For 24 seedlings, 8 from a healthy and 16 from 2 damaged *P. abies* trees (*Fig. 2*) cultivated in the same area within a distance of about 100 m (Selketal of the Harz mountains), the SCE frequency was compared. 960 chromosomes corresponding to 40 metaphases in each variant were evaluated. On average, 38.7±6.1 SCEs/metaphase in seedlings from the healthy and 66.1±13.1 or 83.2±13.4 SCEs/metaphase in seedlings from the damaged trees were scored (*Tab. 1* and *Fig. 2*). The differences between healthy and damaged trees were significant ($P < 0.001$) according to the MANN-WHITNEY test. This may indicate either more DNA lesions or a less efficient removal of lesions during the first postgermination cell cycles in seedlings of damaged as compared to seedlings of healthy spruce trees.

All seedlings descending from one individual showed the same tendency although many of these should be genetically heterogeneous due to cross-pollination. It therefore seems to be probable that the SCE frequency is maternally determined in these cases. For example, limited supply of cations (such as Mg^{2+}) to the mother tree necessary for normal functioning of enzymes responsible for correct repair of DNA damage could result in higher amounts of DNA lesions not removed before DNA replication and thus giving rise to an increased SCE frequency. Experiments are under way to test the validity of this hypothetic explanation.

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Outcrossing Rate of Teak (*Tectona grandis* (L.))

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Summary

The outcrossing rate of (*Tectona grandis* (L.)) was estimated from allozyme segregation in progenies from 15 teak trees collected near Ngao, Thailand. The average single-locus outcrossing rate was found be 0.89, with a standard error based on the variation between loci of 0.08. The multilocus outcrossing rate was estimated to be 0.95 with a standard error based on re-sampling between progenies within families (bootstrapping) of 0.07. The results suggest that teak is mainly a outcrossing species. This result is in agreement with experiences from controlled pollinations.

Key words: Tectona grandis, mating system, allozymes.

FDC: 165.4; 176.1 *Tectona grandis.*

Introduction

Tectona grandis (L.) is a mainly insect pollinated tropical tree species with a large natural distribution in South East Asia (KAOSA-ARD, 1981). It has a long history as a plantation species due to its valuable timber and today it is of major importance in many plantation programs throughout the tropical world. Tree improvement activities were initiated in Thailand (KAOSA-ARD, 1993) and India (KUMERAVELU, 1993) in the 1960s. Today, tree improvement is recognized as an important part of plantation programs in many countries (see KJÆR and FOSTER, 1995, for references).

The flower biology and mating system attracted early attention (GRAM and LARSEN, 1958; BRYNDUM and HEDEGART, 1969; HEDEGART, 1973) because low fruit and seed yield were found to be serious obstacles to large scale propagation in seed orchards. One hectare of clonal seed orchard can for example only produce seed sufficient for a 16 ha teak plantation with the prevailing nursery technique (WELLENDORF and KAOSA-ARD, 1988).

The pollination studies by BRYNDUM and HEDEGART (1969) found that many apparent pollen vectors operated only within the crown (e.g. ants). Controlled crosses showed, however, that the investigated trees were almost self-incompatible. In this study the outcrossing rate is estimated by isozyme markers on trees of the same origin as were used in the BRYNDUM and HEDEGART studies.

Material and Methods

Seeds were collected in January, 1994, by climbing 20 selected trees in the seed stands (seed production areas) close to the Teak Improvement Center, Ngao, Lampang. The stands were planted in the 1940s. They are located close to natural stands and are within the natural distribution area of teak.

The seeds were germinated after pre-treatment (80 °C for 48 hours followed by 6 hours soaking in water) at 34 °C with alternating 12 hours of light and dark. Five families were excluded from the study due to insufficient germination.

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The seedlings were homogenized in an extraction buffer (WENDEL and WEEDEN (1989) no. 2, modified by adding 3 mM Dithiothreitol (DTT) and omitting mercaptoethanol). Allozyme variation at 4 loci (Glutamate-oxaloacetate (GOT, E.C. No. 2.6.1.1.), 2 loci; phosphogluco-mutase (PGM, E.C. No. 2.7.5.1), 1 locus; diaphorase (DIA, E.C. No. 1.6.2.2.), 1 locus) were resolved by starch gel electrophoresis. GOT and DIA were resolved in a TRIS-EDTA-borate buffer, pH=8.6 (WENDEL and WEEDEN, type 7) and PGM in a TRIS-citrate buffer, pH=7.0 (WENDEL and WEEDEN, type 3). A larger number of isozymes was screened, but other isozyme loci were excluded due to lack of heterozygotes among the 15 families, poor resolution of the zymogram, or difficulties in interpretation of the zymogram. A total of 235 seedlings were analyzed.

The maternal genotypes were inferred from the segregation in the progenies. The single locus outcrossing rate (t_s) was estimated for each locus by 2 different maximum likelihood algorithms, where gene frequencies in ovules and pollen were estimated simultaneously with the outcrossing rate through iteration. Estimates were calculated by the MLT-programme (RITLAND, 1990), which finds estimates of the outcrossing rate via the Newton-Raphson method. It is not restricted to estimate parameters within a biological feasible parameter range, and could therefore estimate outcrossing rates larger than 1. Estimates above 1 were therefore, posterior, restricted to be 1.00. New estimates for gene frequencies for these loci were then calculated for t_s = 1.00. For comparison, maximum likelihood estimates of the outcrossing rates were also calculated following the EM (Expectation Maximization)-algorithm as described by WEIR (1990), p.176-179, which estimates outcrossing rates subject to the constraint that the it should not exceed 1.

A multi-locus estimate of outcrossing rate was estimated with corresponding gene frequencies following the procedure by RITLAND and JAIN (1981). Again, calculations were done by the MLT-programme (RITLAND, 1990). The sampling properties of this multi-locus outcrossing estimate were examined by numerical resampling of progenies within families. The distribution of the m genotypes within each family was viewed as a frequency distribution for that particular family. Each of the re-sampled data sets were created by "drawing" m "new" progeny genotypes with probabilities according to this frequency distribution (bootstrapping). 500 bootstrapped multi-locus estimates were calculated, one for each of the numerical re-sampled data sets. Please refer to CROWLEY (1992) for details on bootstrapping.

Mendelian segregation was tested by Chi²-tests of the segregation of heterozygotes which, under the hypothesis of Mendelian segregation, is expected to be 1:1 in homozygotes : heterozygotes.

Results

The inferred maternal genotypes are presented in *table 1* together with the estimates of single-locus outcrossing rates. The single-locus estimates according to both the MLT-programme and EM-algorithm vary from 0.70 (*DIA*) and 0.87 (*GOT1*) to 1.00 (*GOT2* and *PGM*). The average single-locus outcrossing estimate was thus 0.89 with a standard error of 0.08. The estimates for *GOT1* and *PGM* were both estimated by the MLT-programme to be above 1, and therefore posteriori restricted to 1.00.

The multi-locus estimate (t_m) of the outcrossing rate according to RITLAND and JAIN (1981) was found to t_m = 0.95. The distribution of the t_m-estimates from the 500 re-samples of the data are shown in *figure 1*. The estimates arrange around t_m = 0.95 in a Gaussian distributed manner except for a few outliers. 95% of the distribution is located between t_{low} = 0.85 and t_{high} = 1.10. 23% of the bootstrapped estimates were larger than 1. Posteriori restriction of these values was omitted in order to give a better picture of the influence of sampling errors on the location of the maximum of the likelihood function. The standard deviation of bootstrapped t_m-estimates was found to

Table 1. – Maternal genotypes (inferred), estimate of outcrossing rates, allele frequencies and Chi²-test for MENDELian segregation.

Seed tree no	Genotype DIA	GOT 1	GOT 2	PGM
1	22	11	22	12
2	22	11	22	22
3	22	11	22	22
4	22	11	22	22
5	22	11	12	22
6	22	11	12	22
7	22	11	22	22
8	22	12	22	22
9	22	11	22	22
10	22	11	22	22
11	22	11	12	22
12	22	11	22	22
13	22	11	22	22
14	12	11	22	22
15	22	11	22	22
Allele[1] frequency (p) in pollen:	0.99	0.97	0.98	0.99
Allele[1] frequency (p) in ovules :	0.96	0.97	0.90	0.97
Mendelian segregation: Chi²(df=1)	3.20^{NS}	2.78^{NS}	3.13^{NS}	0.80^{NS}
Single-locus outcrossing rate:	0.87	1.00	0.70	1.00
Average single-locus outcrossing rate		0.89		
Standard error[2]		0.08		
Multi-locus outcrossing rate:		0.95		
Standard error[3] (not restricted)		0.22		
Standard error[3] (restricted $t_m \leq 1$)		0.07		

[1]) Most common allele, estimates correspond to the multi-locus outcrossing rate.

[2]) Based on variation between loci.

[3]) Based on numerical re-sampling of progenies within families.

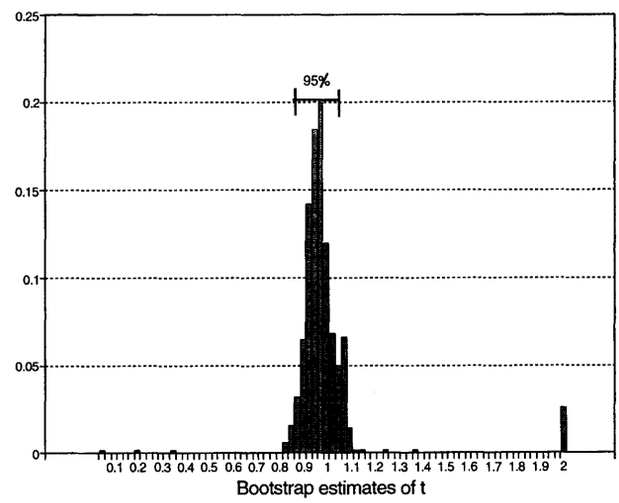

Figure 1. – Distribution of 500 estimates of multi-locus outcrossing rate based on re-sampling of the data (bootstrapping).

be high, 0.22, mainly due to the few outliers. If all estimates above 1 are restricted to 1.00, then the standard deviation will be substantial lower, 0.07. The distribution of the bootstrapped estimates will of course be highly screwed by this restriction.

The Chi^2 tests for Mendelian segregation were accepted for all loci (*Table 1*).

Discussion

The single locus estimates according to the NEWTON-RAPHSON and EM-algorithms gave the same results when the outcrossing estimate according to the NEWTON-RAPHSON algorithm posteriori was imposed the constraint, that they should not exceed 1. The number of iterations used in the 2 algorithms was quite different. The NEWTON-RAPHSON algorithm only required a few iterations before the estimates showed acceptable convergence, whereas the EM-algorithm used hundreds of iterations. The differences were of no practical importance in terms of computer-resources as both algorithms obtained the results within few seconds when calculated on a modern personal computer.

The multi-locus estimate of outcrossing rate was found to be high, 0.95. 13 of the bootstraps (2.6%) resulted in very high t-estimates, $t_m = 2.0$ (*Figure 1*). These high estimates are clearly outside the acceptable parameter range as discussed above. They are of low frequency, but increase the standard deviation of the bootstrapped values significantly. When looking at *figure 1,* it is clear that the large standard error, 0.22, give a poor impression of the actual distribution of the re-sampled estimates. It is much more informative to look at the actual distribution of the bootstrapped values.

The average single-locus outcrossing rate was found to be a little lower than the corresponding multi-locus estimate. This is a common feature, because the multi-locus model is more powerful than the single-locus models in detecting outcrossing progenies.

To conclude, this study suggests that the investigated trees have produced their progenies without any significant amount of selfing. This result is consistent with the earlier observations in Thailand of barriers to self-pollination based on studies of controlled crosses combined with behavior of pollinators (HEDEGART, 1973).

KERTADIKARA (1992) found a similar high outcrossing rate ($t_m = 0.98$) based on an isozyme study of progenies from Karnataka, India. The teak forests of Karnataka are (partly) located within the "Dry Interior" (according to classification of CHAMPION and SETH, 1964). Mating systems of plants are frequently reported to depend on environmental conditions and developmental stages and are subject to genetic variation within and between populations (BROWN et al., 1989; KARRON, 1991). In this case, however, the reproduction in the semi-moist Thai forests has resulted in the same high outcrossing rates as was found in Kertadikara's study.

The few tropical tree species investigated so far, have generally been found to be predominantly outcrossing (LOVELESS, 1992; BAWA, 1992). The result from this study shows that teak fits into the general pattern.

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