Genetic Structure and Mating System in Teak (*Tectona grandis* L. f.) Provenances

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Summary

Genetic variability in teak has been analysed up to now quite exclusively in provenance or progeny tests using quantitative traits. Few data were available on gene diversity revealed by genetic markers. This study dealt with analysis of 14 enzyme systems expressed in leaf parenchyma of teak seedlings.

Gene diversity was assessed in 9 populations including 1 population which consisted of 10 open-pollinated progenies. These 10 progenies from an Indian stand served also for genetic analysis of isozyme patterns. Eighteen of the 20 putative loci investigated were polymorphic. A total of 80 alleles were scored at the polymorphic loci, up to 11 alleles were noticed at a polymorphic locus. At the *Adh* locus, 4 of the 11 alleles were null alleles. The diploid status of the species was confirmed by expression patterns of isozymes.

In the 9 populations, the polymorphic locus rate was on average 79% (at criterion 95%). Considering polymorphic loci, the average number of alleles per locus was 2.8 and the observed heterozygosity 0.32. Populations showed a general lack of heterozygosity (average fixation index: 0.11).

Outcrossing rate was assessed in the provenance consisting of 10 separated progenies, multilocus outcrossing rate based on 10 polymorphic loci was about 0.98, while average single locus outcrossing rate was 0.92. Mother trees showed negative fixation indices at each polymorphic locus. Genetic structure of progenies and of reproductive trees was then discussed.

Key words: allozyme, genetic structure, isozyme, mating system, polymorphism, *Tectona grandis.*

FDC: 165.3; 165.52; 232.12; 176.1 *Tectona grandis.*

Introduction

Teak, *Tectona grandis* L.f., one of the most economically important tropical timber species, is native to the tropical deciduous forests of India, Myanmar (formerly Burma), Laos, Thailand and Indonesia (TROUP, 1921). *Tectona grandis* L.f., a member of *Verbenaceae* family, is diploid, 2n=36 (GILL et al., 1983), monoecious, and insect pollinated (BRYNDUM and HEDEGART, 1969; HEDEGART, 1973; MATHEW, et al., 1987). Selfing is likely possible, as it is thought that insects worked mainly on a single tree, producing inbreed fruits, which generally showed low germination capacities (HEDEGART, 1973).

The tree has been planted world-wide in the tropical regions since the beginning of 19th century, especially in Asia, Africa and Central America (MÉNIAUD, 1930; CHOLLET, 1967; MUNISWAMI, 1977; KEOGH, 1979; DUPUY, 1990). International provenance trials supported mainly by FAO, DANIDA and CIRAD-Forêt (formerly Centre Technique Forestier Tropical, CTFT), have been established extensively in 1970s in Africa, Central America and South-east Asia (DELAUNAY, 1977; PIOT, 1977; EGENTI, 1978; KAOSA-ARD, 1986; KEOGH, 1987), showing an important variation of quantitative traits among provenances. However, genetic markers, which can be used for genetic variation analyses, were poorly developed in teak (KUMARAVELU, 1979; VERHAEGEN, unpublished).

The present study was therefore focused on estimation of genetic structure within stand. The first step was then devoted to the genetic analysis of isozyme patterns because of a lack of related literature on inheritance of isozymes in this species. Ten half-sib progenies of an Indian provenance were analysed for a maximum number of isozyme systems. Genetic inheritance of isozyme patterns were then deduced. Isozyme patterns and gene diversity were also analysed in 8 other teak populations, representing both the 3 native regions mentioned above (India, Thailand and Indonesia) and artificial habitats (West and East Africa). Mating system was finally investigated in the stand where 10 maternal progenies were separately collected.

Materials and Methods

Plant material

Seeds were harvested from 10 mother trees located in Thithimathy Royal Forest (Karnataka, India, seedlot I_3). They were used for the present analysis of isozyme patterns. Eight other teak seedlots of native and artificial habitats were also investigated: 2 Indian populations from Karnataka province (I_1 and I_2), 2 Thai stands (T_1 and T_2, representing natural forest (T_1) and plantation (T_2); 2 Indonesian stands (J_1 and J_2), and 2 stands planted in West (A_1, Ivory Coast) and East (A_2, Tanzania) Africa *(Table 1)*. These 8 seedlots were a bulk collection of seeds from an unknown number of trees (generally more than 50 mother-trees).

Table 1. – Geographic origin of *Tectona grandis* provenances.

Provenance	Province	Country	Longitude	Latitude
I_1 Sakrebail Royal Forest Seed production area	Karnataka	India	75°29' E	13°48' N
I_2 Virnoli Seed production area	Karnataka	India	74°37' E	15°12' N
I_3 Thithimathy Royal Forest	Karnataka	India	76°00' E	12°15' N
J_1 Kandangan	Central Java	Indonesia	110° E	7°00' S
J_2 Saradan	East Java	Indonesia	111° E	7°30' S
T_1 Tam Bah Thai Mae Huat Natural Forest		Thailand	99°55' E	18°40' N
T_2 Mae Huat Seed production area		Thailand	99°55' E	18°40' N
A_1 Kokondekro		Ivory Coast	5°02' W	7°38' N
A_2 Tanzania		Tanzania	37° E	7° S

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Teak fruits were exposed to a heat treatment (80±1 °C for 48 h), just prior to germination in order to break down seed dormancy, it was the most efficient pre-treatment tested. Seeds germinated within a month in a water-saturated sand media, at 30 °C, under a 16 h photoperiod. Seedlings (about 20 days after cotyledon emergence) were thereafter transferred into greenhouse conditions (minimal temperature 27 °C, natural lighting complemented to a 16 h photoperiod). In most cases, the thermic pre-treatment was efficient to enhance the germination. Nevertheless, the germination capacities varied according to the seedlots (from 5% to 95%, number of germinations per fruit). Consequently, the number of seedlings available from each stand varied from only 3 plants in T_2, a Thai provenance, to 263 plants in I_3, an Indian provenance.

Isozyme technics

The enzyme activities were extracted from young leaves in which principal and secondary midribs had been removed, leaves were harvested from at least 2-month-old seedlings. The leaf samples collected were immediately ground in cooled room conditions (5±1 °C) in a mortar containing the extraction buffer (360 µl for 200 mg plant material), which was a sodium tetraborate buffer (50 mM, pH 8.3) supplemented with 0.1% dithiothreitol, 2.0% polyvinyl pyrrolidone 40 000, 1.0% polyethyleneglycol 20 000, 1.0% bovine serum albumine, 0.25 M ascorbic acid, 0.2 mM pyridoxal-5'-phosphate, 0.4 mM nicotinamide adenine dinucleotide, 0.3 mM nicotinamide adenine dinucleotide phosphate, 0.45 M sodium thioglycolate and 0.14 M sucrose. The homogenates were centrifuged at 15 000 g for 20 min at 4 °C.

Fifteen µl of the supernatant were put on each well at the anodic side of gels. The electrophoretic migration took place at 5±1 °C in vertical polyacrylamide gel (stacking gel: polyacrylamide 3%, running gel: polyacrylamide 9%) under an electric field of 12 $V.cm^{-1}$ for 4.5 h to 5 h. The electrode and gel buffers were Tris (90 mM) borate (90 mM), ethylenediamine tetraacetic acid disodium salt (2.5 mM), at pH 8.38.

The standard staining procedures were adapted with some minor modifications from VALLEOS (1983), CHELIAK and PITEL (1986), PASTEUR et al. (1987) and WENDEL and WEEDEN (1989) excepted for Carboxyl Esterase which was revealed in a barbital buffer (50 mM, pH 8.2) containing indoxyl acetate (1.1 mM previously dissolved in acetone), and cupric acetate (0.1 mM).

Sixty-nine enzyme activities had been tested (some were tested with various substrates and staining procedures), only the following 14 were finally retained because of reproducible patterns and of possible genetic analysis of patterns in each individual: Alanine aminopeptidase (E.C. 3.4.11.1) (AAP), Aspartate amino transferase (E.C. 2.6.1.1.) (AAT), Acidic phosphatase (E.C. 3.1.3.2) (ACP), Alcohol dehydrogenase (E.C. 1.1.1.1) (ADH), Diaphorase (E.C. 1.6.4.3) (DIA), Endopeptidase (E.C. 3.4.-.-) (ENDO), Carboxyl esterase (E.C. 3.1.1.-) (EST), Fluorescent β-D-glucosidase (E.C. 3.2.1.21) (β-GLU), Glycerate-2 dehydrogenase (E.C. 1.1.1.29) (G_2DH), Leucine aminopeptidase (E.C. 3.4.11.1) (LAP), Lactate dehydrogenase (E.C. 1.1.1.27) (LDH), Nicotinamide adenine dinucleotide dehydrogenase (E.C. 1.6.99.3) (NADH-DH), Peroxidase (E.C. 1.11.1.7) (PER) and Superoxide dismutase (E.C. 1.15.1.1) (SOD). Alleles were numbered according to the mobility of the protein they encoded from the fastest to the slowest one.

Data processing

Inheritance of each enzymatic system patterns was assessed in the ten half-sib progenies from I_3. Genotypes of all seedlings from each stand, grown in the greenhouse conditions, were then deduced from the banding patterns observed for each enzyme system.

Within population genetic variation was estimated by considering different parameters: allele frequency, average number of alleles per locus, proportion of polymorphic loci (at criterion 95%), observed and expected heterozygosity over all loci, and the χ^2 goodness of fit to test the deviation from HARDY-WEINBERG principle.

Mixed mating system model (RITLAND and JAIN, 1981; RITLAND, 1983) was used for estimation of outcrossing rate at the stand level and at the progeny level according to RITLAND (1990). Each progeny contained at least 20 seedlings. Genotypes of each individual mother tree was assessed, genetic structure of mother trees was also investigated.

Results

Isozyme patterns

Representation of all electrophoretic patterns observed in the seedlings analysed, for the 14 enzyme systems retained with their presumptive genotypes were given in *figure 1.* Each enzyme system is thereafter detailed. Genetic inheritance of enzyme systems elucidated in half-sib progenies was compatible with all patterns observed in each provenance. Provenances showed however some additional alleles not observed in the 10 open-pollinated progenies.

Alanine aminopeptidase (AAP)

Two zones were observed for teak alanine aminopeptidase system. The most cathodic one overlapped that of leucine aminopeptidase patterns, only the slowest one characterised teak alanine aminopeptidase patterns. These patterns can be postulated as controlled by a single gene (*Aap*) encoding a monomeric enzyme, at which 4 active alleles have been observed in all populations studied.

Aspartate amino transferase (AAT)

The patterns of teak aspartate amino transferase activities were very polymorphic, and can be divided into 3 principal activity zones. The most anodic one can be postulated as the expression of a single monomorphic locus, while the 2 others appeared encoded by 2 different loci expressed as dimeric proteins, denoted *Aat-b* and *Aat-c.* Their activity zones might sometimes be overlapped (on electrophoretic gels). Interlocus heterodimer bands with a low activity were sometimes detected (they are not drawn on *Fig. 1*). At *Aat-b* and *Aat-c,* 6 and 5 alleles were respectively scored.

Acidic phosphatase (ACP)

Two regions highly polymorphic have been observed in teak acidic phosphatase. The most anodic zone was however of low activity, indeed non active in many individuals assayed. The slowest region consisted of 3, 4, 5, 6, 7 or 8 thin bands, regularly spaced. Each pattern showed most intensive bands in the middle part. It can be assumed that slow zone of teak acidic phosphatase is encoded by a single locus (*Acp-b*) and have a polymeric molecular structure, and that the genotype with 3 bands represented the homozygous, while the heterozygous genotypes had 4 or more bands, depending on the corresponding alleles involved (the heterozygous ACP-b_1/ACP-b_4 had 8 bands).

Alcohol dehydrogenase (ADH)

The teak alcohol dehydrogenase showed only 1 activity zone, with 1, 2 or 3 bands. The genotypes with 2 bands were, however rare, but existed in different positions. It can be postulat-

ed that ADH occurs in teak as a dimeric enzyme and is apparently encoded by a single locus (*Adh*) with 7 active alleles scored. While the genotype with just 2 bands could only be explained if a silent or null allele was hypothesised. These genotypes were considered heterozygous, the 4 2-banded patterns indicating 4 different null alleles (A to D). The homozygous genotypes for null alleles were not found, the frequency of those alleles was relatively weak (≤0.05) in the populations.

Diaphorase (DIA)

The teak diaphorase enzyme was represented by 2 separated zones. The fastest one, however coincided with the activity of NADH-DH. The slowest zone showed 2, 3 or 4 bands depending on the individuals, and corresponded probably to the expression of 2 different loci, encoding a monomeric enzyme and labelled as *Dia-a* and *Dia-b* respectively, each having 3 recorded alleles. The most common alleles were respectively DIA-a_3 and DIA-b_2.

Endopeptidase (ENDO)

Only one activity zone was observed for teak endopeptidase. Each individual presented 1 or 2 bands, and several electrophoretic mobilities have been observed. This zone can then be assumed as the expression of a single locus (*Endo*) encoding a monomeric enzyme, and with 6 active alleles detected for all populations studied.

Esterase (EST)

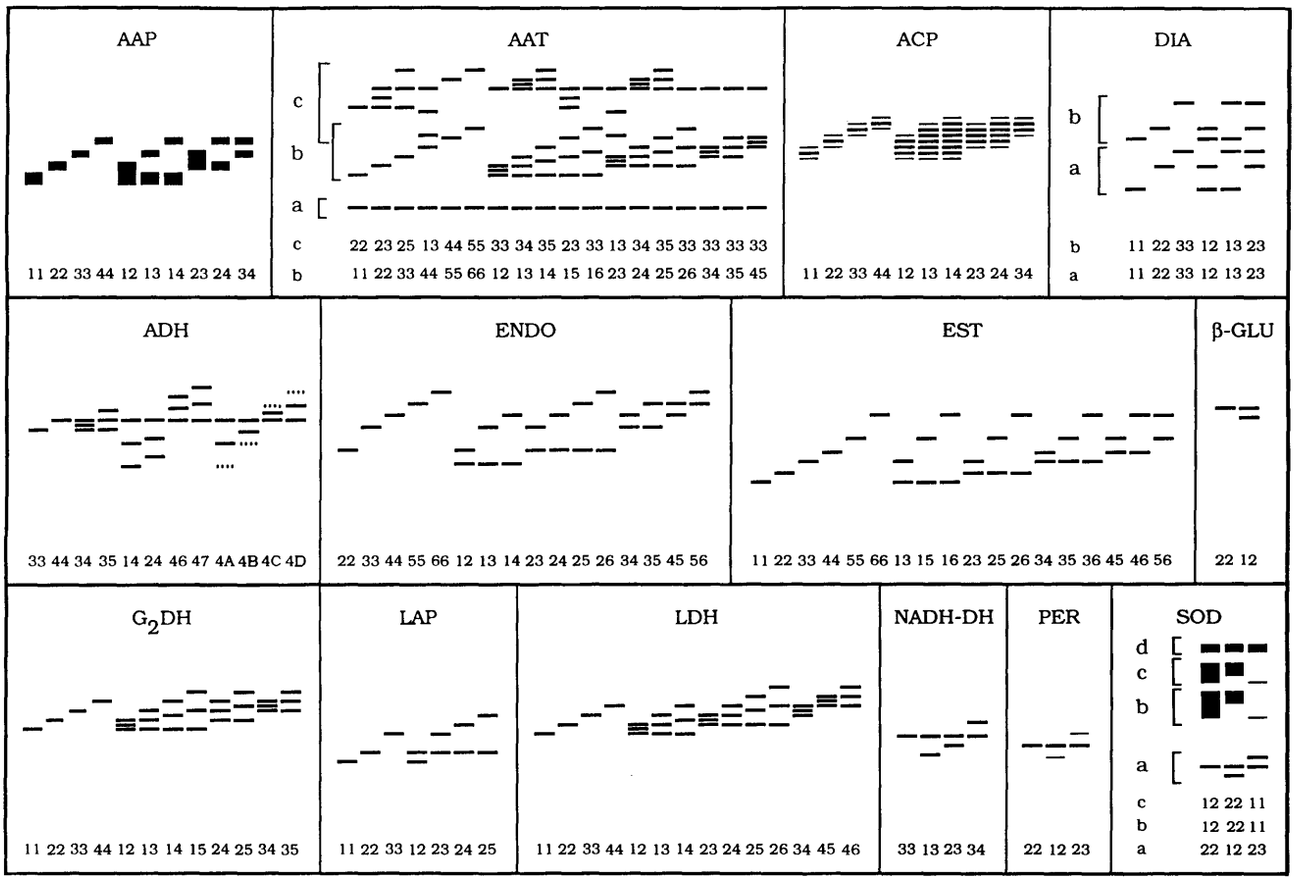
The teak carboxylic and fluorescent esterase systems have exactly the same patterns, the former had however more distinct bands than the latter, and was finally retained. Moreover as the stained bands were stable, it can be recorded a long time after staining. Several activity zones were observed, but only the fastest one can however be scored, since it was the most active. One or 2 bands were observed, giving rise to several phenotypes. It can be postulated that this zone resulted from the activity of a monomeric enzyme and it was controlled by a single gene (*Est*) with 6 active alleles observed in the populations analysed. The most frequent alleles were labelled as EST_3 and EST_5, depending on the populations studied.

Fluorescent-β-D-glucosidase (β-GLU)

This enzyme in teak had only a single zone of activity with 1 or 2 bands, corresponding to a monomeric enzyme. The slowest band was the most frequent, the fastest band was never observed alone and was rare, so that the homozygous genotypes were never found for this allele. This zone then can be assumed to be due to the activity of a single diallelic locus (*β-Glu*).

Glycerate-2 dehydrogenase (G_2DH)

In teak glycerate-2 dehydrogenase system, 3 activity zones of slow migration were observed. The most cathodic patterns was coincident with alcohol dehydrogenase patterns. Only the 2 other zones could be considered as the glycerate-2 dehydrogenase patterns. Each zone was probably controlled by a different gene (*G_2dh-a* and *G_2dh-b*) encoding for a dimeric enzyme, the most cathodic zone (*G_2dh-b*) was not always present. Only 1 locus with 5 alleles observed, was finally retained (*G_2dh-a*) for the present study.



... position of inactive enzyme (encoded by null allele).

Figure 1. – Representation of isozyme patterns for the 14 enzymatic systems tested in *Tectona grandis,* with the corresponding genotypes assessed; only bands specific of the considered enzyme system were drawn, all genotypes recorded at each locus were presented (migration from the top to the bottom).

Leucine aminopeptidase (LAP)

A single zone was observed for teak leucine aminopeptidase system. The genotypes showed either 1 or 2 bands. This zone was probably controlled by a locus (*Lap*) with 5 active alleles detected in all 9 populations. The enzyme structure seemed to be monomeric.

Lactate dehydrogenase (LDH)

The teak lactate dehydrogenase patterns presented 2 zones of activity, the most anodic showed also perfect coincidence with those of alcohol dehydrogenase. The LDH bands were in fact those in the slower zone, which can be assumed as controlled by a single locus (*Ldh*) encoding a dimeric enzyme, with 6 alleles observed in all 9 populations studied.

Nicotinamide adenine dinucleotide dehydrogenase (NADH-DH)

Two activity zones separated by an important electrophoretic distance, were observed for teak Nicotinamide adenine dinucleotide dehydrogenase patterns. The cathodic zone showing several thin bands of irregular activity, was apparently controlled by several genes encoding a monomeric enzyme. As their genetic inheritance was not obvious, they were not thereafter considered. While the anodic zone seemed to be governed by a single locus (*Nadh-dh*), expressed by monomeric enzyme, with 4 active alleles recorded, the second allele being the most common.

Peroxidase (PER)

One activity zone appeared in teak peroxidase enzyme patterns. This zone showed 1 or 2 bands, corresponding to the patterns of a monomeric enzyme encoded by a single locus (*Per*), at which 3 alleles had been scored. The allele PER_2 was the most frequent.

Superoxide dismutase (SOD)

The teak superoxide dismutase enzyme was observed as negative staining on violet background. Several activity zones were revealed, the fastest having 1 or 2 bands, might be governed by a single diallelic locus, denoted *Sod-a*. The slowest bands in the cathodic section had the same electrophoretic mobility in all individuals scored, showing a single monomorphic locus (*Sod-d*). While the other bands in the intermediary zone varied in their intensity and thickness. Two different loci (*Sod-b* and *Sod-c*) respectively diallelic, controlled probably this part of the activity zone. Alleles SOD-b_1 and SOD-c_1 had only a weak activity. *Sod* loci are expressed by monomeric enzymes.

Eighteen isozyme polymorphic loci (*Aap, Aat-b, Aat-c, Acp-b, Adh, Dia-a, Dia-b, Endo, Est, β-Glu, G_2dh-a, Lap, Ldh, Nadh-dh, Per, Sod-a, Sod-b* and *Sod-c*) encoded 14 enzyme systems, were therefore scored. Two monomorphic loci were also noticed: *Aat-a* and *Sod-d*. For each enzyme system observed, the patterns suggested a monomeric molecular structure (AAP, DIA, ENDO, EST, β-GLU, LAP, NADH-DH, PER, SOD), or a dimeric structure (AAT, ADH, LDH, G_2DH) and indeed polymeric systems (ACP). The true enzyme structure can not be assumed since only polymorphic polypeptide of the protein can be detected.

Genetic diversity within provenances

Eighty alleles were scored in the 9 populations of teak at the 18 polymorphic loci. The most common allele at a locus was not the same in all provenances (*Table 2*). The average number of alleles per locus was 2.8 (*Table 3*), ranging from 1.7 (provenance T_2) to 3.7 (provenance I_3). The values were however dependent on the sample size of population.

Table 2. – Allelic frequencies of major alleles (average frequency > 0.10) in 9 teak provenances.

Locus	Allele	Provenances								
		I1	I2	I3	J1	J2	T1	T2	A1	A2
Aap	2	0.056	0.333	0.251	0.091	0.429	0.125	0.333	0.371	0.292
	3	0.444	0.565	0.566	0.500	0.286	0.719	0.333	0.490	0.601
	4	0.333	0.028	0.102	0.364	0.143	0.094	0.333	0.086	0.056
Aat-b	2	0.167	0.211	0.103	0.827	0.538	0.563	0.667	0.818	0.590
	3	0.222	0.035	0.027	0.038	0.385	0.438	0.333	0.042	0.315
	5	0.333	0.553	0.811	0.000	0.000	0.000	0.000	0.000	0.011
Aat-c	3	0.944	0.877	0.877	0.500	0.846	0.750	0.833	0.757	0.787
Acp-b	2	0.000	0.130	0.186	0.143	0.000	0.250	0.333	0.480	0.371
	3	0.500	0.481	0.318	0.643	1.000	0.563	0.500	0.444	0.539
	4	0.500	0.380	0.380	0.071	0.000	0.063	0.167	0.051	0.045
Adh	4	0.944	0.848	0.933	0.904	0.885	0.719	0.333	0.902	0.888
Dia-a	1	0.167	0.179	0.185	0.096	0.077	0.200	0.167	0.290	0.069
	2	0.389	0.098	0.144	0.288	0.346	0.033	0.000	0.232	0.126
	3	0.444	0.723	0.671	0.615	0.577	0.767	0.833	0.478	0.805
Dia-b	1	0.000	0.045	0.030	0.154	0.231	0.133	0.167	0.145	0.075
	2	0.389	0.348	0.541	0.769	0.577	0.833	0.667	0.746	0.799
	3	0.611	0.607	0.429	0.077	0.192	0.033	0.167	0.109	0.126
Endo	3	0.167	0.018	0.014	0.135	0.115	0.000	0.000	0.170	0.318
	4	0.056	0.214	0.145	0.115	0.038	0.000	0.000	0.042	0.023
	5	0.556	0.759	0.687	0.712	0.654	0.938	1.000	0.561	0.619
Est	3	0.000	0.140	0.054	0.635	0.385	0.313	0.500	0.458	0.534
	5	0.278	0.474	0.564	0.212	0.308	0.594	0.500	0.425	0.399
	6	0.444	0.316	0.224	0.000	0.038	0.000	0.000	0.000	0.011
β-Glu	2	1.000	0.907	0.966	1.000	1.000	0.967	1.000	0.981	0.944
G2dh-a	1	0.250	0.107	0.063	0.750	0.654	0.500	0.500	0.457	0.652
	3	0.563	0.714	0.732	0.154	0.192	0.500	0.500	0.319	0.275
Lap	2	0.889	0.861	0.971	0.955	0.786	0.813	1.000	0.911	0.972
Ldh	2	0.250	0.375	0.382	0.981	0.923	0.667	1.000	0.933	0.854
	4	0.625	0.545	0.527	0.019	0.000	0.133	0.000	0.010	0.022
Nadh-dh	3	0.889	0.860	0.986	0.942	0.885	0.844	1.000	0.850	0.747
Per	1	0.300	0.077	0.089	0.100	0.143	0.036	0.000	0.173	0.133
	2	0.700	0.897	0.898	0.900	0.857	0.821	0.833	0.784	0.867
Sod-a	1	0.222	0.176	0.196	0.125	0.071	0.031	0.167	0.071	0.045
	2	0.778	0.824	0.792	0.875	0.929	0.969	0.833	0.929	0.955
Sod-b	1	0.778	0.611	0.316	0.500	0.571	0.344	0.333	0.596	0.489
	2	0.222	0.389	0.684	0.500	0.429	0.656	0.667	0.404	0.511
Sod-c	1	0.556	0.370	0.300	0.375	0.214	0.219	0.000	0.273	0.489
	2	0.444	0.630	0.700	0.625	0.786	0.781	1.000	0.727	0.511

The average percentage of polymorphic loci, by the 95% criterion, was 79%. If the Thai Mae Huat provenance T_2 was excluded (because of low sample size), both Indian provenance I_3 and Indonesian provenance J_1, had thereafter the lowest proportion of polymorphic loci (75%). In contrast, the Indian provenance I_2 had the most important polymorphism (90%) for all genes assessed.

The average observed heterozygosity was 0.285, while the expected value after HARDY-WEINBERG equilibrium (gene diversity) was 0.347. The Indian provenance I_1 showed the highest values for both observed and expected heterozygosity; the Indonesian provenances J_2 and J_1 showed respectively the smallest observed and expected proportion of heterozygotes. Anyway, deficit of heterozygosity was observed in each population, since the observed proportion of heterozygotes was smaller than that expected under HARDY-WEINBERG equilibrium.

Average fixation indices were positive in each provenance, confirming the heterozygotes deficiency trends. This parameter ranged from 0.038 (Indian provenance I_1) to 0.271 (Indonesian J_2 provenance). Negative values of fixation index (mostly not significant) were however found in some loci in all provenances (lowest and highly significant value: F=-0.49 at the G_2*dh-a* locus in the Indian provenance I_3), showing excess of heterozygotes according to HARDY-WEINBERG equilibrium. Other significant negative F were observed in 1 or 2 populations at *Aat-c, Sod-a, Sod-b* and *Sod-c* loci. No heterozygotes deficiency was observed at both *Aat-c* and *Sod-a* loci for any of studied populations ($F_{IS} < 0$).

Table 3. – Genetic diversity within *Tectona grandis* provenances at 20 loci (including 2 monomorphic loci).

Provenance	Sample size	Average number of alleles	Polymorphic loci (%)	Average observed heterozygosity	Gene diversity	Mean fixation index
I_1 Sakrebail	9	2.3	85	0.371	0.406	0.038
I_2 Virnoli	55	3.4	90	0.320	0.372	0.079
I_3 Thithimathy	263	3.7	75	0.306	0.332	0.066
J_1 Kandangan	18	2.5	75	0.267	0.320	0.085
J_2 Saradan	10	2.4	80	0.210	0.340	0.271
T_1 Tam Bah Thai	16	2.6	80	0.225	0.338	0.252
T_2 Mae Huat	3	1.7	60	0.250	0.333	0.071
A_1 Kokondekro	97	3.6	85	0.311	0.351	0.101
A_2 Tanzania	82	3.3	80	0.305	0.334	0.084
Mean	61	2.8	79	0.285	0.347	0.116

The fit between the observed and expected frequencies tested by χ^2, indicated a significant deviation towards an excess of homozygosity in most provenances at *Aat-b, Acp-b, Dia-a, Dia-b,* and *Sod-b* loci. In contrast, the departure of HARDY-WEINBERG equilibrium was not found in cases of *Endo, β-Glu* (showing low polymorphism) and *Per* loci in any provenance. Only Indian provenance I_1, exhibiting the lowest average fixation index, showed no locus with significant excess of homozygosity. The other populations showed a significant excess of homozygosity at several loci.

Mating system

Outcrossing rate was assessed in provenance I_3 at 16 loci (*β-Glu* and *Per* were not enough polymorphic in this provenance and were excluded). Single-locus outcrossing rates showed that teak is an allogamous species (*Table 4*). *Adh* locus led to heterogeneous estimation of outcrossing rate because of the low frequency of minor alleles. Only the 10 most polymorphic loci, with 3 alleles or more, were taken into account for multilocus estimation of outcrossing rate: *Aat-b, Adh, G2dh-a, Sod-a, Sod-b* and *Sod-c* were excluded. When more than 3 alleles were recorded at a locus, they were reclassified into 3 synthetic alleles according to their respective electrophoretic mobility. Allogamy was predominant in each progeny (*Table 5*). The average single-locus outcrossing rate (0.92) was not significantly lower than the multilocus one (0.98).

Genotypes of mother trees were assessed, they showed a high level of heterozygosity (0.64 in average, *Table 4*), which was higher than that expected according to HARDY-WEINBERG equilibrium (0.442). Mother trees were monomorphic at 4 loci, they exhibited a negative fixation index at each other locus (*Table 4*).

Table 4. – Single-locus mating system of 10 progenies and fixation index of mother-trees in provenances I_3.

Locus	Single-locus outcrossing rate	Mother-tree fixation index
Aap	0.84 ± 0.18	-0.38
Aat-b	1.06 ± 0.76	-0.05
Aat-c	0.81 ± 0.14	–
Acp-b	0.71 ± 0.18	-0.52
Adh	2.00 ± 1.20	-0.11
Dia-a	0.93 ± 0.18	-0.30
Dia-b	0.01 ± 0.46	-0.38
Endo	1.09 ± 0.38	-0.24
Est	1.00 ± 0.22	-0.39
G2dh-a	2.00 ± 0.04	–
Lap	0.37 ± 0.32	–
Ldh	0.78 ± 0.52	-0.54
Nadh-dh	0.62 ± 0.38	–
Sod-a	0.95 ± 0.32	-0.18
Sod-b	0.78 ± 0.98	-0.67
Sod-c	1.32 ± 0.32	-0.33

Discussion

Ploidy level

SHARMA and MUKHOPADHYAY (1963) considered that teak might be a tetraploid species although these authors observed only bivalents at meiosis. Teak was even suspected as an hexaploid species by KHOSLA and SAREEN (1980). Our results showed a typical diploid expression of genes, that did not exclude a polyploid origin of the species, the presence of several acting genes with the same activity could result from polyploidy. The diploid status of teak presumed by GILL et al. (1983) is confirmed by the gene expression we observed through enzymatic activities. If teak has a polyploid origin, the evolution of its genome has led to a present diploid expression, at the chromosomal level as well as at the gene expression level.

Genetic structure

Isozymes analyses in teak revealed 20 loci, most of them being polymorphic. Gene diversity studies can be carried out in teak using this kind of genetic markers. There was a general excess of homozygosity within progeny population, as revealed

Table 5. – Outcrossing rates and heterozygosity of 10 mother trees from I_3 provenances determined at 10 polymorphic loci.

Mother tree	Outcrossing rate		Heterozygosity
	single locus	multilocus	
1	0.90	0.93	0.60
2	1.11	0.96	0.80
3	0.93	1.17	0.60
4	0.90	1.04	0.80
5	0.89	0.94	0.50
6	0.76	0.82	0.60
7	0.57	2.00	0.40
8	1.07	2.00	0.60
9	0.97	0.85	0.70
10	1.09	1.29	0.80
Mean[a]	0.92 ± 0.14	0.983 ± 0.080	0.64

a: mean ± confidence interval at 0.95

by the positive index fixation mean value (0.11). Nevertheless, selection at some loci (*G_2dh-a, Aat-c)* in favour of heterozygous individuals should also occurred to explain significant excess of heterozygosity according to HARDY-WEINBERG equilibrium (selection did not act necessarily at isozyme loci, it can do at linked loci). Populations were subjected to selection effects increasing heterozygosity at few loci and to a general deviation from HARDY-WEINBERG in favour of homozygosity. A lack of heterozygosity can be observed in a population because of restriction of gene flow within the whole provenance and of increase of relatedness between neighbour individuals (GREGORIUS and NAMKOONG, 1983). In these conditions, the mother trees should exhibit the same trends.

The lack of heterozygosity (positive fixation index) observed at the progeny level was not found at the reproductive stage in teak. Lower fixation indices in mother trees have been already observed in several forest tree species (YAZDANI et al., 1985; PRAT and ARNAL, 1994). Heterozygous genotypes appeared to be favoured at the reproductive stage, homozygous genotypes were removed by natural selection or thinning of the stand.

Selfing rate is very low (about 2%), as observed by HEDEGART (1976), and cannot be the major reason of the lack of heterozygosity in progenies. Viable seedlings did not generally result from selfing. Self-pollinated seeds had low viability and produced no or few seedlings, as suggested by HEDEGART (1973). Thus, selfing induces fruits with low germination rate or the lack of fruits in teak. A weak fructification, which is a restrictive factor for regeneration and plant production, may reveal a high level of selfing. Insects assure pollination, but they favour selfing because of their long stay on the same tree (MATHEW et al., 1987). Assuming that all populations were mostly allogamous, and that self-pollination did not produce viable seeds an excess of homozygosity detected at the seedling stage (several-month-old) can therefore be expected only if crossing occurred between related or surrounding trees. Outcrossing carried out by insects concern essentially a small group of trees, which might be moreover related, leading to a lack of heterozygosity. A single flower had few occasions of pollination since the pollen receptivity period is short: few hours, during one morning (HEDEGART, 1973).

It may be hypothesised that gene diversity and high heterozygosity in teak population are maintained by early exclusion of selfed material (no embryo development, low germination), and by progressive selection against homozygous genotypes during stand life. Consanguineous trees were suppressed and only most heterozygous genotypes attain the reproductive stage.

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Utility of Random Amplified Polymorphic DNA (RAPD) Markers for Linkage Mapping in Turkish Red Pine (*Pinus brutia* Ten.)

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Summary

We have applied the random amplified polymorphic DNA (RAPD) marker system to estimate linkage relationships in *Pinus brutia* TEN.. We used DNA samples from 30 haploid seed megagametophytes from each of 4 mother trees. Ninety-five 10-base oligonucleotide primers were evaluated and 34 revealed at least one polymorphic RAPD locus. The number of segregating RAPD loci per polymorphic primer varied from 2.32 to 3.25, but when segregating loci per tested primer was considered it was low, ranging on the average, from 0.28 in tree 4 to 0.59 in tree 1. Based on the RAPD loci segregating in 1:1 ratio, genetic linkage groups formed from 6 for genotype-4 (total map distance=163.91 cM) to 13 for genotype-3 (total map distance=511.2 cM). It was also found that a number of segregating loci in all 4 genotypes (ranging from 14 to 21) could not be assigned into any of the constructed linkage groups. It was difficult to compare linkage groups among the genotypes since most of RAPD loci segregating in one genotype were not found in others. Thus, the linkage map provided very little information on the genomic organization of RAPD markers at the species level. The utility of RAPD markers in forest genetics is also discussed in the paper.

Key words: Pinus brutia, RAPD markers, genetic linkage mapping, polymerase chain reaction.

FDC: 165.3; 174.7 *Pinus brutia.*

Introduction

Turkish red pine (*Pinus brutia* TEN.) is found throughout the eastern Mediterranean and is a commercially important timber species in Turkey. Frequent forest fires, excessive utilization of stands and conversions of forest to agricultural have likely impacted the genetic diversity of Turkish populations, however, the magnitude of the impact is unknown. Red pine tree improvement programs have been established (ISIK, 1989; ISIK and KAYA, 1993), but there is an urgent need to assess the genetic diversity in Turkish populations. Inexpensive and easy to apply genetic markers would greatly facilitate this effort.

Since the early 1970's, allozymes have been the most widely used genetic markers in forestry since they are inexpensive, rapid to apply and require only modest technical skills. Examples of such studies include; geographic variation (GURIES and LEDIG, 1982), systematic relationships (CONKLE *et al.,* 1988), within stand variation (NEALE, 1985), mating systems (SHAW and ALLARD, 1982), gene flow (MILLAR, 1983) and estimation of pollen contamination in seed orchards (FRIEDMAN and ADAMS, 1985).

The main limitation of allozyme techniques is the small number of markers available. Linkage studies in conifers based on allozyme markers have mapped no more than about 10% of the genome (226.4 cM) (CONKLE, 1981). DNA markers, however, have numerous advantages over isozymes and have gradually replaced isozymes for many applications in forestry (NEALE and WILLIAMS, 1991; NEALE *et al.,* 1992). However, genetic markers such as restriction fragment length polymorphisms (RFLP) are technically demanding and expensive and would be difficult to apply in Turkey given the current laboratory standards.

Random amplified polymorphic DNA (RAPD) markers are new types of genetic markers which are based on the polymerase chain reaction (PCR) (WELSH and MCCLELLAND, 1990; WILLIAMS *et al.,* 1990). RAPD markers are inherited in a Mendelian manner and can be generated for any species without prior DNA sequence information (WELSH and MCCLELLAND, 1990). One advantage of RAPD markers is the increased speed of analysis and dramatic reduction in the amount of DNA required for analysis. Furthermore, when haploid megagametophyte tissues from conifers are used as the source of template DNA, there is no need to have specific crosses to carry out linkage mapping (CARLSON *et al.,* 1991; TULSIERAM *et al.,* 1992).

In a preliminary study, KAYA and NEALE (1993) assessed the utility of RAPD markers for studying genetic polymorphism in *Pinus brutia* (Turkish red pine) and *Pinus nigra* var *pallasiana* (Anatolian black pine). This study showed that variation could be detected with RAPD markers and it should be possible to construct genetic maps. The objectives of the current study

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