ALLOZYME DIVERSITY IN TWO TANZANIAN AND TWO NICARAGUAN LANDRACES OF TEAK (*TECTONA GRANDIS* L.)

Erik D. Kjær & Hans R. Siegismund

Arboretum, Royal Veterinary and Agricultural University, Kirkegårdsvej 3A, DK–2970 Hørsholm, Denmark

Received October 17, 1995; accepted June 10, 1996

ABSTRACT

Allozyme diversity was evaluated for two Tanzanian and two Nicaraguan landraces of teak (*Tectona grandis* [L.]) and compared to the variation of four stands from within the natural distribution area as reference. Historical records suggest that the genetic processes involved in introductions may have been very different in Tanzania and Nicaragua. Speculations are that the genetic variation in the Tanzanian landraces has been enhanced following provenance hybridization, whereas the diversity in the Nicaraguan landraces may have been reduced because of bottlenecks. The allozyme diversity was found to be lower in the two Nicaraguan populations than in two Tanzanian, supporting these speculations. However, the differences between four natural populations surveyed for comparison were also large.

Key words: teak, *Tectona grandis*, allozymes, genetic diversity, landraces

INTRODUCTION

Tectona grandis (L.) is a tropical tree species with a large natural distribution in South and South East Asia (KAOSA-ARD 1981). It has a long history as a plantation species due to its valuable timber, and has been introduced successfully as an exotic species to many parts of the tropical world (reviewed by WHITE 1991). Today it is considered to be one of the most promising plantation species in the tropics and tree improvement thus promises to be very profitably (KJÆR & FOSTER 1995).

The genetic origin of the stands found outside the natural distribution area is largely unknown, as historical records mostly are inadequate. However, in the case of introductions to Tanzania and Central America, some knowledge is available. Teak was introduced to Tanzania at the beginning of this century from several genetic distant populations (moist, south western part of India ("Nilambur"), Myanmar (formerly Burma) and/or Java (KEIDING 1989; WOOD 1967). Most introductions to Nicaragua came from other central American countries (Panama, Honduras, Trinidad and Guatemala) where teak was introduced earlier (WADSWORTH 1960, here after KEOGH 1980). Historical records suggest, that many of these Central American landraces have passed through very small population sizes as seeds have been collected on a low number of trees (reviewed by KEOGH 1980). The available records thus suggest distinct differences between the introduction history to Tanzania and Nicaragua.x The landraces in Tanzania may be based on genetic material of variable origin and subsequent provenance hybridisation, opposite to the landraces in Nicaragua that may be based on seed sources with reduced genetic variation.

The purpose of this article is to estimate the allozyme variation in four landraces of *Tectona grandis* (L.), two from Tanzania and two from Nicaragua, and compare it to the allozyme level found in four seed sources from within the natural distribution area of teak as reference. The examined landraces are major seed sources used in afforestation of teak in Tanzania and Nigaragua, respectively. Our main effort in this study is to look for empirical evidence pro or contra the speculations that the genetic variation of the Tanzanian landraces have been enhanced following provenance hybridization, whereas the genetic variation in Nicaraguan landraces may have been reduced because of bottlenecks.

MATERIAL AND METHODS

Samples

Seeds from eight stands were included in this study (Table 1), four landraces and four samples from natural populations. The stand at Tecal-El Rececreo (Nic–1) originates from an older stands in the area, which in turn can be traced back to a small seedlot collected from a few trees in a botanical garden in Sri Lanka. Amalia-Chinandega (Nic–2) is founded on seed imported from a stand in Guatemala of unknown origin (Personal information from Hans Roulund, Centro de Mejoramiento Genetico y Banco de Semillas Forestales, Nicaragua). Mtibwa (Tanz–1) is based on a seed

Table 1 Origin of the populations used in this study

Population	Country	Location/region	Latitude	Longitude	Rainfall [mm.y^{-1}]
Thai–1	Thailand	Ban Cham Pui, Lampang	18°29' N	18°29' N	1100
Thai–2	Thailand	Mae Haut, Lampang	18°40' N	18°40' N	1260
Laos	Laos	Khong Island	14°10' N	14°10' N	1925
India	India	Sadiuaval, Tamil Nadu	11°00' N	11°00' N	900
Nic–1	Nicaragua	Recal, El Recreo	12°09' N	12°09' N	3000
Nic–2	Nicaragua	Amalia, Chinandega	12°31' N	12°31' N	1800
Tanz–1	Tanzania	Mtibwa, Morogoro	6°07' S	6°07' S	1200
Tanz–2	Tanzania	Kihuwi, Tanga	5°11' S	5°11' S	1400

collection in 1961 in an older Tanzanian stand at Bigwa. This stand was founded in 1898–1906 based on imports from India or Java. Kihuwi (Tanz–2) is probably first generation planted in Tanzania, probably of Indian or Java origin (based on information from The National Tree Seed Project, Tanzania, and WOOD 1967). Four populations from the natural distribution area (Thai–1, Thai–2, Laos and India) are examples of natural populations. They do not represent the full range of the natural distribution area. They serve the purpose of providing estimates of genetic variation in natural teak populations so that the variation in the landraces can be compared to a sample of natural populations.

In each stand the seeds were collected from the ground in an area sufficiently large to cover a large number of individuals. Approximately 1 kg of seed were provided from each seed source. This is a sufficient quantity to allow a good representation of the populations. Still, effects of sampling properties can not be out-ruled if different individuals are the prevailing gamete donors in different crop years.

The seeds were stored at 5 °C in sealed plastic bags until germination. They were germinated after pretreatment (80 °C for 48 hours followed by 6 hours soaking in water) at 34 °C with alternating 12 hours of light and dark. Leaves were harvested when seedlings were 5–10 cm of height. The leaf samples were stored at ÷80 °C until analysis.

Electrophoresis

The seedlings were homogenized in extraction buffer (No. 2, WENDEL and WEEDEN (1989), which was modified by adding 3 mM Dithiothreitol (DTT)).

Allozyme variation was resolved at nine loci: glutamate-oxaloacetate (GOT, E.C. No. 2.6.1.1), 2 loci; phosphoglucomutase (PGM, E.C. No. 2.7.5.1), 2 loci; diaphorase (DIA, E.C. No. 1.6.99.1), 1 locus; glucose-6-phosphate isomerase (GPI, E.C. No. 5.3.1.9), 1 locus; triose-phoshate isomerase (TPI, E.C. No. 5.3.1.1), 2 loci, esterase (EST, E.C. No. 3.1.1-), 1 locus.

GOT and DIA were resolved by starch gel electrophoresis in a TRIS–EDTA–borate buffer, pH = 8.6 (type 7 of WENDEL & WEEDEN 1989) at 500 V for 4.5 hours. PGM, GPI, TPI and EST were resolved by cellulose acetate electrophoreses (HEBERT & BEATON 1989). Super Z–12® applicator kit and Titan III® cellulose acetate plates of Helena Industries were used for applications and electrophoreses. PGM was resolved in Tris-glycine buffer, pH=8.5 (HEBERT & BEATON 1989). It was carried out at room temperature for 20 minutes at 450V. Separation of allozymes at the GPI, TPI and EST loci was carried out in a Morpholine-citrate buffer (0.040M Citric acid adjusted to pH = 7.2 by N-(3-aminopropyl)-morpholine) for 25 minutes at 350V. PGM, GPI, TPI and EST allozymes were visualized according to the staining recipes of HEBERT and BEATON (1989). GOT was stained with the recipe given in MANCHENKO (1994), method 1, and DIA according to MANCHENKO (1994) modified by adding additional 2 mg 2,6-dichlorophenol indophenol (DCIP) and 5 mg NADPH per 50 ml staining solution. Cellulose acetate electrophoresis in general gave the most clear bands except for DIA which was resolved best by starch gel electrophoresis. The two GOT loci were resolved well by both techniques, but the best separation of the GOT alleles were achieved by starch gel electrophoresis because of longer movement. KJÆR AND VERAPONG (1995) accepted Mendelian segregation in *Got–1, Got–2, Dia* and *Pgm–1* based on studies of half sib families. No formal genetic analysis is available for the rest of the loci included in the present study as these loci were not polymorphic in the progenies included in KJÆR and VERAPONGS study.

A larger number of isozymes was screened with different buffers by both starch and cellulose acetate electrophoresis, but other isozyme loci were excluded due to lack of variation, poor resolution of the zymograms, or difficulties in interpretation of the zymograms. Alcohol dehydrogenase (ADH, E.C. No. 1.1.1.1) and leucine aminopeptidase (LAP, E.C. No. 3.4.11.1) were excluded because the enzymes lost their activity

during storage at –80 °C within a few months generating too many missing observations. A total of 484 seedlings were analyzed.

Data analysis

Allele frequencies were calculated for each population at each locus. Genetic diversity was measured as the expected degree of heterozygosity H_e, the average number of alleles per locus, and the fraction of polymorphic loci (subject to a 99 % criterion).

Genotypic distributions were compared to the expected Hardy-Weinberg proportions. Deviations from Hardy-Weinberg expectations were tested by GUO and THOMPSON'S (1992) Monte Carlo permutation test. Based on 17,000 permutations per test, the probabilities of significance were calculated as the frequency of deviations from Hardy-Weinberg proportions in the permutated data equal to or more extreme than the observed. "Table-wide" levels of significance according to the sequential Bonferroni technique (HOLM 1979; RICE 1989) were applied in multiple comparisons. Following HOLM (1979), the tests were ranked according to their P values. The test corresponding to the smallest P value (P_1) was declared significant on a "table-wide" significance level α if $P_1 < \alpha/n$, i.e. $nP_1 < \alpha$, where n is the number of tests. The second smallest P value (P_2) was declared significant if $P_2 < \alpha/(n-1)$, *i.e.*, $(n-1)P_2 < \alpha$, and so on.

The relationship between the populations was analyzed and quantified by constructing a maximum-likelihood tree. Since some of the populations presumably have been through bottlenecks we have chosen to analyze the relationship among them with the maximum-likelihood procedure of FELSENSTEIN (1981, 1993). This procedure assumes that genetic drift is the cause of the differentiation and does not assume a constant population size over time, which is assumed by the commonly used UPGMA method (NEI 1987).

The distribution of genetic variation within and between populations was analyzed with Wright's F-statistics. Four of the examined populations in this study are artificial composites. F-statistics were therefore calculated for both for the four populations from the natural distrbution area (Thai–1, Thai–2, Laos and India) alone, and for the full data. F_{ST} based on all populations should only be seen as an emperical measure of the distribution of variance within and between population. Calculations follow the procedures described by WEIR and COCKERHAM (1984). Unbiased estimates were used. The standard deviation of the estimates were calculated by jackknifing over the populations.

RESULTS

Genetic variation at single loci

Allele frequencies, Wright's fixation index (F_{IS}), tests for Hardy-Weinberg proportions, and sample sizes are presented in Table 2. Most of the loci showed little variation except *Got–1* where the Tanzanian and the Indian populations were quite variable. Significant deviations from Hardy-Weinberg proportions were found for Thai–1, Laos, Tanz–1, Tanz–2 and India at the *Got1* locus. However, the deviations were not significant for Laos and Tanz–2 on a table-wide level of significance of 5% (test not shown). The deviation from Hardy-Weinberg proportions correspond to a positive F-value in the case of Tanz–2 and Thai–1, *i.e.*, surplus of homozygotes. For the Indian population the significant deviation from Hardy Weinberg proportions was caused by a surplus of heterozygotes compared to the expectations under Hardy Weinberg proportions (a negative F-value).

Genetic diversity

The measures of genetic diversity are presented in Table 3. The average H_e at the nine loci varies between 0.02 (Thai–1) to 0.11 (India). There seems to be a geographic pattern: The Thai and Laos populations have the lowest degree of genetic diversity (0.02, 0.05 and 0.03), the landraces from Nicaragua a little higher (0.05 and 0.08), Tanzanian higher (0.08, 0.10) and the population from India the highest among the investigated populations (0.11). The average number of alleles per locus (N_A) and the fraction of polymorphic loci $P(99\%)$ gave a slightly different picture with highest diversity in the Tanzanian populations. The differences between the populations in N_A and $P(99\%)$ were smaller than the differences in H_e.

Population structure

The relation between the populations are illustrated with a maximum-likelihood tree (Figure 1). A geographical pattern seems to be recognized from Figure 1. The two landraces from Tanzania cluster together as do the two populations from Thailand. The two Nicaraguan landraces seem to be less similar, Nic–2 being close to the Thai-populations, Nic–1 being more unique. The high frequency of the 85-allele at *Got–1* in Nic–1 (Table 2) places this population closer to the Indian, and indicates that this population hardly originates from the two Thai population included in this study (although it is still possible that Nic–1 still may origin from other Thai populations). The 110-allele in *Pgm–2* only appears in the Indian population also indicating that none of the

Table 2 Allele frequencies, Wright's fixation index (*F*), test for deviation from Hardy-Weinberg proportions (P<0.05 corresponds to significant deviations) based on GUO & THOMPSON's (1992) Monte Carlo permutation test, and sample size (N)

Locus	Allele	Population							
		Thai–1	Thai–2	Laos	Nic–1	Nic–2	Tanz–1	Tanz–2	India
Dia	105	0.00	0.01	0.00	0.00	0.00	0.01	0.00	0.00
	100	1.00	0.95	1.00	0.96	0.88	0.99	1.00	0.93
	92	0.00	0.04	0.00	0.04	0.13	0.00	0.00	0.07
	F	–	–0.04	–	–0.04	0.09	–0.01	–	–0.08
	P	–	1.000	–	1.000	0.456	1.000	–	1.000
	N	40	40	36	40	40	40	40	40
Got–1	110	0.00	0.00	0.00	0.01	0.00	0.03	0.03	0.22
	100	0.93	0.94	0.96	0.80	0.97	0.58	0.70	0.46
	93	0.07	0.06	0.04	0.02	0.01	0.37	0.28	0.00
	85	0.00	0.00	0.00	0.17	0.02	0.02	0.00	0.32
	F	0.64	–0.06	0.65	0.13	–0.02	0.26	0.42	–0.15
	P	0.006	1.000	0.043	0.415	1.000	0.043	0.000	0.000
	N	43	60	36	70	56	79	79	60
Got–2	156	0.00	0.00	0.03	0.01	0.00	0.01	0.05	0.00
	130	0.00	0.01	0.00	0.00	0.00	0.05	0.05	0.01
	100	1.00	0.99	0.97	0.99	1.00	0.94	0.90	0.99
	F	–	–0.01	–0.03	–0.01	–	0.18	0.10	–0.01
	P	–	1.000	1.000	1.000	–	0.209	0.242	1.000
	N	43	55	36	70	56	80	63	60
Gpi	103	0.00	0.00	0.00	0.02	0.00	0.00	0.01	0.00
	100	0.99	1.00	1.00	0.98	1.00	1.00	0.99	1.00
	F	–0.01	–	–	–0.02	–	–	–0.01	–
	P	1.000	–	–	1.000	–	–	1.000	–
	N	43	55	36	30	56	47	68	33
Tpi–1	100	1.00	1.00	1.00	1.00	1.00	0.98	1.00	1.00
	86	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00
	F	–	–	–	–	–	–0.02	–	–
	P	–	–	–	–	–	1.000	–	–
	N	43	55	36	30	56	57	68	33
Tpi–2	110	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00
	100	1.00	1.00	0.94	1.00	0.93	1.00	0.99	1.00
	85	0.00	0.00	0.06	0.00	0.07	0.00	0.00	0.00
	F	–	–	0.47	–	–0.08	–	–0.01	–
	P	–	–	0.100	–	1.000	–	1.000	–
	N	43	55	36	30	56	57	68	33
Est	125	0.001	0.07	0.01	0.02	0.03	0.07	0.01	0.00
	100	0.99	0.93	0.99	0.98	0.97	0.93	0.99	0.98
	90	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02
	F	–0.01	–0.08	–0.01	–0.02	–0.03	–0.08	–0.01	–0.02
	P	1.00	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	N	43	55	36	30	56	57	68	33

landraces hardly descent directly from this population. Of course, the four samples from the natural distribution area (Thai–1, Thai–2, Laos and India) cannot be be used to infer on the actual origin of the land races, because they only represent a small fraction of the full

Table 2 *(continued)*

Locus	Allele	Population							
		Thai–1	Thai–2	Laos	Nic–1	Nic–2	Tanz–1	Tanz–2	India
Pgm–1	110	0.00	0.03	0.00	0.00	0.11	0.06	0.00	0.00
	100	1.00	0.97	1.00	1.00	0.89	0.94	1.00	1.00
	F	–	–0.03	–	–	–0.12	–0.07	–	–
	P	–	1.000	–	–	1.000	1.000	–	–
	N	43	51	20	21	55	57	48	60
Pgm–2	*110*	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.10
	100	1.00	1.00	1.00	1.00	0.99	1.00	0.98	0.90
	90	0.00	0.00	0.00	0.00	0.01	0.00	0.02	0.00
	F	–	–	–	–	–0.01	–	–0.02	0.07
	P	–	–	–	–	1.000	–	1.000	0.436
	N	43	51	20	21	55	57	48	60

Table 3 Genetic diversity. Expected heterozygosity (H_e), number of alleles (N_A), and fraction of polymorphic loci (99%)

Locus	Allele	Population							
		Thai–1	Thai–2	Laos	Nic–1	Nic–2	Tanz–1	Tanz–2	India
Dia	H_e	0.00	0.10	0.00	0.07	0.22	0.03	0.00	0.14
	N_A	1	3	1	2	2	2	1	2
Got–1	H_e	0.13	0.11	0.08	0.33	0.05	0.53	0.44	0.64
	N_A	2	2	2	4	3	4	3	3
Got–2	H_e	0.00	0.02	0.06	0.03	0.00	0.11	0.18	0.02
	N_A	1	2	2	2	1	3	3	2
Gpi	H_e	0.02	0.00	0.00	0.01	0.00	0.00	0.03	0.00
	N_A	2	1	1	2	1	1	3	1
Tpi–1	H_e	0.00	0.00	0.00	0.00	0.00	0.04	0.00	0.00
	N_A	1	1	1	1	1	2	1	1
Tpi–2	H_e	0.00	0.00	0.11	0.00	0.13	0.00	0.02	0.00
	N_A	1	1	2	1	2	1	2	1
Est	H_e	0.02	0.14	0.03	0.03	0.05	0.13	0.02	0.03
	N_A	2	2	2	2	2	2	2	2
Pgm–1	H_e	0.00	0.06	0.00	0.00	0.20	0.12	0.00	0.00
	N_A	1	2	1	1	2	2	1	1
Pgm–2	H_e	0.00	0.00	0.00	0.00	0.02	0.00	0.03	0.18
	N_A	1	1	1	1	2	1	2	2
Mean	H_e	0.02	0.05	0.03	0.05	0.08	0.10	0.08	0.11
	N_A	1.33	1.67	1.44	1.78	1.78	2.00	2.00	1.67
	P(99%)	0.33	0.44	0.44	0.44	0.56	0.67	0.44	0.44
Relative to India	H_e(%)	18	42	27	48	67	94	71	100
	N_A(%)	80	100	87	107	107	120	120	100
	P(99%)	75	100	100	100	125	150	100	100

distribution of teak. Studies covering the full genetic variation are needed for that purpose.

Wright's *F*-statistics supports the apparent large population differentiation. The multilocus F_{ST} based on the four natural populations was found to be 0.27 (with standard error of 0.141). The estimated multilocus F_{IS}

Table 4 Estimated frequency of silent allele (*p*) at *Got–2*, test for deviation from expected phenotypic distribution under the assumption of random mating subject to (i) existence ($G_{(+silent)}$), and (ii) non-existence of the silent allele ($G_{(-silent)}$)

Population	*p*	$G_{(+silent)}$	*P(i)*	$G_{(-silent)}$	*P(ii)*
India	0.00	28.0	0.000***	28.0	0.000***
Tanz–2	0.24	12.8	0.005*	16.7	0.001**
Thai–1	0.12	4.2	0.046	5.3	0.033
Laos	0.10	3.1	0.113	15.3	0.021
Tanz–1	0.12	3.1	0.250	8.6	0.032
Nic–1	0.04	2.8	0.419	3.7	0.296
Nic–2	0.00	0.1	0.994	0.1	0.994
Thai–2	0.00	0.4	0.512	0.4	0.512

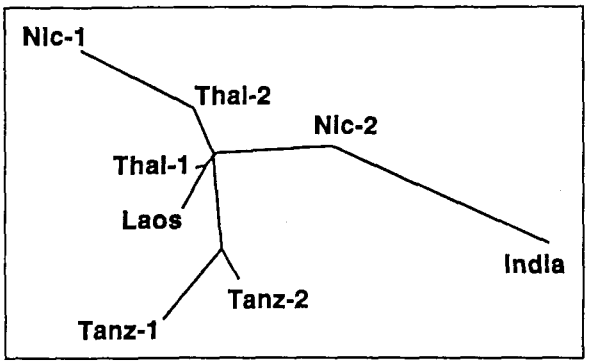

Figure 1 Phylogenetic maximum likelihood tree. The tree is unrooted. Branch lengths indicate the expected accumulated variance due to drift. Angles between branches are arbitrary.

was low, –0.026, but determined with a large standard error, 0.103. The multilocus F_{ST} based on all popula tions, i.e. both landraces and natural populations, was found to be 0.125 with a standard error of 0.030. The multilocus F_{IS} for this mixture of populations is 0.101 with and standard error of 0.063, *i.e.*, not significantly different from zero.

DISCUSSION

Deviation from Hardy-Weinberg proportions

Three of the examined populations were found to deviate significantly from Hardy-Weinberg proportions at the *Got–1* locus. Two of the populations deviated due to surplus of homozygotes (positive *F*-value), one due to deficit of homozygotes (negative *F*-value). Deviation from random mating such as selfing or close-neighbour mating will result in positive *F*-values. It can also be a result of seed contamination, *i.e.*, if the examined population actually are mixed from two or more separate population (Wahlund effect), large sampling units that actually covers more than one panmictic unit, and – theoretically – natural selection at the locus.

Another explanation can be the presence of a silent allele at the *Got–1* locus, which is supported by *one* observation of such an apparent homozygote (missing band) in *one* population, Tanz–1 (stained normally at the *Got–2* locus). If a silent allele is present, but neglected in the calculations, this will result in a positive *F*-value even in the presence of random mating (BROWN 1979). The presence of a silent allele was examined further by assuming its existence, and estimating the expected frequency from the phenotypic distribution assuming random mating. Maximum likelihood estimates were obtained by so-called "gene counting", calculations follow ØSTERGÅRD *et al.* (1985). The estimated allele frequencies calculated this way are high, up to 0.24 for Tanz–2 (0.12 for Thai–1, 0.12 for Tanz–1, 0.10 for Laos, 0.04 for Nic–1, and 0.00 for India, Thai–2, Nic–2). The deviations between the expected and observed phenotypic distributions remained significant in India and Tanz–2 even when a silent allele were assumed to be present in these frequences. We do not consider such high frequencies to be very likely. We therefore reject the hypothesis that the positive *F*-values are generated merely by silent alleles.

Two recent studies of teak have shown that teak is a mainly outcrossing species. KERTADIKARA and PRAT (1995) found a high outcrossing rate (t_m = 0.98) based on a sample from India, and KJÆR and VERAPONG (1995) found similar high outcrossing rate (t_m = 0.95) in a Thai population. This small amount of selfing corresponds to an equilibrium *F*-value of $F = s/(2–s)$ = 0.01–0.05 (HARTL & CLARK 1989, p. 262). Selfing can therefore not explain the high *F*-values found in this study, if we assume that the breeding system of the investigated populations do not differ significantly from the populations studied by KERTADIKARA and PRAT (1995) and KJÆR and VERAPONG (1995). Variation between teak populations in mating parameters is of course a possibility, as this is known from other species (KARRON 1992). The highly significant surplus of heterozygotes in the Indian population is more puzzling.

Maybe the negative *F*-value is due to pollen-flow from a genetic distant population into the examined stand. This will generate a surplus of heterozygotes depending on the differences in allele frequencies in pollen and the mother population (JONG *et al.* 1994).

Genetic Diversity

Differences in genetic diversity measured as average expected heterozygosity was found in this study. The main differences were found between the three populations from the Laos-Thailand part of the natural distribution area on the one hand and the population from India on the other. The Thai-Laos populations had only 18–42% of the expected heterozygosity found in the Indian population. Similar low diversity of a Thai population compared to populations from India was found by KJÆR, SIEGISMUND and VERAPONG (1996). KERTADIKARA & PRAT (1995) also relativ high heterozygosity in Indian populations, but the differences found in their study were much smaller. Also, the general level of diversity in their study was much higher. The origin of the apparently relatively lower diversity of the Thai-Laos populations compared to the Indian populations found in the present study is not known. The Indian and Thai parts of the natural distribution area are geographically separated and probably have been so for thousands of years (KERTADIKARA 1992). Distinct morphological characteristics between populations from the two areas (BINGCHAO and ZHENG 1986) support the theory that they have a genetically distant relationship. Thai and Laos provenances are well adapted to their natural habitats. World wide transplanting experiments have shown, however, that Thai- and especially Laos-provenances generally perform poorly compared to Indian provenances when used outside the natural distribution areas (KJÆR *et al.* 1995, Figure 63, 71 and 72). Whether this has any connection to the general level of genetic diversity remains an open question that cannot be answered by the present study. Other studies of forest trees have shown little concordance between adaptive traits and biochemical markers (SAVOLAINEN 1994), though some empirical evidence support a connection (BUSH & SMOUSE 1992).

The landraces were found to have genetic diversity intermediate between Thai-Laos and the Indian populations, the Tanzanian populations being more variable than the Nicaraguans. Among the Tanzanian populations, the 2nd generation in Tanzania (Tanz–1) was found to have more genetic variation than the first generation stand (Tanz–2). We do not know the exact origin of these landraces, but the results are in concordance with the historical record of interprovenance hybridisation in Tanzania as described in the introduction. Also, our results indicate, that the diversity of the Nicaraguan populations may have been reduced during introduction, if the starting point has been a level of diversity comparable to the Indian. However, bearing in mind the differences in diversity between the natural populations, we can not exclude the possibility that the observed differences between Nicaraguan and Tanzanian landraces are merely founder effects.

The general level of allozyme diversity in this study is low compared to the diversity found in the comparable study by KERTADIKARA and PRAT (1995). Especially, the very low diversity in the Thai/Laothian populations were not observed in their study. Kertadikara used polyacrylamide gel electrophoresis (KERTADIKARA 1992), but we used a combination of starch and cellolose acetate gel electrophoresis. It would seem that the former technique gives the best resolution in the case of teak, as several of the loci found polymorphic by Kertadikara appeared monomorphic when studied on both starch and cellulose acetate. The developmental stage were almost the same in the two studies, although Kertadikara used seedlings that were a little older.

The multilocus F_{ST} based on the four natural populations only, was found to be high, 0.21, and 0.13 based on all populations. This is in good concordance with results of KERTADIKARA & PRAT (1995), who found a F_{ST} value of 0.12 based on 8–9 teak populations including both natural populations and landraces. These F_{ST} values are relative high for tree species in general (HAMRICK & GODT 1989), although not unusual for tropical tree species (LOVELESS 1992; HAMRICK 1994).

ACKNOWLEDGEMENTS

We thank Mrs. Kirsten R. Sørensen, Bente Rasmussen, and Merete Linnet for excellent assistance with the laboratory work. The staffs at Centro de Mejoramiento Genetico y Banco de Semillas Forestales, Nicaragua, The National Tree Seed Project, Tanzania, The Teak Improvement Centre, Thailand, Institute of Forest Genetics and Tree Breeding, Coimbatore, India, and Danida Forest Seed Centre, Denmark, were most helpful in providing seed and information. Verapong Suangtho and Apichard Kaosa-Ard are acknowledged for important discussions in Thailand. Tim Boyle and an anonymous reviewer contributed with valuable suggestions. This study was supported by Danida Forest Seed Centre and The Danish Research Council for Development Research.

REFERENCES

BINGCHAO, K. & ZHENG, S. Z. 1986: Studies on morphology and anatomy of teak (*Tectona grandis* L.) provenance. Mimeographed. 22p. The Research Institute of Tropical Forestry. Chinese Academy of Forestry.

BROWN, A. H. D. 1979: Enzyme polymorphism in plant populations. *Theoretical Population Biology* **15**:1–42.

BUSH, R. H. & SMOUSE, P. E. 1992: Evidence for the adaptive significance of allozymes in forest trees. *New Forests* **6**:179–196.

FELSENSTEIN, J. 1981: Evolutionary trees from gene frequencies and quantitative characters: finding maximum likelihood estimates. *Evolution* **35**:1229–1242.

FELSENSTEIN, J. 1993: PHYLIP (Phylogeny Inference Package) version 3.5c. Distributed by the author. Department of Genetics, University of Wastington, Seattle.

GUO, S. W. & THOMPSON, E. A. 1992: Performing the exact test for Hardy-Weinberg proportion for multiple alleles. *Biometrics* **48**:361-372.

HAMRICK, J. L. 1994: Genetic diversity and conservation in tropical forests. *In:* Proceedings from The International Symposium on Genetic Conservation and Production of Tropical Forest Tree Seed. ASEAN–Canadian Forest Tree Seed Centre Project, Muak-Lek, Saraburi, Thailand.

HAMRICK, J. L. & GODT, M. J. W. 1989: Allozyme diversity in plants. *In*: Plant Population Genetics, Breeding and Genetic Resources (eds. Brown, A. H. D., Clegg, M. T., Kahler, A. L. & Weir, B. S.), p.282–298. Sinauer Associates Inc. Massachusetts.

HARTL, D. L. & CLARK, A. C. 1989: Principles of Population Genetics. 2nd edition. Sinauer Associates, Inc. Sunderland. Mass.

HEBERT, P. D. N. & BEATON, M. J. 1989: Methodologies for Allozyme Analysis Using Cellulose Acetate Electrophoresis. A Practical Handbook. Department of Biological Sciences. Great Lake Institute, University of Windsor, Windsor, Ontario.

HOLM, S. 1979: A simple sequentially rejective multiple test procedure. *Scandinavian Journal of Statistics* **6**:65–70.

JONG, G., RUITER, J. R. & HARING, R. 1994: Genetic structure of a population with social structure and migration. *In*: Conservation Genetics (eds. Loeschcke, V., Tomiuk, J. & Jain, S. K.). pp 147–164. Birkhäuser Verlag. Basel.

KAOSA-ARD, A. 1981: Teak, *Tectona grandis*, its natural distribution and related factors. *Nat. Hist. Bull. Siam.Soc.* **29**:54–74.

KARRON, J. D. 1992: Patterns of genetic variation and breeding systems in rare plant species. *In*: Genetics and Conservation of Rare Plants (eds. D. A. Falck & K. E. Holsinger). Oxford University Press. pp. 87–98.

KEIDING, H., WELLENDORF, H. & LAURIDSEN, E. B. 1986: Evaluation of an International Series of Teak Provenance Trials. Danida Forest Seed Centre. DK–3050 Humlebæk, Denmark.

KEIDING, H. 1989: Seed collection from teak plantations at Mtibwa and Longuza, Tanzania. Mimeographed 7pp. Danida Forest Seed Centre. DK–3050 Humlebæk, Denmark.

KEOGH, R. M. 1980: Teak provenances of the Carribian, central America, Venezuela and Colombia. *In*: Proceedings from IUFRO Symposium on wood production in the neotropics. Institute for Tropical Forestry, Rio Piedras, Puerto Rica.

KERTADIKARA, A. W. S. 1992: Variability Genetique de Quelques Provenances de Teck (*Tectona grandis* L.f.) et leur Aptitude a la Multiplication Vegetive. These de Docteur. L'Universite de Nancy, France.

KERTADIKARA, A. W. S. & PRAT, D. 1995: Genetic structure and mating system in teak (*Tectona grandis* L.f.) provenances. *Silvae Genetica* **44**:104–110.

KERTADIKARA, A. W. S. & PRAT, D. 1995: Isozyme variation among teak (*Tectona grandis* L.f.) provenances. *Theoretical and Applied Genetics* **90**:803–810.

KJÆR, E. D. & FOSTER, G. S. 1996: The economics of tree improvement of teak. *DFSC Series of Technical Notes* (in press). Danida Forest Seed Centre. DK–3050 Humlebæk, Denmark.

KJÆR, E. D., LAURIDSEN, E. B. & WELLENDORF, H. 1995: Second Evaluation of an International Series of Teak Provenance Trials. Danida Forest Seed Centre. DK–3050 Humlebæk, Denmark.

KJÆR, E. D., SIEGISMUND, H. R. & SUANGTHO, V. 1996: A multivariate study on genetic variation in teak (*Tectona grandis* L.). (submitted).

KJÆR, E.D. & VERAPONG, S. 1995: Outcrossing rate of teak (*Tectona grandis* L.). *Silvae Genetica* **44**:145–147.

LOVELESS, M. D. 1992: Isozyme variation in tropical trees: patterns of genetic diversity. *New Forests* **6**:67–94.

MANCHENKO, G. P. 1994: Handbook of Detection of Enzymes on Electrophoretic Gels. CRC Press, Florida, 341 pp.

MADOFFÉ, S. S. & MAGHEMBE 1988: Performance of teak (*Tectona grandis L.f.*) provenances seventeen years after planting at Longuza, Tanzania. *Silvae Genetica* **37**:175–178.

NEI, M. 1987: Molecular Evolutionary Genetics. Columbia University Press.

ØSTERGÅRD, H., NIELSEN, G. & JOHANSEN, H. 1985: Genetic variation in cultivars of diploid ryegrass, *Lodium perenne* and *L. multiflorum*, at five enzyme systems. *Theoretical and Applied Genetics* **69**:409–421.

RICE, W. R. 1988: Analyzing tables of statistical tests. *Evolution* **43**:223–225.

SAVOLAINEN, O. 1994: Genetic variation and fitness: Conservation lessons from pines. *In*: Conservation Genetics (eds. Loeschcke, V., Tomiuk, J. & Jain, S. K.). pp. 27–36. Birkhäuser Verlag. Basel.

SOKAL, R. R. & ROHLF, F. J. 1981: Biometry. 2nd edition. W.H. Freeman and Company. San Francisco.

WADSWORTH 1960: Datos de crecrecimiento de plantaciones forestales en Mexico, Indias Occidentales y Centro y Sur America. *Carribian Forester* **21** (supl). Here cited after Keogh (1980).

WEIR, B. S. & COCKERHAM, C. C. 1984: Estimating *F*-statistics for the analysis of population structure. *Evolution* **48**: 1358–1370.

WENDEL, J. F. & WEEDEN, N. F. 1989: Visualization and interpretation of plant isozymes. *In*: Isozymes in Plant Biology (eds. Soltis, D. E & Soltis, P. S.), pp.5–45. Chapman and Hall. London.

WHITE, K. J. 1991: Teak. Some aspects of research and development. RAPA publication 1991/17. FAO Regional Office for Asia and Pacific. Bangkok.

WOOD, P. J. 1967: Teak planting in Tanzania. Proceedings from FAO world symposium on man-made forests. Document 3:1631–1644. Canberra, Australia.