Callusing occurred on all media used, although amounts increased with increasing concentrations of IBA. The survival of *in vitro*-rooted shoots following weaning, however, indicates that the callus did not interfere with vascular connections within the plants.

Under non-sterile conditions over 90% of *C. sempervirens* shoots rooted, regardless of the use of NAA-based rooting powder. FRANCO and SCHWARZ (1985) reported *ex vitro* rooting of *C. lusitanica*, although repeated application of a solution containing 2.8 mg.l^{-1} NAA was necessary to induce 30% rooting in this species. The use of an *ex vitro* rooting step greatly improves the economic efficiency of micropropagation (CONSTANTINE, 1987), and increases the likelihood of such a system proving viable for *Cupressus* spp. and *Chamaecyparis* spp..

Multiplication (6fold to 10fold), rooting (up to 95%) and weaning survival (>90%) rates obtained with *C. sempervirens* and *Chamaecyparis lawsoniana* were acceptable for commercial production. *C. sempervirens* also rooted in compost and survived weaning in acceptable numbers. The methods described in this paper provide a sound basis for the development of efficient techniques for the production of *Cupressus* species using micropropagation, and for the rapid increase of genotypes showing resistance to *Seiridium* canker in screening programmes (SPANOS, 1995).

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Genetic Variation Across the Natural Distribution of the South East Asian Pine, *Pinus kesiya* ROYLE ex GORDON (Pinaceae)

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Summary

Allozyme variation has been assessed in 9 populations (172 families) of the economically important south east Asian pine, *Pinus kesiya* ROYLE ex GORDON, from across its entire geographical range, using 12 isozyme loci identified by 7 enzyme systems. The mean percentage of polymorphic loci per population, the mean number of alleles per locus and the mean genetic diversity within populations were 45.4%, 1.6 and 0.153 respectively. Genetic diversity was greatest in populations from the south-eastern populations and lowest in north-western populations. In the present study G_{ST} (0.121) was high compar-

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ed to other studies (G_{ST} = 0.023 and 0.039), that sampled a restricted part of the geographic range. Two theories have been proposed to explain the present distribution of *P. kesiya*: (i) migration from the south through Malaysia and the Philippines; (ii) migration from the north. Data based on the analysis of isozymes would support migration from the south.

Key words: Pinaceae, *Pinus kesiya*, isozymes, genetic variation, geographic variation.

FDC: 165.5; 181.1; 174.7 *Pinus kesiya*; (59).

Introduction

Pinus kesiya (ROYLE ex GORDON) is part of a complex of taxonomically poorly known south east Asian tropical pines that also includes *P. tabulaeformis* CARR., *P. yunnanensis* FRANCH and *P. langbianensis* A. CHEV. *Pinus kesiya* has a wide, but fragmented distribution, in China, India, Laos, Myanmar, Philippines, Thailand, Tibet and Vietnam, with altitudinal ranges from 350 m to 2900 m above sea level (a.s.l.). ARMITAGE and BURLEY (1980) recognised 9 groupings of populations, including the Chin Hills – Naga Hills (Myanmar – India border) region; the Shan Hills – Chiang Mai (Myanmar – Thailand border) region; the north-central Thailand region; the southern Vietnam region and the Philippines region. The largest area of *P. kesiya* distribution is the Shan Hills – Chiang Mai region, with much smaller areas elsewhere. The species occurs in a wide range of forest types from groups of trees scattered through temperate or subtropical forests dominated by evergreen broad-leaved species, through pure stands on steep slopes and ridges to savannah forest. This pattern appears to reflect both climatic and edaphic conditions and the influence of man through fire and shifting cultivation (ARMITAGE and BURLEY, 1980).

Pinus kesiya is a moderately resinous species and has properties that make it useful for a wide range of either specific and general purposes when sawn or for pulp production. Although it is not decay resistant it does season and finish well. The species has been particularly popular in Zambia and Madagascar, where tens of thousands of hectares of plantation have been established. However, nothing is known of the genetic variation that is represented within these plantations, although the Madagascan introductions are thought to be derived from Vietnamese provenances (ARMITAGE and BURLEY, 1980).

The ecological amplitude of *Pinus kesiya* might suggest that genetic diversity within and between populations should be similarly diverse (HAMRICK *et al.*, 1992). However, allozyme studies from limited parts of the geographical range (Chiang Mai region of Thailand) reveal that there was a high level of genic diversity (He = 0.148; BOYLE *et al.*, 1991b; SZMIDT *et al.*, 1996), but low differentiation between populations (G_{ST} = 0.023; BOYLE *et al.*, 1991b; SZMIDT *et al.*, 1996) compared to other pine species with similar distribution patterns (e. g. *P. merkusii*; CHANGTRAGOON and FINKELDEY, 1995; SZMIDT *et al.*, 1996). Unfortunately, whilst these studies have focused on the main part of the *P. kesiya* distribution, potentially important populations in extreme north west and south east of the mainland distribution have been overlooked. The aim of the present investigation was to estimate allozyme variation within and between 9 natural populations of *P. kesiya* that represent the complete mainland south east Asian range of the species.

Material and Methods

Materials

One hundred and seventy two families (5 seeds per family) from 9 populations sampled from across the range of *Pinus kesiya* were studied to assess genetic variation (*Table 1*, *Figure 1*).

Electrophoresis

Electrophoresis was carried out on one day old megagametophyte extracts. The testa of the seed was nicked, sown on

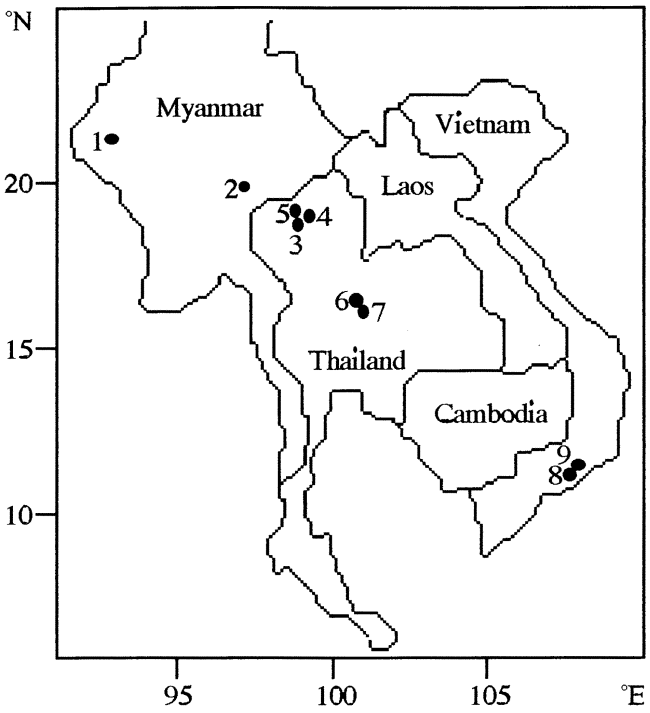

Figure 1. – Distribution of 9 sampled populations of *Pinus kesiya* in south east Asia. Population numbers as in *table 1*.

Table 1. – Localities and environmental data for 9 populations of *Pinus kesiya* populations. MAR – mean annual rainfall. 'n' – number of individuals.

No.	DANIDA No.	Region	Country	n	Lat. (°N)	Long. (°E)	Alt. (m)	MAR (mm)
1	01772	Zokhua	Myanmar	12	22°25'	93°40'	1600	2335
2	01773	Aungban	Myanmar	20	20°41'	96°37'	1350	1303
3	01521	Nong Krating	Thailand	20	18°05'	98°35'	1080	1769
4	01522	Doi Suthep	Thailand	20	18°46'	98°53'	1300	1769
5	01523	Doi Inthanon	Thailand	20	18°32'	98°35'	1000	2084
6	01524	Phu Kradung	Thailand	20	16°51'	101°47'	1300	1813
7	01525	Nam Now	Thailand	20	16°40'	101°33'	800	1316
8	01516	Xuan Tho	Vietnam	20	11°55'	108°32'	1400	1769
9	01519	Lang Hahn	Vietnam	20	11°37'	108°16'	1000	1769

to damp cotton wool in a plastic culture dish, germinated at approximately 25 °C and the diploid embryo separated from the haploid megagametophyte. Megagametophytes were crushed in c. 120 μL extraction buffer (50 ml lithium-borate gel buffer, 37 mg KCl, 10 mg $MgCl_2$, 18 mg EDTA, tetrasodium, 25 mg PVPP, 0.5 ML Triton-X-100, 2 ml β-mercaptoethanol). Enzyme extracts were absorbed onto filter-paper wicks and loaded onto 12% starch gels. Sixteen enzyme systems were tested for activity: aspartate aminotransferase (AAT; 2.6.1.1); aconitase (ACO; 4.2.1.3); alcohol dehydrogenase (ADH; 1.1.1.1); aldolase (ALD; 4.1.2.13); diaphorase (DIA; 1.8.1.4); formate dehydrogenase (FDH; 1.2.1.2); glutamate dehydrogenase (GDH;1.4.1.2); glucose-6-phosphate dehydrogenase (G6PDH; 1.1.1.49); hexokinase (HEX; 2.7.1.1); isocitrate dehydrogenase (IDH; 1.1.1.42); malate dehydrogenase (MDH; 1.1.1.37); malic enzyme (ME; 1.1.1.40); 6-phosphogluconic dehydrogenase (6PGD; 1.1.1.44); phosphoglucose isomerase (PGI; 5.3.1.9); phosphoglucomutase (PGM; 2.7.5.1); shikimate dehydrogenase (SDH; 1.1.1.25). Of these, seven enzyme systems (AAT; IDH; PGM; G6PDH; ALD; 6PGD; MDH) were used to screen all of the populations. Electrophoresis was carried out in either histidine-citrate gels (AAT; IDH; PGM: electrode buffer; 10.09 g L-histidine in 1 L deionised water, pH to 5.7 with citric acid: gel buffer; 1 part electrode buffer to 6 parts deionised water) or tris-citrate gels (ALD; G6PDH; 6PGD; MDH: 16.35 g tris-base in 1 L deionised water, pH to 8.3 with citric acid: gel buffer; 1 part electrode buffer to 14 parts deionised water).

Enzyme nomenclature followed international rules. Upper case letter codes refer to enzyme systems. Mixed upper and lower case codes refer to enzyme loci. For systems controlled by more than one locus, the isozymes were numbered from anode to cathode and each allozyme was also numbered from anode to cathode.

Genetic data analysis

The genotype of each diploid mother tree analysis of 5 megagametophytes per progeny array. The mean number of alleles per locus (A), the percentage of polymorphic loci (P), the observed heterozygosity (H_o) and the expected panmictic heterozygosity (H_e) were calculated using BIOSYS-1 (SWOFFORD and SELANDER, 1981). NEI's unbiased genetic distances (GREGORIUS, 1984; NEI, 1978) were calculated for each pair of populations and the values clustered using the unweighted pair-group mean analysis (UPGMA) of SNEATH and SOKAL (1973). Inter- and intra-population genetic diversity was partitioned using WRIGHT's F-statistics (WRIGHT, 1978). The fixation index (F_{IS}) for polymorphic loci in each population and its averages across both populations and loci were determined. Deviations from HARDY-WEINBERG equilibrium were tested using the χ^2 test. Genetic differentiation between populations was analysed using G-statistics (NEI, 1973, 1987) defined by the formula, $H_T = H_S + D_{ST}$, where H_T is the total gene diversity and H_S is the average gene diversity within populations. D_{ST}, the average gene diversity among populations, was obtained as the difference $H_T - H_S$. The genetic differentiation between populations was obtained as $G_{ST} = D_{ST}/H_T$. The statistical significance of the deviation of G_{ST} from 0 was tested using χ^2 (WORKMAN and NISWANDER, 1970). Significance tests in

Table 2. – Allele frequencies in the 9 *Pinus kesiya* populations. N = sample size. '–' indicates locus not scored. Population designations as in *table 1.*

Locus	Allele	Population								
		1	2	3	4	5	6	7	8	9
Aat-1	(N)	12	20	20	20	20	20	20	20	20
	a	0.000	0.075	0.025	0.025	0.050	0.125	0.050	0.600	0.525
	b	1.000	0.925	0.975	0.975	0.950	0.875	0.950	0.400	0.475
Ald-2	(N)	9	4	15	14	20	16	18	9	13
	a	0.167	0.500	0.700	0.571	0.500	0.594	0.750	0.556	0.731
	b	0.833	0.500	0.300	0.429	0.500	0.406	0.250	0.444	0.269
G6Pdh-1	(N)	12	20	20	20	20	20	20	20	20
	a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Idh-1	(N)	12	20	20	20	20	20	20	20	20
	a	1.000	1.000	1.000	1.000	1.000	1.000	0.950	0.925	0.950
	b	0.000	0.000	0.000	0.000	0.000	0.000	0.050	0.075	0.050
Mdh-1	(N)	–	–	20	20	20	20	17	12	20
	a	–	–	0.000	0.029	0.075	0.000	0.000	0.000	0.000
	b	–	–	1.000	0.971	0.925	1.000	1.000	1.000	1.000
Mdh-2	(N)	–	–	20	17	20	20	17	12	20
	a	–	–	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Mdh-3	(N)	–	–	20	17	20	20	20	12	20
	a	–	–	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Mdh-4	(N)	–	–	20	17	20	20	20	12	20
	a	–	–	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Mdh-5	(N)	–	–	20	16	20	20	20	11	20
	a	–	–	0.050	0.094	0.125	0.175	0.225	0.000	0.025
	b	–	–	0.250	0.875	0.800	0.775	0.775	1.000	0.975
	c	–	–	0.600	0.031	0.075	0.050	0.000	0.000	0.000
	d	–	–	0.100	0.000	0.000	0.000	0.000	0.000	0.000
6Pgd-1	(N)	12	20	20	20	20	20	20	20	10
	a	0.042	0.100	0.000	0.125	0.075	0.125	0.025	0.200	0.105
	b	0.958	0.900	0.975	0.850	0.925	0.825	0.950	0.800	0.895
	null	0.000	0.000	0.025	0.025	0.000	0.050	0.025	0.000	0.000
6Pgd-2	(N)	12	20	20	20	20	20	20	20	19
	a	0.292	0.350	0.675	0.525	0.725	0.475	0.550	0.575	0.447
	b	0.708	0.650	0.325	0.475	0.275	0.525	0.450	0.425	0.553
Pgm-1	(N)	12	20	20	20	20	20	20	20	20
	a	0.125	0.075	0.175	0.075	0.125	0.175	0.150	0.200	0.050
	b	0.875	0.925	0.825	0.925	0.875	0.875	0.700	0.800	0.950
	c	0.000	0.000	0.000	0.000	0.000	0.000	0.150	0.000	0.000

parametric linear regression (t test; SOKAL and ROHLF, 1981) were performed for the regression coefficients of the parameters (allele frequencies and H_e) against latitude, longitude, mean annual rainfall (MAR) and altitude of the nine populations to identify relationships between genetic variation in populations and geographical and ecological gradients.

Results

Loci descriptions

One region of AAT activity, with 2 alleles (A*at*-1a and A*at*-1b), was observed. Two regions of 6PGD activity were detected (*6Pgd*-1 and *6Pgd*-2) and 2 alleles recorded at each locus. Allele *6Pgd*-1b overlapped allele 6*Pgd*-2a and a null allele was detected at *6Pgd*-1. One stained zone of PGM activity, with 2 alleles (P*gm*-1a and P*gm*-1b) was observed. Two regions of IDH activity (*Idh*-1, *Idh*-2) were observed, but only *Idh*-1 was scored since *Idh*-2 was unclear. Two alleles were observed at *Idh*-1. Four zones of MDH activity were observed, which were interpreted as 5 loci (*Mdh*-1, *Mdh*-2, *Mdh*-3, *Mdh*-4, *Mdh*-5). Two alleles were scored at both *Mdh*-1 and *Mdh*-5. Allele *Mdh*-1b overlapped the *Mdh*-2 locus and allele *Mdh*-5a overlapped the *Mdh-4* locus. Two zones of ALD activity were observed (*Ald*-1, *Ald*-2), but *Ald*-1 was unclear. Only *Ald*-2 was scored and 2 alleles were observed.

Number of alleles at each locus and patterns of allele distribution

A total of 24 alleles, at 12 loci, across 9 populations were detected. The number of alleles detected at each locus ranged from 1 at *G6Pdh*-1, *Mdh*-2, *Mdh*-3 and *Mdh*-4 to 4 at *Mdh*-5. The differences among populations are the result of differences in the frequencies of shared alleles and the presence and absence of rare alleles (*Table 2*). Only 2 (8%) of the alleles (*Aat*-1a and *Aat*-1b) were significantly correlated with latitude of origin ($r^2 = -0.888$, $p < 0.001$ and $r^2 = 0.892$, $p < 0.001$, respectively). All but 2 alleles (*Pgm*-1c and *Mdh*-5d, Nam Now and Nong Krating, Thailand, respectively) were found in 2 or more populations. Allele *Idh*-1b was restricted to populations from Vietnam (Xuan Tho and Lang Hahn) and the Vietnamese-Thai border (Nam Now) and *Mdh*-1a was restricted to the Thai populations from Doi Suthep and Doi Inthanon. A putative null allele was found in Thai populations from Doi Suthep, Doi Inthanon, Phu Kradung and Nam Now.

Agreement with HARDY-WEINBERG expectations

For estimating F_{IS}, 55 genotype distributions for each polymorphic locus in each population were used and compared with the expected genotype distributions under HARDY-WEINBERG equilibrium. χ^2 comparisons showed that 50 of the 55 (90.9%) loci tested did not deviate from the HARDY-WEINBERG equilibrium. Deviations observed at the 5 loci (*6Pdg*-2, Aungban; *Idh*-1, Xuan Tho and Lang Hanh; *Mdh*-5, Nong Krating; *Pgm*-1, Nam Now) appeared to be associated with sample size (*6Pdg*-2) and the occurrence of rare alleles (*Idh*-1; *Mdh*-5; *Pgm*-1), such that an increase in the number of individuals sampled may bring these loci to HARDY-WEINBERG equilibrium.

Genetic variation within populations

The percentage of polymorphic loci per population (P; 95% criterion) ranged from 25.0% (Zokhua, Myanmar) to 58.3% (Nam Now, Thailand; Doi Inthanon, Thailand), with a mean of 45.4% (*Table 3*). The mean number of alleles per locus (A) within populations ranged from 1.3 (Zokhua, Myanmar) to 1.8 (Nam Now, Thailand; Doi Suthep, Thailand), with a mean of 1.6 (*Table 3*). The mean genetic diversity within populations (H_e) was 0.153, but substantial differences were observed

Table 3. – Genetic diversity within 9 populations of *Pinus kesiya*. 'A' – Mean number of alleles per locus; 'P' – Percentage of polymorphic loci; H_o – observed heterozygosity; H_e – expected heterozygosity. Figures in parentheses are standard deviations.

No.	Population	A	P*	Mean Heterozygosity	
				H_o	H_e**
1	Zokhua	1.3	25.0	0.090 (0.044)	0.086 (0.043)
2	Aungban	1.4	41.7	0.142 (0.067)	0.126 (0.057)
3	Nong Krating	1.7	33.3	0.128 (0.051)	0.155 (0.063)
4	Doi Suthep	1.8	41.7	0.161 (0.062)	0.148 (0.056)
5	Doi Inthanon	1.7	58.3	0.175 (0.057)	0.156 (0.052)
6	Phu Kradung	1.7	50.0	0.176 (0.057)	0.185 (0.060)
7	Nam Now	1.8	58.3	0.161 (0.058)	0.168 (0.058)
8	Xuan Tho	1.5	50.7	0.162 (0.063)	0.193 (0.065)
9	Lang Hahn	1.6	50.0	0.135 (0.056)	0.156 (0.059)
	Mean	1.61	45.44	0.148 (0.057)	0.153 (0.057)

*) A locus is considered polymorphic if the frequency of the most common allele does not exceed 0.95.
**) Unbiased estimate (NEI, 1978).

Table 4. – Comparison of H_e between 4 population regions identified by ARMITAGE and BURLEY (1980). 'sd' – standard deviation; 'na' – not applicable.

Region	Population number	H_e (sd)
Chin Hills, Myanmar	1	0.086 (na)
Shan Hills, Myanmar - Chiang Mai, Thailand	2, 5, 3, 4	0.146 (0.014)
South Vietnam	8, 9	0.175 (0.026)
North Central Thailand	6, 7	0.177 (0.012)

between populations (*Table 3*). No significant differences were found between H_o and H_e. Genetic diversity, H_e, was greatest (mean 0.176) in populations from Central Thailand and Vietnam and lowest (0.086) in populations from the Chin Hills, Myanmar, with the eastern Myanmar and Chiang Mai populations intermediate (0.146; *Table 4*). H_e tended to decrease with decreasing longitude and increasing latitude (*Fig. 2*). However, in regression tests no significant relationship was found between H_e and either longitude ($r^2 = 0.575$, $t = 1.859$, non-significant) or latitude ($r^2 = 0.570$, $t = 1.835$, non-significant).

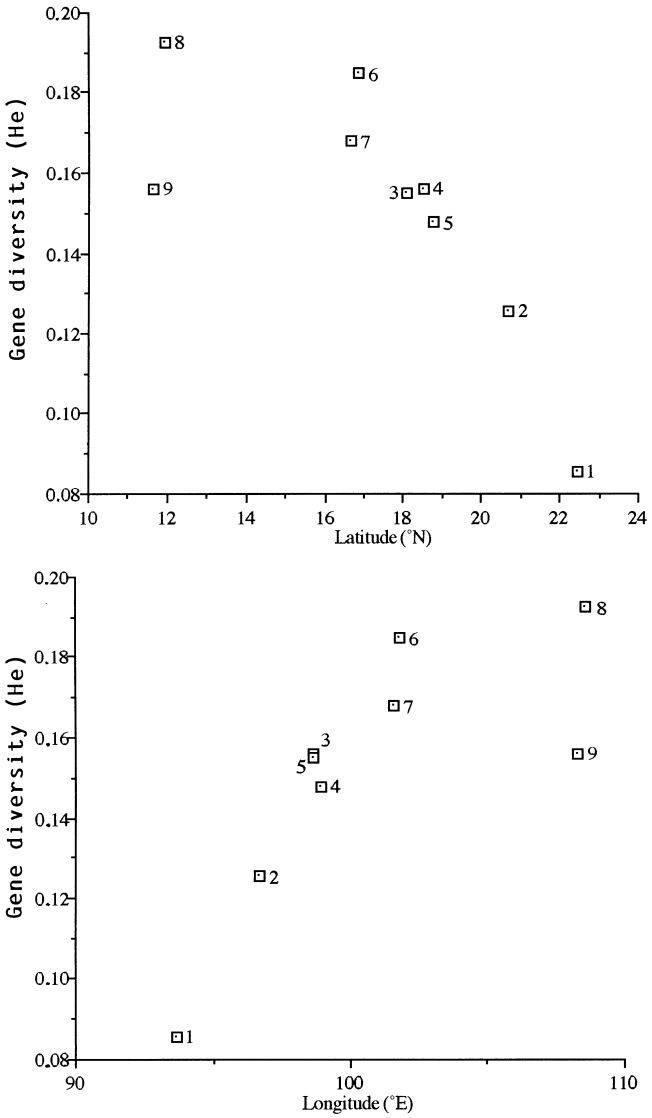

Figure 2. – Relationship between gene diversity in 9 *Pinus kesiya* populations and: (a) latitude; (b) longitude.

Population differentiation

The mean genetic differentiation between populations (G_{ST}) was 0.121, indicating that approximately 88% of the enzyme variation resided within populations. G_{ST} values for individual loci were low, except at *Ald*-2, *Aat*-1 and *Mdh*-5 (*Table 5*). However, apart from *Aat*-1 and *Mdh*-5, the G_{ST} values were not statistically significant ($P < 0.001$ and $P < 0.05$ respectively), indicating that there were no significant heterogeneities among populations at these loci; mean G_{ST} value was also statistically significant. NEI's unbiased genetic distances were calculated between all *Pinus kesiya* populations (NEI, 1978) and clustered using UPGMA (*Figure 3*). However, the groupings identified neither corresponded to the geographical distribution of the populations (except that the 2 Vietnamese populations are very similar to each other) nor the regional areas identified by ARMITAGE and BURLEY (1980).

Discussion

Over its entire mainland south east Asian range the genetic diversity of *Pinus kesiya* (mean $H_e = 0.153$) is somewhat lower than the mean for 103 other isozyme studies in the family Pinaceae ($H_e = 0.176$; HAMRICK and GODT, 1996). However, H_e ranges from 0.000 in North American *P. torreyana* (LEDIG and CONKLE, 1983) to 0.270 in the neotropical *P. oocarpa* (MILLAR *et al.*, 1988). Unfortunately in many studies of genetic variation within pine species studies have concentrated on either relatively small parts of the distributional range, e.g. *P. oocarpa* (2 populations; MILLAR *et al.*, 1988) or on introduced material from potentially unknown origin. Previous studies of *P. kesiya* genetic variation have been restricted to parts of the distribution (particularly the area around Chiang Mai in Thailand; BOYLE *et al.*, 1991a and b; SZMIDT *et al.*, 1996). However, *P. kesiya* has a much wider, though disjunct, distribution than northern Thailand. Whilst similar levels of genetic diversity have been found in this study ($H_e = 0.153$) and the studies by SZMIDT *et al.* (1996; $H_e = 0.148$) and BOYLE et al. (1991b; $H_S = 0.166$), the distribution of the variation between populations is very different in the three studies. In the present study G_{ST} (0.121) was high compared to both the studies of SZMIDT *et al.* (1996; $G_{ST} = 0.023$) and BOYLE *et al.* (1991b; $G_{ST} = 0.039$), and high compared to the average value across the family Pinaceae ($G_{ST} = 0.073$; HAMRICK and GODT, 1996).

Despite the disjunct distribution and investigations into

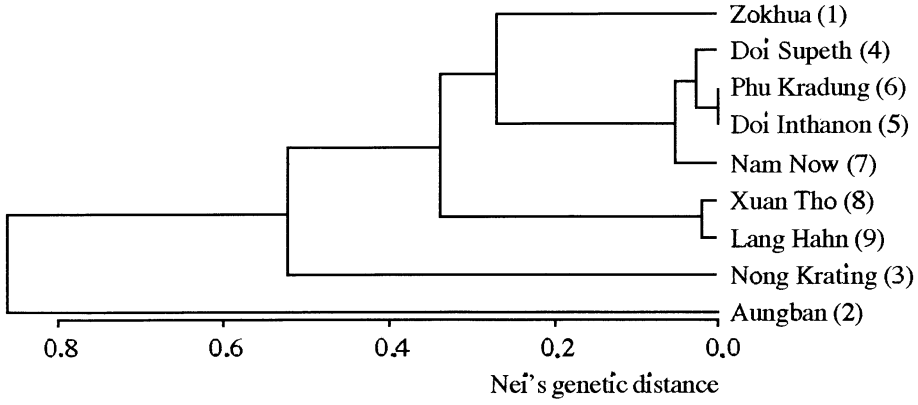

Figure 3. – UPGMA clustering of 9 populations of *Pinus kesiya* based on NEI's unbiased genetic distance.

Table 5. – Comparison of G-test results of 9 populations of *Pinus kesiya*.

	Ht	Hs	Gst†	Dst
Aat-1	0.274	0.180	0.342***	0.094
6Pgd-1	0.186	0.179	0.037ns	0.007
6Pgd-2	0.500	0.465	0.070ns	0.035
Pgm-1	0.242	0.232	0.041ns	0.010
Idh-1	0.038	0.037	0.042ns	0.001
Mdh-1	0.029	0.028	0.048ns	0.001
Mdh-5	0.372	0.270	0.274**	0.102
Ald-2	0.492	0.437	0.112ns	0.055
mean	**0.267**	**0.229**	**0.121*** **	**0.038**

†) ns – non significant; **)$0.01 < p < 0.001$; ***)$p \geq 0.001$

morphological (BURLEY and BURROWS, 1972), secondary products (GREEN *et al*., 1974) and growth rate variation (SHELBOURNE, 1963) there is little current evidence for major patterns of geographical variation. SHELBOURNE (1963) found the greatest growth rate in Philippine and Vietnamese provenances compared to Myanmarese and Assam provenances, whilst BURLEY and BURROWS (1972) showed the apparent occurrence of a latitudinal gradient in stomatal row number; stomatal row number decreasing from south to north. On the basis of terpene data ARMITAGE and BURLEY (1980) considered that the *Pinus kesiya* complex may be divided into 2, possibly 3, races that comprise: (i) Assam; (ii) Thailand, Vietnam, Philippines; (iii) Myanmar. However, the use of resin composition data has recently been criticised (BIRKS and KANOWSKI, 1988, 1993).

Cluster analysis of NEI's genetic distances failed to confirm either the geographical distribution of the species or that genetic diversity was associated with the groups identified by ARMITAGE and BURLEY (1980). However, consideration of the genetic diversities within ARMITAGE and BURLEY's (1980) geographical regions showed that there was significantly lower diversity in the north-western part of the range (Chin Hills and Shan Hills Myanmar and Chiang Mai, Thailand) compared to the south-eastern part of the range (North Central Thailand and South Vietnam). Furthermore, there is some evidence that the genetic diversity was clinal across both longitude and latitude.

Clinal patterns of genetic diversity have been identified in a number of species, e. g. *Fagus sylvatica* (LEONARDI and MENOZZI, 1995), *Picea abies* (LAGERCRANTZ and RYMAN, 1990), *F. crenata* (TOMARU *et al*., 1997) and *Quercus petraea* (ZANETTO and KREMER, 1995). Such patterns have often been explained on the basis of post-glacial migration and founder effects. *Pinus kesiya* is distributed mainly in seasonally dry continental south east Asia, with extreme eastern populations in the Philippines. ARMITAGE and BURLEY (1980) have speculated that paleogeographical and floristic evidence would indicate a Tertiary or mid-Pleistocene migration of *P. kesiya* through Malaysia and the Philippines. If such a hypothesis is correct then the greatest genetic diversity might be associated with the south-eastern part of the present *P. kesiya* distribution. The evidence presented here would suggest that this might be the case, but relatively few populations have been sampled from the south east of the distribution and no populations from the Philippines. The alternative hypothesis of migration of *P. kesiya* from the north was specifically excluded by ARMITAGE and BURLEY (1980), but was considered important by SZMIDT *et al*. (1996). Additional data from a wide range of different sources will be needed to distinguish between these competing hypotheses of *P. kesiya* colonisation of south east Asia. However, detailed understanding of patterns of genetic diversity (POWELL *et al*., based on the paternally inherited *Pinus* chloroplast genome (DONG *et al*., 1992) would allow pollen distribution patterns to be determined and the potential for pollen flow (ASMUSSEN and SCHNABEL, 1991; ENNOS, 1994; PETIT *et al*., 1993) mapped within and between these populations.

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Ex Vitro Survival, Rooting and Initial Development of *in Vitro* Rooted *vs* Unrooted Microshoots From Juvenile and Mature *Tectona grandis* Genotypes

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Summary

The influence of *in vitro* formed adventitious roots on acclimatization and initial *ex vitro* development of microshoots from juvenile and mature teak (*Tectona grandis*) genotypes was investigated. Overall, the *in vitro* rooted microshoots gave rise to higher survival and *ex vitro* rooting rates 7 weeks after transfer than those not rooted *in vitro*. The age difference resulted in higher mortality rates 7 and 15 weeks after transfer for the microshoots of mature origin. The number of roots produced *in vitro* was observed to have a strong influence on the number of roots formed *ex vitro* 7 weeks after transfer and on the height of the microshoots at the time of transfer, 7 weeks later and to a lesser extent after 15 weeks. Differences in height at transfer between microshoots from the two origins of plant material tended to disappear during the acclimatization process. Overall, more than 80% of the microshoots that were initially transplanted from the various categories tested were successfully acclimatized to *ex vitro* conditions. These results are discussed considering mainly the influence of the maturation process on the formation of *ex vitro* roots in *in vitro*-derived microshoots and emphasizing the role of some basic exogenous factors.

Key words: acclimatization, adventitious rooting, age, *ex-vitro* development, microshoots, *Tectona grandis*.

FDC: 165.44; 181.65; 176.1 *Tectona grandis*.

Résumé

L'influence de l'appareil racinaire de type adventif formé *in vitro* sur l'acclimatation et les premiers stades de développement *ex vitro* de microboutures de génotypes juvéniles et mature de teck (*Tectona grandis*) a été etudiée. Globalement, 7 semaines après leur transfert, les microboutures enracinées *in vitro* se distinguent par des taux de survie et d'enracinement *ex vitro* supérieurs à leurs homologues non enracinées *in vitro*. L'influence de l'âge se ressent au niveau des taux de mortalité plus élevés 7 et 15 semaines après le transfert pour les microboutures provenant du clone mature. Le nombre de racines formées *in vitro* influe sur le nombre de racines développées en conditions *ex vitro* 7 semaines après le transfert, ainsi que sur la hauteur des microboutures à la date du transfert, après 7 semaines, et dans une moindre mesure après 15 semaines. Les différences de hauteur mises en évidence entre les deux origines à la date du transfert tendent à s'estomper durant l'acclimatation. Plus de 80% de l'ensemble des microboutures transférées *ex vitro* ont pu être acclimatés avec succès. Ces résultats sont discutés en considérant principalement l'impact du phénomène de maturation sur la rhizogenèse *ex vitro* de microboutures issues d'*in vitro,* ainsi que l'influence de certains facteurs exogènes prépondérants.

Mots clés: acclimatation, âge, développement *ex-vitro*, enracinement adventif, microboutures, *Tectona grandis.*

Introduction

Tectona grandis, commonly known as teak, has gained a worldwide reputation as a high quality timber on account of the attractiveness and durability of its wood. This arborescent species occurs naturally although discontinuously in deciduous

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