Clonal Seed Orchard of Teak (Tectona grandis L.f.): Genetic Diversity Measures Primary Basis for Future Environmental Uncertainty

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Abstract To gain understanding of the importance of genetic diversity, seed source and ecotype variation of clonal seed orchard (CSO) of teak, the lower genetic diversity might be causing, to some extent inbreeding in CSO affecting seed set, seed germination and seedling health. However, the genetic diversity forms primary basis for future environmental uncertainties, these factors is insufficient to explain the poor performance of seed orchards with respect to seed production and viability. Future CSOs may be established using genetically diverse clones selected from same provenance and showing profuse synchronized flowering and seed set. These attributes have to be ensured while selecting candidate plus trees or plus trees from which the clones are derived.

Keywords Clonal seed orchard, Genetic diversity, Teak, Tectona grandis L.f.

1 Introduction

Teak (Tectona grandis L.f.) is one of the preeminent durable timbers of the world known to perform well in plantations under favorable conditions. Global demand of products from teak has got very good prospects for propagating them in plantations. With the diminishing availability of teak from natural forest, plantations are important sources of timber to meet the mounting demand. About 94 per cent of global teak plantations are in tropical Asia, with India (44%) and Indonesia (31%) accounting for the bulk of the resource. In India and Myanmar the first systematic efforts to establish teak plantations were made more than 150 years ago (FAO, 1993). The first teak plantation in India was established in 1842 at Nilambur, Kerala, Southern India with the purpose of enriching the teak forests (Katwal, 2005). At the moment, teak plantations exist around 1.5 million hectare in India and around 50 000 hectare of teak plantations are propagated annually (Subramanian et al., 2000). In Kerala, teak plantations cover about 50 per cent of the total man made forests of which 32 per cent are of site quality II and above, while the rest are of quality III and IV (George, 1961). Teak plantations are raised in Nilambur, Wayanad, Ranni, Konni, Chalakkudy, Thrissur and Palakkad forest divisions. Apart from Kerala, teak plantations also exist in other states such as Karnataka, Tamil Nadu, Andhra Pradesh, Maharashtra, Madhya Pradesh, Uttar Pradesh, West Bengal, Andamans and Assam. Organized teak planting programme started in Tamil Nadu since the first five – year plan. Teak was planted for high quality timber. Currently teak plantations are available in 20 000 ha in Tamil Nadu, which also includes 2 000 ha along canal banks in the Tanjore district (Kala et al., 2005). The history of teak planting in Andhra Pradesh is over a century old and total planted area up to 2000-2001 is 111 931 ha. The oldest artificially raised teak is through seed dibbling in Marriapala RF in Vishakapattanam district, followed by wood plantations in Nallamalai catchments of Kurnool district (Rao, 2005). A good deal of planting has been done from time to time in different localities of Maharashtra (Tewari, 1992). Maharasthra forest department has established teak plantations in about 2, 75 907 ha. Private companies and progressive farmers also started raising teak plantations from 1990 onwards (Gogate et al., 2005). Teak plantations in Madhya Pradesh dates back to 1891 in North Raipur Forest Division. Large scale teak plantations were taken up in 12 districts by teak development corporation of
Madhya Pradesh and so far 114,000 ha teak plantation has been raised (Gangopadhyay, 2005).

In 1891, T.F. Bourdillon introduced traditional stump planting technique for teak planting. After hundred years Maharashtra Forest Development Corporation, in 1991 introduced a new nursery technique for raising nursery stock of teak in root trainers (Khedkar and Subramanian, 1995). The root trainer technology for teak was developed in 1988 by Kerala Forest Department initiated by Prabhu (1998). Since then, millions of teak seedlings have been raised in root trainers.

Teak was raised on plantation scale in Kerala by British rulers and subsequently after independence was taken over by Kerala Forest Department (KFD). These plantations are established based on various age groups and site quality classes. Seed orchards form an important connection between ongoing tree improvement programme and profitable planting. Seed orchards are intended to supply superior seeds in ample quantity (Zobel, 1984).

Vegetative propagation can play a key role in tree improvement programme as a means of large scale multiplication of planting material for plantation forestry and agro-forestry in the tropics. It is the method of rising planting stock not involving seed; but, includes cuttings, layering, budding, grafting and recently micropropagation. Propagating plants vegetatively results in clones, with each clone retaining the genetic characteristics of the tree at the point from which the buds or cuttings were obtained (Evans and Turnbull, 2004). Vegetative propagation by grafting has been suggested as a possible method for raising plants for the establishment of teak seed orchards. This technique has been standardized in teak by Kedharnath and Venkatesh (1963). Clonal seed orchards (CSO) are established through planting vegetative propagated plants of phenotypically superior trees and managing them as isolated plantations to get genetically improved seed lot, through intermating of these superior types.

Teak genetic improvement was started in India in the year 1954. Teak improvement was limited to establishing seed production areas (SPAs) and clonal seed orchards (CSO). CSOs are established with grafted plants of superior phenotypes (plus trees) selected from natural forests and plantations. These “plus trees” were chosen at a very high intensity (often one in several hectares of forests) with rigorous selection criteria (Emmanuel and Bagchi, 1988; Kumar et al., 1998). CSOs are the only source of ex-situ conservation for teak in India. Although, CSOs are good collection for static conservation, they represent only a fraction of the total genetic variation present within the species. One objective of tree improvement program is mass production of genetically superior seed. Such seeds are usually collected from CSOs or seedling seed orchards (SSOs). The seed orchards are always involved in major breeding programs all over the world. Estimation of genetic gain and gene diversity aspect of seed orchard crops is crucial for management and conservation of the clones in the clonal seed orchard. Clonal breeding value, fertility variation, pollen contamination and seed production are the major factors for consideration when genetic gain and diversity are estimated.

More than 1,000 ha of CSOs have been established in India with 450 ha in Maharashtra, 240 ha in Madhya Pradesh, 120 ha in Karnataka, 92 ha in Andhra Pradesh, 35 ha in Kerala, 30 ha in Orissa and Tamil Nadu, and 25 ha in Arunachal Pradesh (Katwal, 2005). CSOs are the main focus of genetic improvement of teak in India; but the output from these orchards has been far from satisfactory. Poor flowering, asynchrony in flowering phenology, low fruit and seed set were the major problems faced (Nagarajan et al., 1996; Indira and Basha, 1999; Vasudeva et al., 2001; Mathew and Vasudeva, 2003; Gunaga and Vasudeva, 2005). Teak is the most widely planted and researched tropical hardwood species that is propagated and domesticated through plantations for one and a half centuries. Significant research and development had taken place in the standardization of techniques/methods applied in raising plantations, harvesting and post-harvesting utilization and tree improvement activities. Despite these efforts, teak resources of the world need instant attention for their sustainable management. Raising teak monoculture on the basis of ecological and economic aspects has to be considered for long term execution of supply of timber. At the same time
bottlenecks in tree improvement and seed production have to be removed through improved basic research, the potential of leading edge areas like biotechnology must also be used for speedy improvement of this plantation species. Since teak is a highly cross pollinated species, one of the reasons for low fruit and seed set and poor seed viability could be the low genetic variability between the clones which are assembled in clonal seed orchards. The natural crossing between genetically similar or related genotypes may be causing poor seed set because of self- incompatibility and inbreeding depression. The most common problem encountered in teak seed orchards in India is the asynchrony of flowering among different clones assembled in the orchard. Some clones flower at an early age (e.g. 4–5 years) whereas some others flower only after 10–15 years. Sometimes when a clone belonging to one particular ecotype (e.g. dry/moist) is included in a seed orchard established in an area of another ecotype it may not flower at all for 40 years or more. The review provides the the status and importance of genetic diversity studies on Clonal seed orchard rose by Kerala Forest Department during 1985 at Kulathupuzha in South Kerala.

2 Depletion of teak forests
During the past 50~100 years teak genetic resources have been drastically distressed because of uncontrolled logging and mixing of germplasm. Habitat ruin and disintegration have constrained the distribution of species to undersized and secluded populations. Although, comprehensive studies on genetic variability in teak are limited, significant variation in quantitatively inheritable characters have been reported in provenances of natural populations from India, Thailand and Laos (Kjaer, 1996).

3 Plus tree Selection
As a part of teak improvement programmes recognition of plus trees from natural forests and plantations has been carried out. A plus tree is a morphologically exceptional individual combining a number of enviable traits. The criteria adopted for the selection of plus trees of teak, each dominant tree is compared with at least 5 trees within a radius of 50 meters from the plus tree. In order to determine the dominance of the plus tree, a scoring system is adopted for characters like height and girth at breast height of the tree, clear bole height, straightness of stem, pattern of branching, resistance to pests and diseases, and seed production.

4 Establishment of Seed Orchard
The grafts of selected plus trees were used to establish seed orchards.

4.1 Collection of scion-wood
Scion wood for grafting was collected during the month of February-April from the selected plus trees. Small branches bearing suitable bud from the upper one third of the tree crown were collected. Bud-wood cuttings, 10–30 cm in length were prepared from these branches. These were bundled together, wrapped in polythene bags and transported to the grafting sites. The cuttings remained viable for 2~3 days.

4.2 Preparation of stock for grafting
One or two year old teak stumps about 15~25 mm in diameter at the collar region were used as stock for grafting. Such seedlings were collected from the nursery and stumps 15~20 cm long were prepared out of it.

4.3 Grafting technique
Though two types of grafting such as cleft grafting and bud grafting have been experimented in teak the latter was preferred because it ensures greater economy of bud-wood material. Depending upon the number of good buds on the bud-wood, it is possible to make 3~5 grafts from a single bud-wood instead of only one as in cleft grafting, it is easier and quicker than cleft grafting, it suits for grafting on naked stumps. Budding was done in the collar region of the stump. A rectangular patch of the outerbark hearing the bud was removed from scion-wood, An equal sized bark was removed from the collar region of the stump, and bark with the bud from the scion-wood was fixed there and tied with polythene tape. The upper cut-end of the stump was covered with wax. Grafted stumps were then labelled and planted in polythene containers (25×15 cm) filled with sieved soil. The containers were kept under shade or a thatch. Unwanted sprouts arising directly from the stump, other than the affixed bud, were removed. Successful grafts sprouted in 10~20 days giving out first pair of leaves. Established buldlings were kept in the nursery
throughout the summer.

Planting of grafts in the orchard site and planted in previously prepared pits of size 50×50×50 cm. Location of orchards Forest Circles of Kerala With the onset of monsoon showers the grafts were transferred to the orchard Three representative site were located in Southern, Central and Northern was implemented by Kerala Forest Research Institute and another one was implemented by Kerala Forest Department at Kulathupuzha in Southern region. Lay out design of the grafted ramets were planted in the orchard site in a randomized polycross design. This is to ensure maximum degree of intercrossing among the assembled plus tree clones and reduce inbreeding between ramets belonging to the same clone. Spacing in all the orchards an espacement of 8×8 m (quincuncial) was adopted. Such wider espacement is meant to ensure open sun-light condition which is essential for good flowering and seed production. Isolation of all the four orchards is isolated by more than 200 meters from the nearest teak stands.

5 Studies on genetic diversity

Genetic diversity forms the base of biodiversity hierarchy (Namkoong et al., 1996) and it serves as building blocks in future selection and breeding (FAO, 1989). In recent years, biochemical and molecular markers are widely used to study the extent and pattern of genetic variation in tree species. A few studies have been conducted to estimate genetic diversity and outcrossing rates in selected populations from natural and cultivated range in teak using isozyme and Random Amplified Polymorphic DNA (RAPD) markers (Changtragoon and Szmidt, 2000; Nicodemus et al., 2005; Lowe et al., 2005). However, genetic diversity information of teak in clonal seed orchard (CSO) has not been reported.

AFLP marker study on genetic variation in a clonal seed orchard established in 1985 by Kerala Forest Department at Kulathupuzha in Kollam district of Southern Kerala, India consisting of 1 200 trees of 31 clones in the orchard, planted in 8×8 m spacing. These clones were raised from 31 plus trees selected from natural teak forests as well as plantations raised in main teak growing forest divisions of Kerala. Clones were out planted in clonal seed orchard in randomized polycross design. Phenological events and seed setting of each clone were taken. Fruits collected from each clone were dried and cleaned by removing calyx and other debris. Fruits were subjected to pre-sowing treatment of alternate wetting and drying for seven days. Immediately after pre-sowing treatment, fruits were sown in germination trays filled with vermiculate. Each tray contained the fruits from each clone. Regular watering was provided and daily germination count was recorded.

DNA was extracted from 0.5 to 1 g of fresh leaf tissues using the modified CTAB procedure (Doyle and Doyle, 1990) and AFLP method was carried out following the standard protocol of (Vos et al., 1995). The reactions were carried out according to the manufacturer’s protocol (AFLP® Analysis System 1 and Starter Primer Kit; Invitrogen Life Technologies, Inc.,USA). AFLP® DNA ladder of size 30~300 bp (Invitrogen Life Technologies, Inc., USA) was run on either side of the denaturing gel as the standard marker. The ladder was labeled with γ-p32 ATP using T4 polynucleotide kinase according to the manufacturer’s protocol. The autoradiogram developed was scanned using HP Scanjet 3 770 digital flatbed scanner and transferred each scanned autoradiogram to Kodak Digital Science 1D Image Analysis Software. These bands were scored manually as ‘1’ for the presence of loci and ‘0’ for the absence of loci. Both polymorphic and monomorphic bands were included in the final data sets forming a binary matrix.

The total number of DNA bands formed from 31 clones was 653 of which 651 were polymorphic (99.69%). At the population level, i.e. considering clones from the same location of origin as separate group analysis was done using POPGENE 1.32 version software. The polymorphism varied from 71.67 (Arienkavu) to 86.37 percent (Nilambur). Gene diversity index (H) varied from 0.2007 (Arienkavu) to 0.2208 (Nilambur). The mean total genetic diversity (HT) was 0.2274 and the relative magnitude of genetic differentiation among population (GST) was 0.0783 indicating that 7.83 per cent of the total
diversity was between the populations in the orchard while rest 92.17 per cent of total variations occurred within the clones. The genetic distance varied from 0.012 0 (between Nilambur and Konni) to 0.025 1 (between Konni and Areinkavu). An unweighted pair group method with arithmetic means (UPGMA) dendrogram was constructed to represent the genetic distances between groups of clones. One main cluster consisted of three populations, of which Nilambur and Konni origin trees formed one sub cluster which in turn was linked to Arienkavu trees. The total genetic diversity among the clones in the clonal seed orchard was found to be 0.228 2 with 99.69 per cent of polymorphism. The cluster analysis based on genetic similarity coefficients of all combinations of the thirty one clones using NTSYSpc 2.1 software generated a unique dendrogram with six clusters. Bootstrap values are provided at the corresponding node for each cluster. We were unable to trace out the exact origin of seeds used to raise those plantations as there was no record available. The dendrogram constructed with genetic similarity coefficient data did not yield a firm pattern with respect to the geographic location of the mother trees. The PCoA analysis of clones were scattered along the coordinate axes thus, indicating error in labeling, or mix up of stumps or suppression of scion by root stock. A co-phenetic correlation value (r=0.73) with bootstrap confidence values ranging from 3.2 to 99.8 per cent for the clones in defined clusters provided an additional support for this labeling errors of clones in the orchard. Out of 578 ramets, there were 51 flowering ramets and 35 seed setting ramets. The percentage of flowering was 8.8 and seed setting was 6 per cent respectively. Clone 11 and clone 31 gave germination percentage of 6.6 and 3.3 respectively. The results on seed germination further showed that seeds from only two clones with clone number (clone 11 and clone 31) out of thirty five seed setting trees from 20 clones were able to germinate. But in the present study a positive correlation is observed between genetic distance and morphology, negative correlation between dissimilarity based on the relation of two morphological characters (height of tree and Gbh) of clones observed in the orchard. The relation between genetic distance and phenology were negative, dissimilarity to phenology were positive. In the CSO at Kulathupuzha, flowering (8.8%) of trees from 27 clones and fruiting (6%) of trees from 20 clones were comparatively very low. All flowered clones were unable to produce seeds. Close observation of flowering patterns within each clone revealed that there were some odd performers with respect to flowering and fruiting behavior of teak. From the present study, it can be suggested that while selecting clones for future seed orchard establishment, it may be important to select genetically diverse clones within a broad provenance region, such that clones show profuse flowering and seed set. These attributes have to be ensured while selecting candidate plus trees or plus trees from which the clones will be developed. Clone and ramet number should be labeled properly during future clonal seed orchard establishment. Information on exact origin of the ortet and data on flowering, seed setting, seed viability, germination of the ortet should be considered as essential requirement for selecting a clone for establishing new CSO. The genetic diversity among clones of Nilambur origin was higher than that among teak genotypes in Nilambur natural forests and SPAs.

Hence, the genetic diversity factor is inadequate to explain the poor performance of seed orchards with respect to seed production and viability. Inbreeding (sexual reproduction among closely related individuals) leads to homozygosity at key gene loci. Undersized populations of different species (that are not separated well or are constrained by uneven landscape, due to the expression of unfavorable or deleterious alleles) were particularly vulnerable to inbreeding depression due to unexchange of genes with other populations. This results in low heterozygosity leading to considerable reduction in the survival and reproduction rate of organisms. The organism neither has the potential to foresee the future nor can be optimally personalized for all ecological conditions. But the current genetic composition of a species controls how well its associates will adapt to future environment. Thus, genetic variation plays a key role in all populations and species to endure over evolutionary time through altering environments.

When structuring a breeding program a breeder needs to judge both short and long term objectives. Short
term objectives usually include obtaining significant gains in present characters of interest in the first few generations of breeding while maintaining well-adapted trees. Long term goal include the preservation of low frequency alleles and control of inbreeding. A major conflict arises between short and long term principle. Selection strength must be high to obtain significant genetic gains; yet maintaining uncommon alleles, requires keeping a large breeding population in successive generations. However, there are traditions to structure the breeding population and make selections to reduce this conflict. The gene resource population represents all of the accessible genetic variation that could contribute to the breeding population. This includes indigenous stands, provenance trials, seed orchard parents, progeny in progeny tests, and operational plantations. Breeding population at next level must have adequate genetic variation to maintain genetic gain for many generations. It has a propensity to be more improved than the gene resource population. At the top is the production population, consisting of seed orchard candidates or clones used for operational deployment. These selections are the best selections from the breeding population and provide diversity and genetic increase to operational plantations.

There are different types of gene resource populations, traditionally categorized as either in situ or ex-situ. In situ conserving techniques involve on-site conservation of genetic resources in native habitats; while ex situ techniques involve storing genetic resources in special collections such as seed banks, progeny or provenance tests, and seed orchards. Both in situ and ex situ management are important in maintaining genetic diversity for a breeding program. They vary in effectiveness depending on objective, origin, species, intensity of management, size of a population, etc. One important measure of efficiency is whether a group has a power over the particular gene resource population. Clonal Seed Orchards are long term populations; one needs some control over these populations to ensure they will be obtainable in the future.

**Authors Contribution**

P.M. Sreekanth made substantial contributions to conception and design, or acquisition of data, or statistical analysis and interpretation of data; have been involved in drafting the manuscript or revising it critically for important intellectual content; and have given final approval of the version to be published. M Balasundaran made acquisition of funding, collection of data, design of study and general supervision of the research group and analysis and interpretation of data.

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