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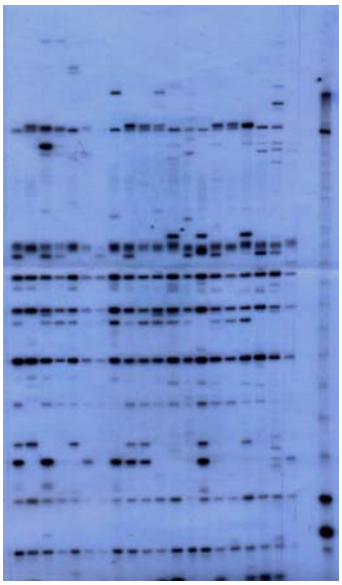

Forest Genetics and Biotechnology Division
Kerala Forest Research Institute
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(Final Report of the Project KFRI 381/02

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Abstract of the Project Proposal

1. Project No.	: KFRI 381/02
2. Title	: Studies on Genetic Diversity of Teak Using AFLP Markers
3. Principal investigator	: Dr. M. Balasundaran, Scientist, KFRI
4. Associate investigators	: Dr. E.P Indira, Scientist, KFRI Dr. P.A. Nazeem (Professor, Kerala Agricultural University)
5. Research Fellows	: Dr. T.B. Suma (Research Associate) : Mr. P.M. Sreekanth (Junior Research Fellow)

6. Objectives :

i. To estimate the genetic diversity in natural teak populations and teak provenances of the Western Ghats region through AFLP technique.

ii. To estimate the genetic variation existing in teak seed stands located in different parts of Kerala

iii. To estimate the genetic variation existing in teak clones being used for raising clonal teak plantation and clonal seed orchards

8. Duration	: 3 Years
9. Funding Agency	: Department of Biotechnology, Government of India

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CONTENTS

ABSTRACT

One hundred and eighty genotypes from 9 teak populations of natural forests (20 trees from each population) of the Western Ghats were evaluated for genetic diversity. The teak populations selected for the study belonged to forest divisions of Konni, Peechi-Vazhani Wildlife Sanctuary, Parambikulam Wildlife Sanctuary, Nilambur South, and Wayanad Wildlife Sanctuary from Kerala State, natural forests of Shimoga, Virnoli and Barchi of Karnataka State and one population from Indira Gandhi Wildlife Sanctuary from Tamil Nadu State. Out of 64 AFLP primer combinations screened, ten best primer combinations were used for the study. Sample collection, DNA extraction, pre-selective amplification, data analysis and report preparation were done at Molecular Biology laboratory of KFRI while the selective amplification reaction of the AFLP protocol and PAGE separation of the amplified products were done at CPBMB laboratory and Radiotracer laboratory of KAU.

Of the nine natural populations, Konni and Wayanad populations had the highest genetic diversity as measured from per cent of polymorphic loci and gene diversity index. Gene diversity (h) varied from 0.139 (Barchi) to 0.244 (Konni and Wayanad). Kerala and Tamil Nadu populations showed higher genetic diversity than Karnataka populations. The gene diversity indices of the nine populations showed higher genetic diversity values for populations from protected forests than populations from territorial forests. Cluster analysis done using genetic similarity coefficients between the populations showed grouping of nine populations into two main clusters; the Karnataka populations (Shimoga, Barchi and Virnoli) forming a single cluster. The Kerala populations (*viz*. Konni, Peechi-Vazhani Wildlife Sanctuary, Parambikulam Wildlife Sanctuary, and Wayanad Wildlife Sanctuary) and Indira Gandhi Wildlife Sanctuary from Tamil Nadu formed a separate cluster. The Nilambur natural forest population stood out separately but joined the cluster of Kerala populations indicating uniqueness of Nilambur populations among the natural teak of Kerala.

Eighty genotypes from 4 Seed Production Areas (SPAs) (20 teak trees from each SPA) *viz.* Konni, Parambikulam, Nilambur and Wayanad were selected for studying the genetic diversity in SPAs. The genetic diversity of the SPAs varied from 0.169 (Konni

SPA) to 0.203 (Wayanad SPA). The percentage of polymorphic loci ranged from 74.42 (Parambikulam SPA) to 84.06 (Konni SPA). The genetic similarity coefficients and clustering of 80 genotypes in the NTSYSpc-generated dendrogram indicated a pattern in accordance with the origin and geographic location of SPAs but with small discrepancies. All the 20 samples from each of Konni and Parambikulam SPAs clustered into respective groups clearly justifying their respective geographic origin. However, five genotypes from Nilambur namely N2, N3, N9, N12 and N14 and one Wayanad genotype W5 intruded into the Konni cluster besides the 20 genotypes of Konni origin. This indicated that these trees might have their origin in Konni and they might have reached Nilambur and Wayanad plantation as stumps for planting or there could be mixing of Konni seeds into Nilambur and Wayanad seed lots.

The study of the genetic diversity of teak Clonal Seed Orchard (CSO) was carried out in a 21-year-old CSO established in 1985 by the Kerala Forest Department at Kalluvettankuzhi (8.58ha) in Thenmalai Forest Division in Kollam District of Southern Kerala. Clones of Nilambur origin showed highest genetic diversity (0.2208) followed by Konni (0.2074) and Arienkavu (0.2007). The dendrogram generated by cluster analysis showed that most of the genotypes clustered in accordance with their geographic origin. However, clustering of a few genotypes of different geographic origin in one cluster indicated possible error in clone labeling, or mix up of stumps or suppression of scion by root stock growth. In the CSO, flowering (8.8%) and fruiting (6%) were very low. All flowered clones were unable to produce seeds. 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.

1. INTRODUCTION

1.1. TEAK

Teak (*Tectona grandis* L. f.) which belongs to the family Verbenaceae, is predominantly tropical and subtropical in distribution. It grows in regions having annual rainfall of 600mm to 3000mm and grows up to 1300m altitude. It reaches its best development on deep well drained foot hills with an annual rainfall of 1200 to 2000mm and a pronounced dry season. Teak occurs in natural forest between 9^0 to 26^0 North latitude and 73^0 to 104^0 East longitudes, which includes southern and central India, Myanmar, Laos Peoples Democratic Republic, and Northern Thailand. In Indonesia, teak is grown in extensive areas. Demand for quality timber has made teak the most widely planted hardwood species even in areas outside its natural distribution.

1.2. DEPLETION OF TEAK FORESTS

Teak genetic resources have been drastically disturbed during the past 50-100 years because of uncontrolled logging and mixing of germplasm. Habitat destruction and fragmentation have restricted the distribution of species to small and isolated populations. Although, detailed studies on the distribution of genetic variability in teak are limited, considerable variation in quantitatively inheritable traits have been reported in provenances from natural populations of India, Thailand and Laos (Kjaer, 1996).

1.3. GENETIC IMPROVEMENT OF TEAK

Teak has a long generation interval and hence prior information on genetic diversity can hasten the progress of implementing conservation measures and tree breeding programmes. Teak improvement is constrained by low genetic variability of genotypes, delayed and inadequate flowering, asynchrony in flowering phenology, low fruit setting, limited seed production, poor seed germination, variability in growth and wood quality, prolonged time requirement for multi-year progeny test, etc.

1.3.1. Seed production area and clonal seed orchards

In order to meet the annual planting requirement, seed production areas (SPAs) are developed by culling inferior trees from even- aged high quality plantations and retaining sufficient number of superior and healthy trees with more than average seed production (Zobel and Talbert, 1984). One of the major reasons for low productivity may be poor quality planting stock (stump / root trainer seedlings) raised out of poor quality seeds collected from different SPAs. Prabhu (2007) also reported considerable variations in various tree and fruit parameters of different SPAs from Kerala. Low fruit production in teak clonal seed orchard was reported by Gunaga and Vasudeva (2005).

1.4. 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 in teak using isozyme and random amplified polymorphic DNA (RAPD) markers to estimate genetic diversity and outcrossing rates in selected populations from natural and cultivated range (Changtragoon and Szmidt, 2000; Nicodemus *et al*., 2005; Lowe et al., 2005). However, studies on genetic diversity of teak in clonal seed orchard (CSO) and seed production areas (SPs) have not been reported. Only a limited natural population from India, particularly in Kerala has been covered in these studies.

1.5. OBJECTIVES OF THE STUDY

The natural forests of the Western Ghats region, an important biodiversity hotspot of South India, is highly vulnerable for destruction. Teak is one of the species adversely affected by forest destructions and for which information on genetic diversity is urgently needed. Loss of natural teak population, due to illicit felling, forest encroachment, severe logging, diseases and pests, fragmentation, genetic drift and skewed gene flow have adverse effect on genetic diversity of the species.

The SPAs located in different climatic regions of Kerala show considerable amount of variations not only due to genetic factors but also due to edaphic factors, which greatly influence their performance. CSOs were planned and established for raising the production of high quality seeds from fast growing superior plantations. But these clones are not tested for genetic variability in order to avoid planting of genetically related clones for avoiding inbreeding in the clonal seed orchard. Among several other factors, the cause of poor seed quality is presumed to be narrow genetic base of the SPAs and the seed orchards. The present study is an attempt to evaluate the genetic variation in teak in the Southern part of the Western Ghats falling within the states of Kerala, Karnataka and Tamil Nadu.

The major objectives of the project are:

iv. To estimate the genetic diversity in natural teak populations and teak provenances of the Western Ghats region through AFLP technique.

v. To estimate the genetic variation existing in teak seed stands located in different parts of Kerala

vi. To estimate the genetic variation existing in teak clones being used for raising clonal teak plantation and clonal seed orchards

2. MATERIALS AND METHODS

2.1. SAMPLE COLLECTION FROM NATURAL FORESTS

Leaf samples were collected from nine natural teak growing forests of the Western Ghats belonging to Kerala, Karnataka and Tamil Nadu states (Fig.1 and Table 1). These locations are Kattathi natural reserve of Konni Forest Division in Pathanamthitta District, Thamaravellachal natural reserve of Peechi – Vazhani Wildlife Sanctuary in Thrissur District, Vengoli and Chettuara reserves in Parambikulam Wildlife Sanctuary in Palghat District, Padkka natural reserve in Nilambur Forest Division in Malappuram District and Tholpetty natural reserve of Wayanad Wildlife Sanctuary, all from Kerala state, Shimoga, Barchi and Virnoli natural reserves from Karnataka State and Pachathalisaraham natural reserve near Sarkarapathi power house in Indira Gandhi Wildlife Santuary from Tamil Nadu State. From each location, 20 trees were identified using transect method of sampling. Expanding leaves were collected from the trees for extracting DNA.

2.1.1. DNA extraction

DNA was isolated from 300 mg juvenile leaf samples following modified CTAB protocol (Doyle and Doyle, 1990). The concentration of DNA in the aqueous solution was estimated at A_{260} (1OD=50 μg ml^{-1}) (Gallagher, 1996) and subjected to AFLP analysis.

2.1.2. AFLP analysis

AFLP analysis was performed as described by Vos *et al*. (1995). AFLP reagents were purchased from M/s. Invitrogen Corporation, USA, and the reactions were carried out according to manufacturer's protocol. The pre-amplification reaction of the AFLP protocol was carried out in Molecular Biology laboratory of Kerala Forest Research Institute while selective amplification reaction was done at the Centre for Plant Biotechnology, Molecular Biology and Radiotracer Laboratory of Kerala Agricultural University, Thrissur. Selective amplification was done with ten primer combinations: E-AGG/M-CTT, E-AAC/M-CTG, E-AAC/M-CTT, E-ACT/M-CAG, E-ACC/M-CTT, E-ACA/M-CTT, E-ACC/M-CTA, E-

AGG/M-CAG, E-AGG/M-CTG and E-AGG/M-CAT selected out of sixty-four possible combinations.

Table. 1. Details of natural teak populations from Western Ghats sampled for AFLP analysis.

Sl.No.	Name of geographic area or locality	Forest Division	State	Latitude (N)	Longitude (E)
1	Konni	Konni Division	Kerala	09° 55′	76° 67′
2	Thrissur	Peechi- Vazhani Wild life Sanctury	Kerala	10° 26′	76° 58′
3	Parambikulam	Parambikulam Wild life Sanctury	Kerala	10° 25′	76° 45′
4	Nilambur	Nilambur South Division	Kerala	11° 80′	76° 10′
5	Wayanad	Wayanad Wild life Sanctury	Kerala	11° 02′	76° 41′
6	Pollachi	Indira Gandhi Wildlife Sanctury (IGWLS)	Tamil Nadu	10° 35′	76° 52′
7	Shimoga	Shimoga Division	Karnataka	13° 55′	75° 38′
8	Barchi (Dandeli)	Haliyal Division	Karnataka	15° 17′	74° 38′
9	Virnoli	Haliyal Division	Karnataka	15° 43′	74° 73′

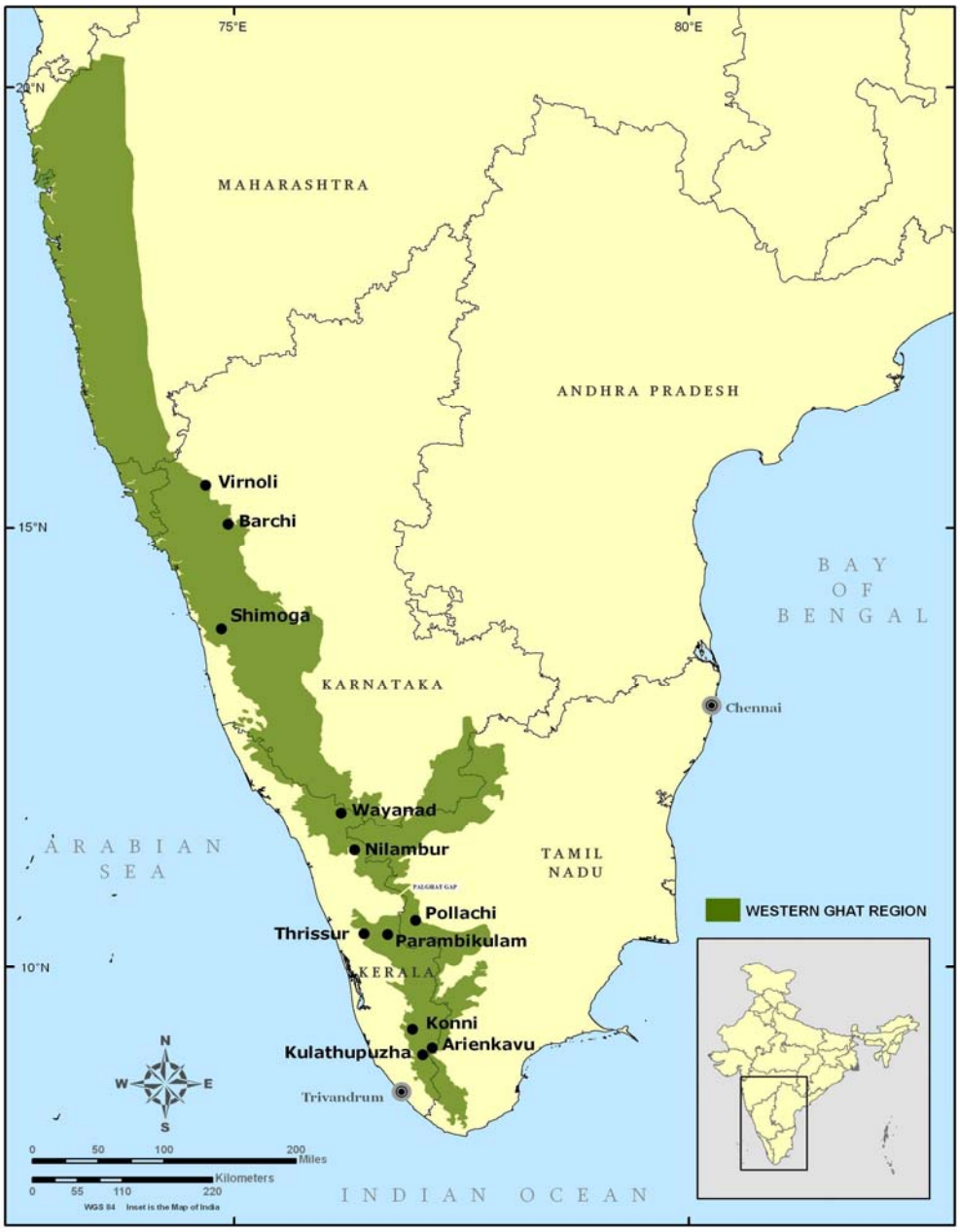

Fig. 1. Map showing the locations of natural populations, seed production areas and clonal seed orchards of teak in the Western Ghats region selected for AFLP analysis

2.1.3. Separation and visualization of amplified products

After PCR amplification, the amplified products were electrophoresced on 6% Polyacrylamide gel with 0.4mm spacers and shark tooth combs in a Sequi – Gen GT Nucleic Acid electrophoresis cell (Biorad, USA). The gel was transferred to chromatographic paper,

and exposed the gel to X–ray film (Kodak®) for overnight. Manual processing of X-ray film was done at the dark room in infra red light.

2.1.4. Data analysis

PCR products as visualized on the film were scored manually as '1' for the presence of band (DNA band) and '0' for absence of band. Each PCR product was assumed to represent a single locus. Both, polymorphic and monomorphic bands were included in the final data sets forming a binary matrix.

The data matrices were grouped into nine populations and analyzed using POPGENE, Version 1.32 package and a pair-wise comparison of population was made (Yeh *et al*., 1999). The genetic diversity parameters within population viz., gene diversity (h) and percent of polymorphic loci (ppl), and pair-wise comparison of genetic distances between populations were estimated. The pair-wise genetic distances obtained was subjected to clustering using Unweighted Pair Group Method with Arithmetic means (UPGMA).

AFLP binary matrix of 180 individual trees was subjected to population structuring. A matrix of genetic distance between individual genotypes of each population based on shared amplification products was calculated. This was also used to construct a UPGMA dendrogram.

2.2. GENETIC DIVERSITY IN SEED PRODUCTION AREAS

Four younger SPAs located in four important geographic areas of teak plantations namely Konni, Parambikulam, Nilambur and Wayanad were selected for the study (Table 2). From each SPA, 20 trees were selected at random using transect method. From these trees, expanding leaves were collected for DNA extraction. The methods and protocols for DNA extraction, AFLP reactions, electrophoretic separation and visualization of amplified products, and data analysis were same as those described for natural forests.

Table 2. List of Seed Production Areas (SPAs) selected for AFLP analysis.

Forest division	Locality	Forest Section	Year of Plantation	Area (ha)	Latitude (N)	Longitude (E)
Nilambur	Kombankallu	Edacode	1955	10	11° 18′	76° 46′
Wayanad North	Tholpetty	Kaimaram	1963	10.63	11° 47′	76° 84′
Parambikulam Wildlife Sanctuary	Thoonakadavu	Anappady	1955	27	10° 62′	76° 55′
Konni	Kondodi	Karippanthode	1965	16.96	09° 42′	76° 55′

2.3. GENETIC DIVERSITY IN CLONAL SEED ORCHARD

AFLP analysis was carried out in a 21-year-old clonal seed orchard of 8.58 ha area established in 1985 by Kerala Forest Department at Kulathupuzha, (Kalluvettankuzhi) in Thenmalai Forest Division in Kollam District of Southern Kerala. There were 1200 trees of 31 clones in the orchard, planted in 8m X 8m spacing. These clones were raised from 31 plus trees selected from natural teak forests and plantations raised in main teak growing forest Divisions of Kerala. Of these, 15 clones originated from Nilambur, 9 clones from Konni and 7 clones from Arienkavu under Thenmalai Forest Division in Kollam District. The clones were raised by grafting buds from bud wood cuttings of healthy branches of plus trees to one-year-old teak stumps. Successful grafts were outplanted in clonal seed orchard in randomized design and details of individual clones and its origin are given in Table 3.

2.3.1. Sample collection, phenology, seed setting and germination parameters

To study the genetic variation of clones in the clonal seed orchard, we selected half the portion of layout comprising 578 healthy trees (ramets of 31 clones) and left out rest of the layout due to damage and stunted nature of the trees. The selected area included all the 31 clones repeated as per randomized design. Juvenile expanded leaves of 31 clones were randomly collected from this plot for DNA extraction. Observations on phenological events and seed setting of each clone were recorded. Fruits collected from each clone were dried and cleaned by removing calyx and other debris. Immediately after pre- sowing treatment, fruits were sown in germination trays filled with vermiculite.

The methods and protocols for DNA extraction, AFLP analysis, separation and visualization of amplified products adopted for genetic diversity studies of nine natural teak populations of the Western Ghats were followed for studies on clonal seed orchard also.

Table 3. List of Clones planted in clonal seed orchard, Kulathupuzha (Kalluvettankuzhi).

SL.No.	Clone ID	Clone Number	Total number of ramets	Locality of origin
1	NIL 1	clone 1	17	Nilambur
2	NIL 2	clone 2	16	Nilambur
3	NIL 3	clone 3	16	Nilambur
4	NIL 4	clone 4	19	Nilambur
5	NIL 5	clone 5	13	Nilambur
6	NIL 6	clone 6	14	Nilambur
7	NIL 7	clone 7	19	Nilambur
8	NIL 8	clone 8	14	Nilambur
9	NIL 9	clone 9	17	Nilambur
10	NIL 10	clone 10	15	Nilambur
11	NIL 11	clone 11	20	Nilambur
12	NIL 12	clone 12	18	Nilambur
13	NIL 13	clone 13	18	Nilambur
14	KON 14	clone 14	21	Konni

15	KON 15	clone 15	20	Konni
16	KON 16	clone 16	23	Konni
17	KON 17	clone 17	22	Konni
18	KON 18	clone 18	19	Konni
19	KON 19	clone 19	18	Konni
20	KON 20	clone 20	19	Konni
21	KON 21	clone 21	25	Konni
22	KON 23	clone 23	21	Konni
23	NIL 24	clone 24	22	Nilambur
24	NIL 25	clone 25	18	Nilambur
25	ARK 26	clone 26	18	Arienkavu
26	ARK 27	clone 27	23	Arienkavu
27	ARK 28	clone 28	19	Arienkavu
28	ARK 29	clone 29	20	Arienkavu
29	ARK 30	clone 30	19	Arienkavu
30	ARK 31	clone 31	17	Arienkavu
31	ARK 32	clone 32	18	Arienkavu

Each DNA band was treated as separate putative locus and scored as "1" for the presence of locus and "0" for the absence of locus in each clone to create binary data matrices. Genetic diversity within clonal populations of different geographic origin namely Konni, Areinkavu and Nilambur and genetic distance among clonal populations were estimated. Genetic diversity was quantified as the percentage of polymorphic loci and Nei's gene diversity (Nei, 1973) assuming Hardy-Weinberg equilibrium. Genetic distance between the clonal populations of distinct origin of mother trees were obtained from POPGENE software and the resultant distance matrices were used to construct a UPGMA dendrogram.

The genetic similarities among the thirty-one clones were calculated using NTSYSpc 2.02 software. The resultant similarity matrix was subjected to cluster analysis. Genetic similarities based on simple matching coefficient (Sokal and Sneath, 1963) were calculated between all possible pairs of clones.

3. RESULTS AND DISCUSSION

Teak trees in the Western Ghats forests have been depleted considerably from the early British period due to illicit felling, repeated fires, excessive grazing and enormous soil erosion caused by torrential rain (Prabhu, 2007). Earlier genetic studies on natural teak populations of the Western Ghats had been mainly confined to Kerala portion only. The present study using AFLP analysis provided considerable information on the magnitude and pattern of genetic variation existing in nine natural teak populations from the Western Ghat forests covering three different States viz. Kerala, Karnataka and Tamil Nadu.

3.1. GENETIC DIVERSITY IN NATURAL POPULATIONS

DNA was obtained from leaf samples collected from all the 180 genotypes (9 populations X 20 trees) which originated from nine natural teak populations of the Western Ghats located in the three states of Kerala, Karnataka and Tamil Nadu. The AFLP analysis of the 180 DNA samples, using ten primer combinations involved 1800 reactions. The average number of DNA bands per AFLP fingerprint of a tree per primer pair combination was 67. A representative AFLP autoradiogram samples from Peechi – Vazhani WLS is provided in Fig. 2.

At the population level, polymorphism varied from 56.84 per cent (Virnoli) to 87.97 per cent (Konni) (Table 4). Similarly Nei's (1973) gene diversity index (h) varied from 0.1387 (Barchi) to 0.2449 (Konni). The gene diversity and per cent of polymorphic loci for Konni and Wayanad were almost similar and these two Kerala populations showed the highest genetic diversity. Karnataka populations showed the lowest genetic diversity index varying from 0.1387 (Barchi) to 0.1605 (Shimoga) and per cent of polymorphic loci ranging from 56.84 (Virnoli) to 68.42 (Shimoga).

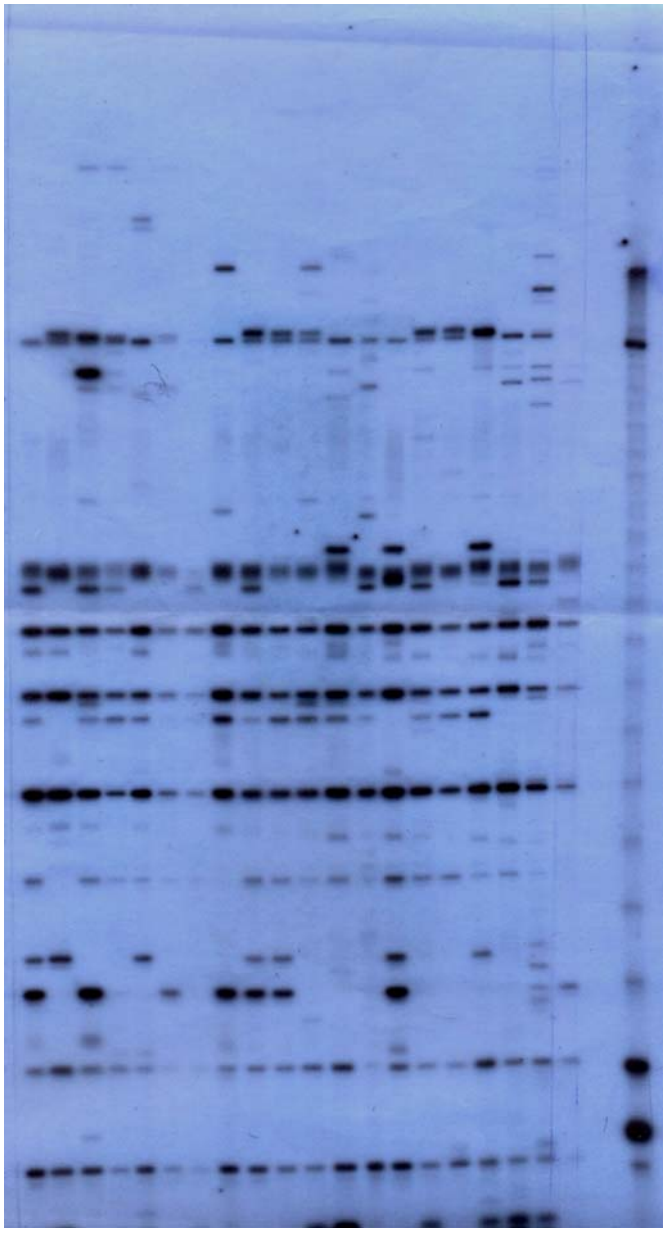

Fig. 2. AFLP profile of twenty natural teak trees from Peechi-Vazhani WLS; DNA amplified using primer combination E-ACT + M-CAG; M: DNA marker 30-330 bp AFLP ladder (Invitrogen Life Technologies, USA); 1-20: tree numbers.

Table 4. Comparison of nine natural populations of teak for various genetic diversity measures.

Teak Populations	Nei's (1973) gene diversity index (h)	Per cent of polymorphic loci (ppl)
Konni RF	0.2443	87.97
P-V WLS, Thrissur	0.2334	81.50
Parambikulam WLS	0.2336	81.35
Nilambur RF	0.1980	75.49
Wayanad WLS	0.2449	86.77
Indira Gandhi Wildlife Sanctuary (IGWLS) Tamil Nadu	0.1980	73.98
Shimoga NF	0.1605	68.42
Barchi NF	0.1387	64.06
Virnoli NF	0.1428	56.84

The standard genetic distances (D) between all pair-wise population comparisons varied from 0.0554 (between Barchi and Virnoli) to 0.1166 (between Tamil Nadu and Barchi). The UPGMA dendrogram showed the genetic relationship between the populations. The dendrogram showed two main clusters with clear separation (Fig. 3). The larger cluster comprised of all the Kerala populations (viz. Konni, Thrissur, Parambikulam, Wayanad and Nilambur) along with Tamil Nadu population (IGWLS) while the second cluster consisted of Karnataka populations viz., Shimoga, Barchi and Virnoli natural forests.

The present study showed that the overall gene diversity of nine teak populations were comparable with mean values obtained in outcrossing woody perennials studied through

RAPD markers. But these estimates are slightly lower than values reported for teak populations screened in earlier studies using isozyme and RAPD markers (Changtragoon and Szmidt, 2000; Nicodemus *et al*., 2005).

In Kerala, gene diversity was higher in protected forests such as wildlife sanctuaries than in territorial forests except Konni natural forest. This might be due to less human disturbances in wildlife sanctuaries when compared to that of other natural forests. Karnataka populations viz., Shimoga, Barchi and Virnoli showed the lowest gene diversity. These populations might have undergone widespread logging, illicit felling, augmentation planting or severe fragmentation of the natural teak forest during the past.

3.1.1.Genetic distance measures between populations

The nine populations selected for the study covered a broad geographic range differing in rainfall and altitude. Teak populations from Virnoli and Barchi of Karnataka State were genetically and geographically closer than any other pairs of teak populations. These two populations showed the lowest genetic distance and they formed a single cluster to which the third population from Karnataka, Shimoga area joined. However, a complete correlation between genetic distance and geographic distance between populations was not seen. Even though teak populations from IGWLS of Tamil Nadu and Barchi of Karnataka showed the highest genetic distance coefficients, these populations were not farthest apart. But, in general, there is a relation between genetic distance and geographic distance.

The Kerala populations viz., Konni, Thrissur, Parambikulam Wayanad and Tamil Nadu (Pollachi) made a separate cluster with its own sub clusters with Nilambur standing out separately from all these populations. The uniqueness of Nilambur teak with respect to growth and wood quality is world famous. The Malabar teak (Nilambur, Kerala) from the Western Ghat region in India, generally displaying good growth and log dimensions with desired wood figure (golden yellowish brown colour), has a wide reputation in the world trade for ship-building (Bhat and Priya, 2004). Nilambur valley is reported to have the most suitable alluvial soil and climatic condition required for good quality teak. The unique edaphic and climatic factors might have resulted in a different type of evolution in the natural teak populations.

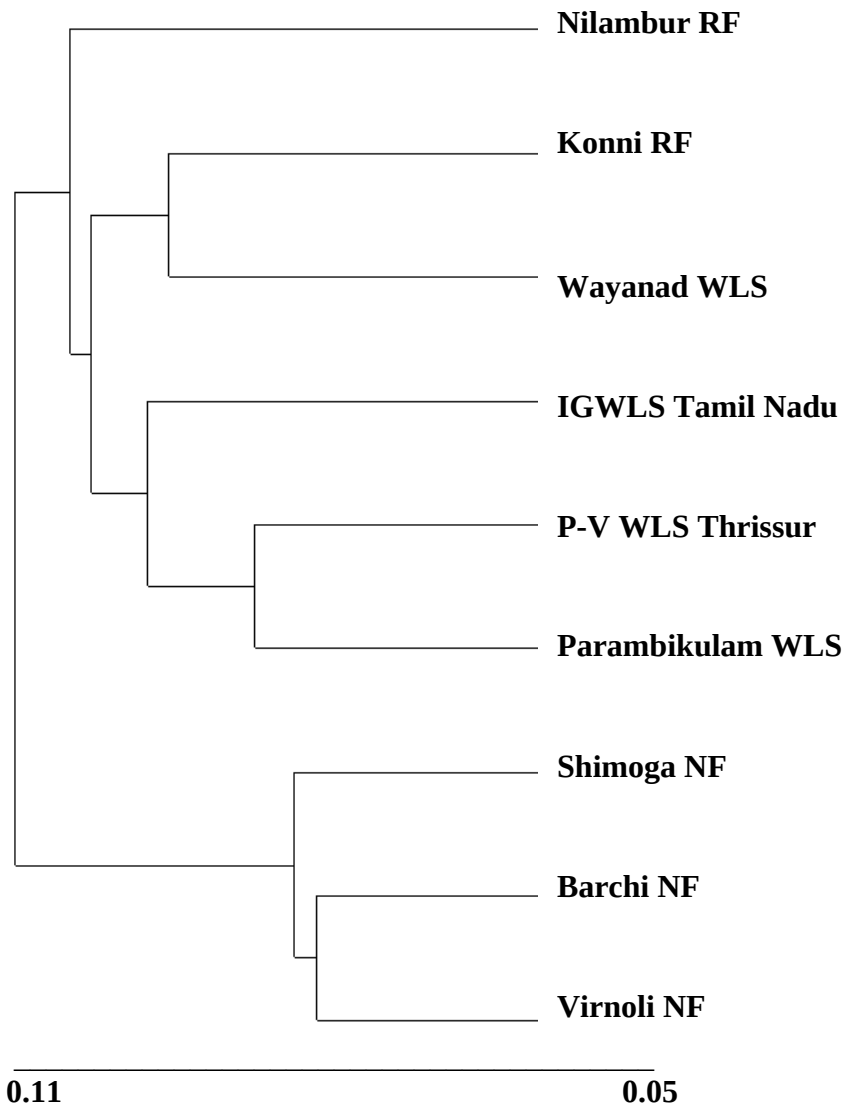

Fig.3. Dendrogram constructed based on Nei's (1978) genetic distance coefficients of nine natural populations of teak.

RF: Reserve Forests; WLS: Wildlife Sanctuary; IGWS: Indira Gandhi Wildlife Sanctuary; P-V WLS: Peechi – Vazhani Wildlife Sanctuary; NF: Natural Forests.

The genetic distance coefficients and the UPGMA dendrogram comprising 180 samples revealed the genetic relatedness of the 180 genotypes from the nine populations of the Western Ghats. All the 20 genotypes of the same geographic origin grouped themselves into the first primary cluster. Thus 9 primary clusters were formed for the nine populations showing genetic fidelity of the provenance. The Karnataka populations viz., Shimoga NF, Barchi NF and Virnoli NF formed a single secondary cluster indicating their genetic relatedness. The genetic separation was partly in agreement with geographic separation.

3.2. GENETIC DIVERSITY IN SEED PRODUCTION AREAS

DNA was obtained from leaf samples collected from the four SPAs (4 populations X 20 trees). AFLP analysis of the 80 DNA samples, using ten primer combinations involved eight hundred reactions. The average number of bands per AFLP primer pair combination was 60.

Six hundred and two DNA bands were scored for statistical analysis. Polymorphism was very pronounced, with 100 per cent polymorphic markers across all ten primers. The per cent of polymorphic loci (ppl) varied from 74.42 (Parambikulam SPA) to 84.06 (Konni SPA) (Table 5). The gene diversity (h) ranged between 0.1692 (Konni SPA) and 0.2034 (Wayanad SPA).

Table 5. Genetic diversity measures of seed production areas (SPAs).

Populations	Nei's (1973) gene diversity (h)	Per cent of polymorphic loci (ppl)
Wayanad SPA	0.2034	80.40
Nilambur SPA	0.1972	81.73
Parambikulam SPA WLS	0.1915	74.42
Konni SPA	0.1692	84.06
Total	0.2296 ±0.1418	100

The estimates of Nei's genetic distance (D) between populations were calculated for all pair-wise SPA comparisons. While Konni and Nilambur SPAs showed the least genetic distance (0.0389), Parambikulam and Nilambur SPAs showed the highest genetic distance (0.0881). UPGMA dendrogram displayed two main clusters with Nilambur and Konni SPAs in one cluster while Parambikulam and Wayanad formed another cluster.

Gene diversity (h) estimated for seed production plantations was generally poor ranging from 0.16-0.20 in the four SPAs from four geographic areas. The diversity values were lower than the diversity values for natural teak populations of the Western Ghats revealed in the present study. Also the values were poorer than those reported for teak plantations by Nicodemus *et al.* (2005). The exact reason for low diversity could not be identified. No information was available from the forest offices regarding the origin of seed lot used for raising the plantations. Records such as plantation Journal was also unavailable for reference. Probably plantations might have been raised using seeds of a couple of mother trees of narrow genetic base. There are about 32 teak SPAs in Kerala and only 4 SPAs have been subjected to genetic diversity estimation in the present study. Understanding the genetic diversity status of each SPAs will be useful in deciding strategies for management of SPAs and for ascertaining the quality of seeds for raising future plantations.

Considering the lower level of genetic diversity within the SPAs, for raising future plantations seeds have to be collected from as many trees as possible and bulked to maintain a broad genetic base preferably with in the same provenance.

3.2.1Cluster analysis individual genotypes

The cluster analysis comprising 80 trees from the four SPAs without grouping them into separate populations (of each SPA) revealed a unique dendrogram (Fig.4).

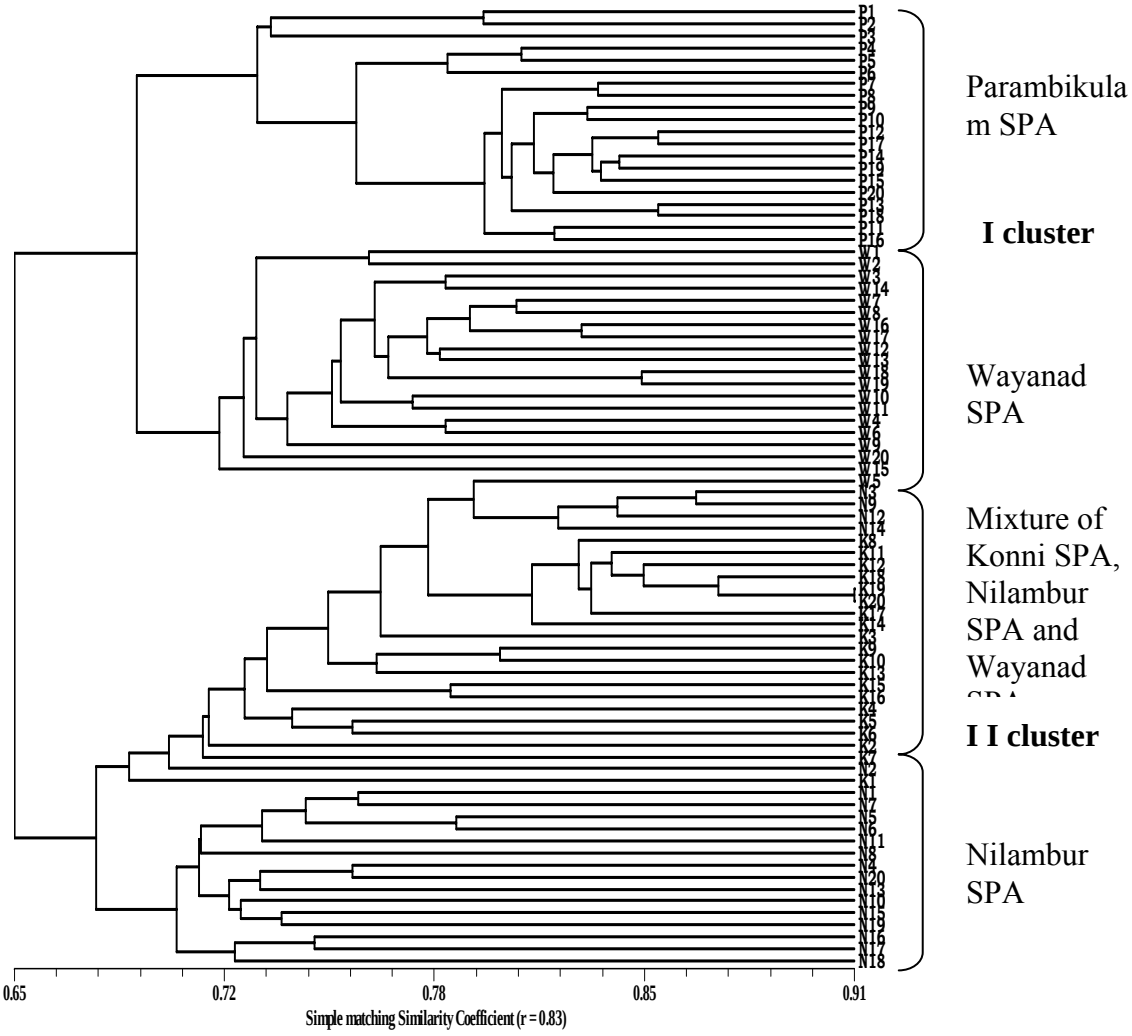

Fig. 4. UPGMA dendrogram of 80 genotypes of teak from four seed production areas. (K1 – K20 Konni SPA), (P1- P20 Parambikulam SPA), (N1 – N20 Nilambur SPA), (W1 – W2 Wayanad SPA).

The dendrogram divided 80 genotypes into two main clusters. The first cluster was shared between genotypes from Parambikulam and Wayanad. The SPAs formed their own sub clusters, the former with 20 genotypes and the latter with 19 genotypes. The second cluster was shared between Nilambur and Konni SPA genotypes but with mixing of genotypes from three geographic areas in one sub cluster. One of the cluster comprised of five genotypes from Nilambur (N2, N3, N9, N12, and N14) and 1 from Wayanad (W5), besides all the 20 genotypes from Konni. This showed that these five genotypes from Nilambur and one from Wayanad might have originated from Konni.

Generally the pattern of clustering of trees was in accordance with the origin and location of SPAs. However, the five genotypes from Nilambur and one Wayanad genotype intruded into the Konni cluster raising the number of trees in Konni cluster to 26. This indicated that these trees might have their actual origin in Konni. They might have reached Nilambur and Wayanad plantations respectively as stumps for planting or as mixed seeds. Transport of teak stumps from Konni to Nilambur and Wayanad could have been resorted when sufficient quantity of stumps were not available from Nilambur nursery for completing the planting work. Sometimes, stumps might have been brought from Konni for casualty replacement. Another probability is the mixing of seeds from Konni, Nilambur and Wayanad before sowing seeds for producing stumps.

3.3. GENETIC DIVERSITY IN CLONAL SEED ORCHARD

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 geographical location of origin as separate groups, the percentage of polymorphism varied from 71.67 per cent (Arienkavu) to 86.37 per cent (Nilambur). Gene diversity index (h) varied from 0.2007 (Areinkavu) to 0.2208 (Nilambur) (Table 6). The genetic distance varied from 0.0120 (between Nilambur and Konni) to 0.0251 (between Konni and Areinkavu).

3.3.1. Cluster analysis of individual clones

Clonal seed orchard is a plantation of vegetatively propagated genotypes or plantlets of plus trees, which are previously selected for their superiority from natural populations or plantations. The cluster analysis based on genetic distance coefficients of all combinations of the thirty one clones generated a unique dendrogram with six clusters (Fig. 5). The first cluster comprised NIL 1 and NIL 2, second of NIL 3, NIL 4, NIL 5, NIL 6, NIL 7, NIL 8, NIL 9 along with KON 18 and KON 20. Third cluster was formed by NIL 11, NIL 12 and KON 14, KON 17 and KON 21. Fourth cluster comprised KON 15, KON 23, KON 16 and KON 19. Fifth cluster was formed by NIL 10, ARK 32, NIL 24, NIL 25, ARK, 26, ARK 27, ARK 28. Sixth cluster was

Table 6. Comparison of genetic variation and origin of clones used for raising clonal orchard at Kulathupuzha (Kalluvettankuzhi).

Clonal population	Gene diversity index (h)	Per cent of polymorphic loci (ppl)
Nilambur	0.2208	86.37
Konni	0.2074	73.66
Arienkavu	0.2007	71.67
Overall	0.2274 ±0.0227	99.69

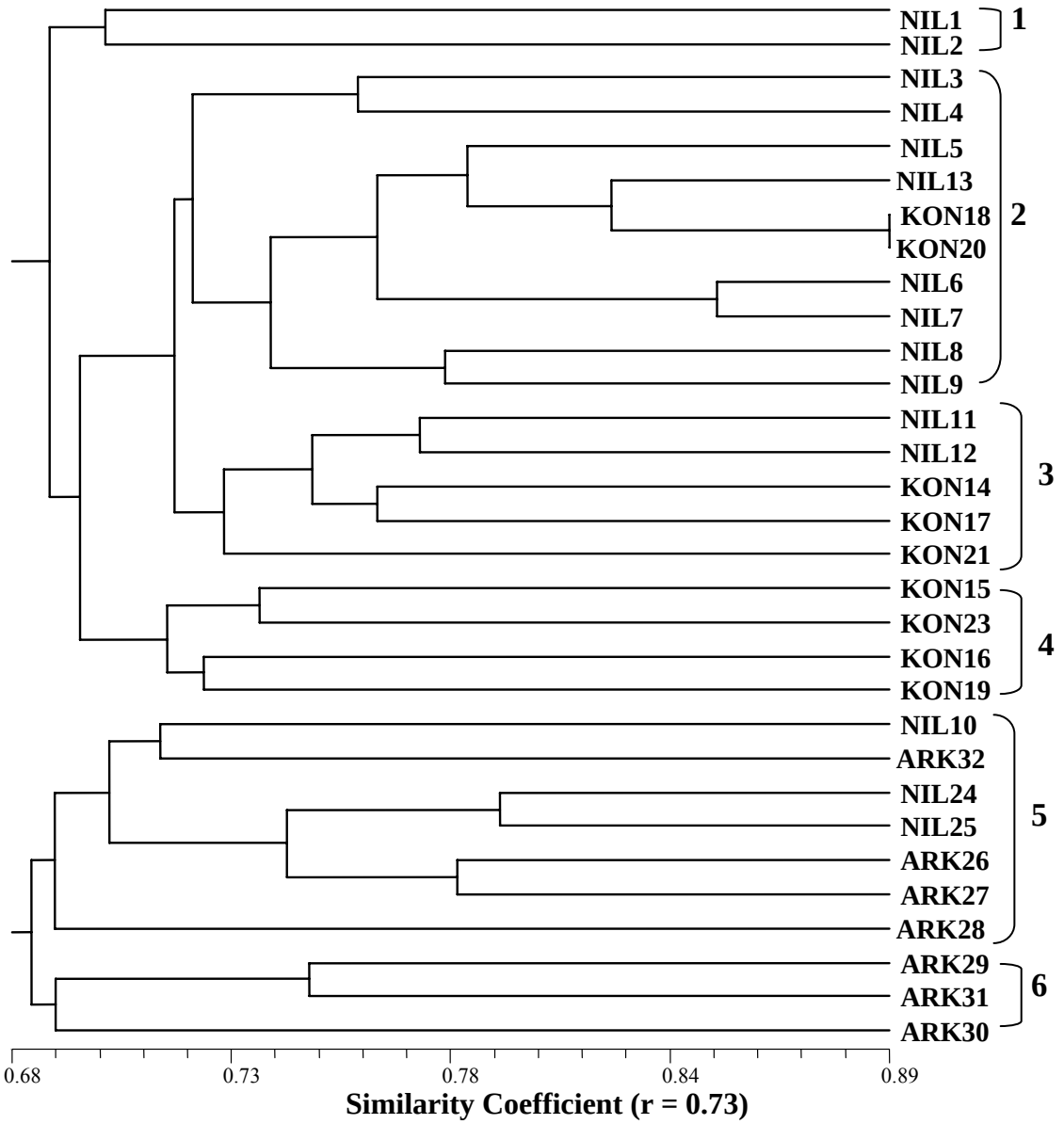

Fig. 5. UPGMA dendrogram of genetic similarity between thirty one clones using NTSYSpc software.

formed by ARK 29, ARK 31 and ARK 30. The pattern of clustering indicated that contrary to the general expectation, a few clones of different geographic origin had come under same cluster. For example the second major cluster comprised of eight clones from Nilambur and two clones of Konni. Such unexpected pattern indicated an error in identity of the clones. The error could be during the time of labeling of clones at some occasion during the period of grafting or planting or due to inadvertent mixing of ramets. The clones had originated from

plantations and not from natural forests (Venkatesh *et al*., 1986). Hence, transporting of teak genotypes from one place to another as seed or stump might have taken place at the time of plantation establishment as suspected for SPAs. Yet another chance is the destruction of scion and establishment and growth of root stock of unknown origin. Contrary to these confusions on genotype identity within SPAs and clones, the samples from natural forest had showed strict genetic fidelity with respect to origin.

3.3.2. Phenology, seed setting and germination parameters

The data on flowering and fruit setting of clones were recorded for 578 trees (ramets) of 31 clones in the orchard for one year. Out of 578 trees, there were 51 flowering trees and 35 seed setting trees. The percentage of flowering was 8.8 per cent and seed setting was 6 per cent showing very low percentage among the clones in the orchard. Out of 31 clones 27 clones were able to flower and 20 clones were able to produce seeds. Low percentage of germination of the collected seeds was observed. Seeds from only two clones were able to germinate. Clone 11 and clone 31 gave germination percentage of 6.6 and 3.3 respectively (Table 7).

Fruit set in teak is influenced by several factors such as genetic diversity of clones, presence and activity of pollinators, weather conditions, etc. The total genetic diversity among the clones in the clonal seed orchard was found to be 0.23. Nicodemus *et al*. (2005) estimated total genetic diversity of 0.3 in same species using RAPD markers in samples from natural forests and plantations of the Western Ghats and Central India. When compared to the genetic diversity of samples from natural forests, the genetic diversity of clonal seed orchard is not too much lower. However, the mixing of genotypes from different areas might have vitiated the estimation to some extent. But the decrease in genetic diversity is insufficient to account for the dismal performance of seed orchards with respect to seed production. Lack of flowering is seen as the major cause of poor seed set. Though we have monitored the flowering and seed setting for one year only, records indicated continued absence of flowering and seed setting in the seed orchards located at Kulathupuzha.

While selecting clones for future seed orchard establishment, it may be important to select clones within a broad provenance region, such that their flowering time could be

matched or CSOs should be established using genetically diverse clones showing profuse synchronized flowering and seed set, selected within a provenance. While raising seed orchards in future, ramet's number should be labeled properly and information on origin of plus trees, consistency of flowering, seed setting, seed viability and germination percentage, etc. are to be recorded properly.

Table 7. Flowering, fruit setting and seed germination in clonal seed orchard at Kalluvettankuzhi.

SL.No.	Clone ID	Clone Number	Total number of (trees) ramets observed	Number of trees flowered	Number of seed setting trees	Germination percentage
1	NIL 1	clone 1	17	5	3	nil
2	NIL 2	clone 2	16	2	2	nil
3	NIL 3	clone 3	16	3	3	nil
4	NIL 4	clone 4	19	2	nil	nil
5	NIL 5	clone 5	13	1	1	nil
6	NIL 6	clone 6	14	3	1	nil
7	NIL 7	clone 7	19	1	1	nil
8	NIL 8	clone 8	14	1	1	nil
9	NIL 9	clone 9	17	2	nil	nil
10	NIL 10	clone 10	15	nil	nil	nil
11	NIL 11	clone 11	20	2	1	6.6 %
12	NIL 12	clone 12	18	1	1	nil
13	NIL 13	clone 13	18	2	nil	nil
14	KON 14	clone 14	21	3	3	nil
15	KON 15	clone 15	20	1	1	nil
16	KON 16	clone 16	23	nil	nil	nil
17	KON 17	clone 17	22	2	1	nil
18	KON 18	clone 18	19	2	2	nil
19	KON 19	clone 19	18	2	1	nil
20	KON 20	clone 20	19	2	1	nil
21	KON 21	clone 21	25	nil	nil	nil
22	KON 23	clone 23	21	2	2	nil
23	NIL 24	clone 24	22	1	1	nil
24	NIL 25	clone 25	18	1	1	nil
25	ARK 26	clone 26	18	nil	nil	nil
26	ARK 27	clone 27	23	1	nil	nil
27	ARK 28	clone 28	19	1	nil	nil
28	ARK 29	clone 29	20	1	nil	nil
29	ARK 30	clone 30	19	1	nil	nil
30	ARK 31	clone 31	17	5	5	3.3 %
31	ARK 32	clone 32	18	3	3	nil
TOTAL		**31**	**578**	**51**	**35**	

4. CONCLUSIONS AND RECOMMENDATIONS

Natural teak population of Kerala and Tamil Nadu part of the Western Ghats in the Indira Gandhi Wildlife Sanctuary showed higher genetic diversity than the Karnataka populations. The lower genetic diversity of teak from Nialmbur natural forest compared to other areas of the Western Ghats of Kerala necessitates immediate attention for its conservation. Nilambur teak forests showed its separate genetic identity in cluster analysis. Considering the genetic divergence of Nilambur teak from other teak populations of Kerala and their unique superior timber quality reported in several other studies, mixing of genotypes from other provenances, especially in breeding populations such as SPAs and CSOs should be avoided in order to maintain genetic purity of Nilambur teak. Centuries of glorious tradition associated with tree form and colour of Nilambur teak, and the separate genetic identity revealed by the present study suggest the possibility of considering geographic indicator registration for Nilambur teak.

Understanding the genetic diversity status of each SPAs will be useful in ascertaining the quality of seeds used for raising future plantations. The genetic diversity of SPAs is lower than that of natural teak populations. This could be due to the narrow genetic base of the parent trees from which seeds for raising the plantation (SPA) might have been collected. Hence, sufficient genetic diversity of a plantation has to be ensured before converting plantations to SPAs in order to avoid inbreeding and poor seed quality.

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 factor 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.

Geographical and genetic distances were significantly correlated showing genetic divergence between distant natural populations. Hence, while mixing clones from different provenances for establishing CSOs, phenologically unmatching clones should be avoided.

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