species is limited. Studies on parent and progeny generations in sexually reproducing species indicate that progeny gene frequencies usually follow Hardy–Weinberg expectations. However, there are virtually no earlier studies in teak on these lines in India. In our study, interestingly, progenies of unimproved populations were genetically more diverse on account of higher values of per cent polymorphic loci as well as Shannon index compared to their parent populations. This may suggest the influence of outcrossing from larger distances in these teak-dominated forests (Tangmitcharoen *et al.* 2006). However, the sitewise comparison of levels of genetic diversity of progenies of SPAs with their respective parental populations did not show a

Table 3. Analysis of molecular variance for unimproved stands and seed production areas of parent and progeny populations of teak from three regions in Karnataka.

Population	Among population (%)	Within population (%)	*P*
Parent			
Unimproved	39	61	0.01
SPA	32	68	0.01
Progeny			
Unimproved	28	72	0.01
SPA	31	69	0.01

consistent pattern. At some sites (Verrampali and Tithimathi) the changes were only marginal; while in Kunehusur, per cent polymorphic loci was substantially lower. Maintaining moderate tree densities of the SPAs (about 200 per hectare compared to 1200 per hectare in control plantations) and other silvicultural practices increase light reception which results in profuse flowering and increased pollinator visits. Consequently, SPAs may experience a higher level of random mating than the unmanaged controls. Hence it is reasonable to assume that management practices that encourage flowering and gene flow among the SPAs could offset the influence of lower densities and contribute positively to the higher genetic diversity levels. However this general assumption was not completely upheld in all the sites. In both the northern and sourthen CSOs, progenies had marginally higher Shannon index compared to their parent populations (table 2).

Till date such comparisons of genetic diversity of progenies of SPA with those from native stands are still lacking in teak, probably due to the fact that progeny testing of SPAs is rarely attempted. Through parent–progeny comparisons, Adams *et al.* (1998) and Medri *et al.* (2003) showed that genetic diversity of progeny populations obtained from managed stands have lower genetic diversity compared to the respective parent population in *P. menziesii* var. *menziesii* and *Araucaria angustifolia*, respectively.

This study has given an insight into the extent of change in genetic diversity following tree improvement practices in teak. Thinning activities in SPA do not necessarily reduce the genetic diversity of the populations, but can severely affect the diversity of successive generations. Further, it is clearly felt that the 49 clonal selections may be inadequate to constitute a first-generation clonal orchard. Keeping in view the out crossing behaviour of teak (Prabha *et al.* 2011) and that much of genetic variation resides within populations, the number of plus trees selected from a population and number of clones/ramets maintained in a CSO or size of seed production areas of teak should be higher or larger than the conventionally done in order to capture maximum genetic diversity for improvement or conservation purpose. However, it would be not be appropriate to regard the observed changes of genetic structure induced by a specific genetic improvement practice as evidence to suggest violation of a principle of tree improvement. A careful and thorough interpretation of marker-based studies taking into account their respective limitations regarding representativeness for the genome and other genes is mandatory in order to assess adaptive potentials of tree populations as suggested by Finkeldey and Mátyás (1999).

However, the selections have to be wide enough to capture major part of the variations present in undomesticated stands of teak. Incorporating a large number of individuals at the initial stages in the breeding population circumvents the possibility of rapid genetic erosion in advanced stages. For example more than 600 selections have been made for a eucalyptus breeding programme in Australia ensuring that adequate genetic diversity of native gene pool is captured in those selections (Jones *et al.* 2006). While infusing additional variation into the population may seem reasonable, it could aggravate the problem of flowering asynchrony, which has been extensively documented in the case of teak (Gunaga *et al.* 1999). Therefore as far as possible much of the clones need to be retained during genetic thinnings in successive generations. Also during the thinning process, orchard designing based on reproductive phenology of clones would greatly reduce mating barriers. Alternatively, collaborative efforts with forest institutes may be initiated for establishing of new orchards.

Acknowledgement

We thank Director, Group Coordinator (Research), and Head of Department, Tree Improvement and Propagation Division, Institute of Wood Science and Technology, Bangalore, for the facilities provided, and Council for Scientific and Industrial Research (CSIR) for providing scholarship to the first author. Assistance and field support provided by research wing of Karnataka Forest Department is acknowledged.

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Received 3 August 2012; Revised 4 November 2012; accepted 20 January 2013
Published on the Web: 10 April 2013