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GENETIC VARIATION IN GROWTH AND OIL CHARACTERISTICS OF MELALEUCA CAJUPUTI SUBSP. CAJUPUTI AND POTENTIAL FOR GENETIC IMPROVEMENT

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Received November 2001

SUSANTO, M., DORAN, J., ARNOLD, R. & RIMBAWANTO, A. 2003. Genetic variation in growth and oil characteristics of *Melaleuca cajuputi* subsp. *cajuputi* and potential for genetic improvement. Patterns of variation in growth and foliar oil traits in *Melaleuca cajuputi* subsp. *cajuputi* were studied in a two-year-old provenance/progeny trial at Paliyan, Indonesia and genetic parameters were estimated. Growth (height and diameter) and oil characteristics (oil concentration and 1,8-cineole per cent) amongst the *M. cajuputi* subsp. *cajuputi* families in the Paliyan trial were found to be highly variable, moderately to strongly inherited and independent of each other genetically. Individual heritabilities ranged from 0.38 for height growth to 0.54 for 1,8-cineole per cent, while genetic correlation between growth and oil traits were very low (-0.07 to 0.10). This bodes well for the capture of gains from selection and breeding of *M. cajuputi* for increased production of higher quality oil in Indonesia.

Key words: *Melaleuca cajuputi* subsp. *cajuputi* Powell - cajuput oil - provenances - heritability - Indonesia

SUSANTO, M., DORAN, J., ARNOLD, R. & RIMBAWANTO, A. 2003. Variasi genetik dalam pertumbuhan dan ciri minyak *Melaleuca cajuputi* subsp. *cajuputi* serta potensi perbaikan genetik. Corak variasi pertumbuhan dan ciri minyak daun *Melaleuca cajuputi* subsp. *cajuputi* dikaji dalam ujian provenans berusia dua tahun/progeni di Paliyan, Indonesia dan parameter genetik dianggarkan. Pertumbuhan (ketinggian dan diameter) serta ciri minyak (kepekatan minyak dan peratus 1,8-sineol) di kalangan famili *M. cajuputi* subsp. *cajuputi* di ujian Paliyan didapati sangat pelbagai, terwaris

dengan sederhana sehingga kuat dan bebas secara genetik antara satu sama lain. Keterwarisan individu berjulat antara 0.38 untuk pertumbuhan ketinggian hingga 0.54 untuk peratus 1,8-sineol. Korelasi genetik antara pertumbuhan dan ciri minyak adalah sangat rendah (-0.07 hingga 0.10). Ini merupakan petanda baik bagi perolehan daripada pemilihan dan pembiakbakaan *M. cajuputi* untuk meningkatkan hasil minyak berkualiti tinggi di Indonesia.

Introduction

Melaleuca cajuputi subsp. *cajuputi*, commonly referred to as cajuput, is the source of the medicinal cajuput oil which is used as an inhalant for relief of coughs and colds and as a balm for relief of rheumatism (Doran 1999).

Indonesia is the major supplier of the oil, which is extracted from the leaf by steam distillation. The oil is produced from plantations in Java and from natural stands in Maluku Province. The seed for the original planting in Java, which is the basis for the present day plantation production, is reported to have been imported from Buru Island in Maluku Province by the Dutch in the 18th century. The cajuput oil industry has over 9000 ha of plantations supporting 12 distilleries in various parts of Java and produces 300 tonnes of oil annually, mostly for local consumption. An estimated 90 tonnes of oil are also produced by traditional landowners from natural stands on the Maluku islands of Ambon, Buru and Ceram. The oil is used in a number of other Asian countries including Malaysia, Thailand and Vietnam. Vietnam is also a centre for oil production and produces an estimated 100 tonnes of oil per year.

Establishment of new plantations, replacement of aged, moribund plantations and planting of gaps in existing plantations are regular activities taking place in each centre of production in Java. The seeds for plantation establishment and replacement are taken from unselected mature trees, which are allowed to grow on to produce seed. This is mainly because facilities and technical expertise required for the detailed testing of oil characteristics have not been available. As a consequence, yield and quality of oil from existing plantations of *M. cajuputi* subsp. *cajuputi* are highly variable and below potential. To address these problems a selection and breeding programme for *M. cajuputi* subsp. *cajuputi* has recently commenced in Indonesia for the first time.

Yield of oil per hectare is a combination of leaf oil concentration and leaf biomass per hectare. Evidence from other *Melaleuca* species is that oil traits (e.g. oil concentration and 1,8-cineole per cent) are highly variable and highly heritable while traits associated with leaf biomass production like diameter and height (Doran *et al.* 1997) and coppicing ability are moderately heritable (e.g. *M. alternifolia*, Butcher *et al.* 1996). Selection for these traits, therefore, can give large and rapid gains in plantation productivity.

In 1995 and 1996 samples of seed and leaves for oil analyses of *M. cajuputi* subsp. *cajuputi* were collected in the Maluku islands of Indonesia and in northern Australia, in a collaboration between Indonesia's Centre of Forest Biotechnology

and Tree Improvement and Australia's CSIRO Forestry and Forest Products. In March 1998 a range of seedlots, including some individual mother tree seedlots (families) with good oil properties (high concentration and high 1,8-cineole per cent) and one seedlot used routinely for establishment of plantations in Java, were planted in a provenance/progeny trial at Paliyan near Yogyakarta, Indonesia.

This paper reports the results from assessment of growth and oil traits in the Paliyan trial at two years from which genetic parameters (heritability and genetic correlation) were estimated and potential gains in oil production from selection and breeding were determined. These are believed to be the first published genetic parameter estimates for *M. cajuputi* subsp. *cajuputi* and are essential baseline data for the newly established selection and breeding programme with this species in Indonesia.

Materials and methods

Trial site, genetic material and experimental design

The progeny trial was established in March 1998 at Paliyan (latitude 7° 59' S, longitude 110° 29' E, elevation 190 m asl) near Yogyakarta, on a loamy latosol soil, which was first cleared of an existing *Gliricidia* plantation. The mean minimum temperature of the coolest month is 23 °C and the mean maximum temperature of the hottest month is 33 °C. Annual rainfall is 1894 mm with a seven to eight months of dry season (< 40 mm per month).

The trial comprised a total of 20 seedlots (Table 1). Of these, 18 represented individual mother tree seedlots (i.e. open-pollinated families) from six geographically-separated provenance groups of *M. cajuputi* subsp. *cajuputi*, all of which represented natural stands. These were mainly families that gave better than average values for oil characteristics from an extensive oil survey and seed collection of the species in the Maluku islands and northern Australia. The exceptions were families 18 and 25 which gave only average values for oil traits. Also included in the trial for comparison was a commercial bulked seed collection from plantation trees at Gundih, Java (Group 6) which was being used to establish new plantations and a single family of *M. viridiflora* from Tanimbar (Group 7, Selaru Island). See Figure 1 for a map of seedlot origins.

The trial layout contained ten complete replications with a row-column structure within replicates providing two-dimensional incomplete blocking. Individual plots comprised single rows of ten trees each with a spacing of 3 m between rows and 1.5 m between trees within rows.

Height and diameter measurements

The trial was assessed for total tree height and diameter at 30 cm above ground level in February 2000, 23 months after planting.

Table 1 Origins and oil characteristics of mother trees of *Melaleuca cajuputi* subsp. *cajuputi* whose families were represented in the progeny trial at Paliyan near Yogyakarta, Indonesia. Treatment 15 (provenance group 7) is *M. viridiflora* from Tanimbar.

Provenance group No.	Family Name	Family no.	Family identity Seedlot & collection no.	Provenance name	Oil concentration (W/W % DW)	1,8-cineole (%)	Latitude (S)	Longitude (E)	Altitude (m)	
1	Buru, Island, Indonesia	1	19534-MM2033	Ratgelombang	3.88	47	03° 08' 33"	126° 54' 36"	40	
		2	19539-MM2054	Masarete	2.53	62	03° 22' 38"	127° 08' 12"	20	
		3	19539-MM2057	Masarete	2.45	59	03° 22' 38"	127° 08' 12"	20	
		5	19539-MM2060	Masarete	2.84	59	03° 22' 38"	127° 08' 12"	20	
		25	19539-MM2064	Masarete	1.01	32	03° 22' 38"	127° 08' 12"	20	
2	Ceram Island, Indonesia	8	19542-BVG2913	Waipirit	3.35	47	03° 19' 43"	128° 20' 20"	10	
		9	19541-BVG2919	Pelita Jaya	3.02	57	03° 03' 00"	128° 08' 00"	100	
		10	19541-BVG2920	Pelita Jaya	2.79	52	03° 03' 00"	128° 08' 00"	100	
		11	19541-BVG2923	Pelita Jaya	1.82	67	03° 03' 00"	128° 08' 00"	100	
		12	19540-BVG2936	Cotonea	2.82	64	03° 04' 22"	128° 08' 00"	30	
		13	19540-BVG2937	Cotonea	1.82	54	03° 04' 22"	128° 08' 00"	30	
		14	19540-BVG2941	Cotonea	2.93	60	03° 04' 22"	128° 08' 00"	30	
3	Ambon Island, Indonesia	18	19543-BVG2976	Suli	1.41	44	03° 37' 02"	128° 18' 40"	60	
4	Northern Territory, Australia	19	18898-DL786	Wangi	2.78	59	13° 09'	130° 35'	30	
		20	19568-DL1705	Port Keats	2.96	55	14° 14' 02"	129° 31' 11"	5	
5	Western Australia	21	19576-DL1787	Beagle Bay	4.85	47	16° 58' 33"	122° 40' 04"	10	
		22	19576-DL1797	Beagle Bay	3.47	52	16° 58' 33"	122° 40' 04"	10	
		23	19578-DL1803	Waterbank	3.59	58	17° 46'	122° 16'	10	
6	Gundih, Java, Indonesia	24	Bulk (Control)	Gundih Plantation	-	-	07° 11' 07"	110° 54' 19"	60	
<i>M. viridiflora</i>	7	Tanimbar, Indonesia	15	19545-BVG2970	Sclaru Island	-	-	08° 12' 24"	130° 59' 48"	15

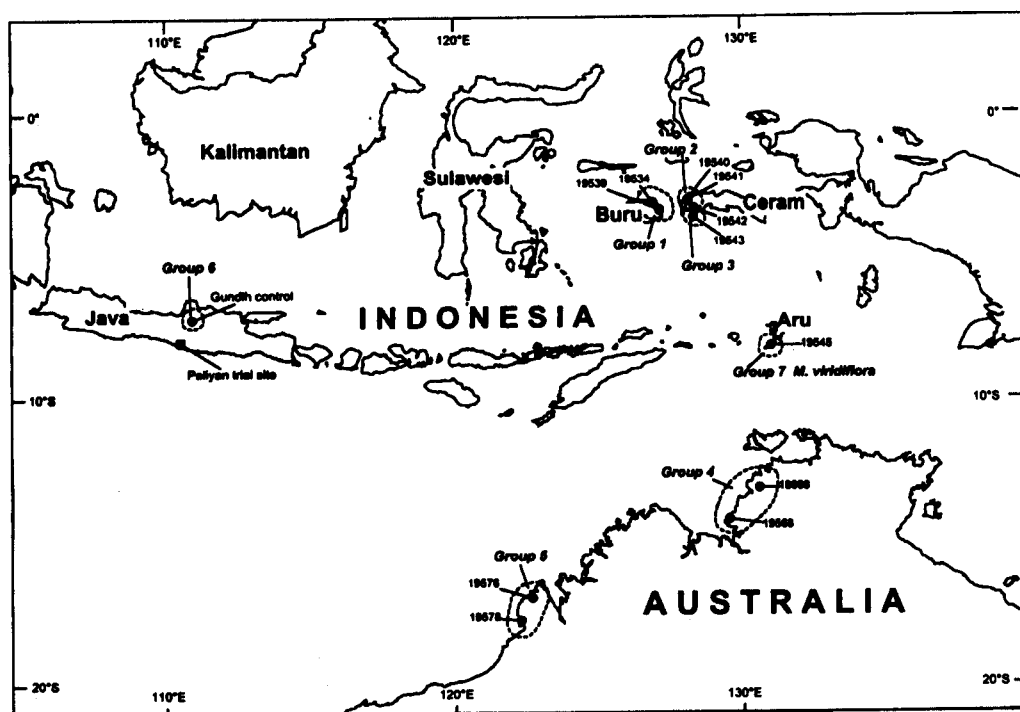


Figure 1 Geographical origins of *Melaleuca cajuputi* subsp. *cajuputi* families and location of Paliyan trial site near Yogyakarta, Indonesia

Determination of oil concentration

In April 2000, leaf samples of *M. cajuputi* subsp. *cajuputi* were collected from the five fastest-growing trees within a plot. Samples comprising about 100 g of mature leaves per tree were taken at random from the lower crown. After collection, the leaf samples were air dried at room temperature in Yogyakarta (approximately 28 °C) for one month. They were then air-freighted to Canberra where, after fumigation in methyl bromide, they were released to CSIRO for oil extraction. This treatment is not known to effect oil properties.

The foliar essential oils of each selected tree were obtained by steam distillation of a subsample (usually 25 g) of each air-dried sample of leaves for two hours with cohobation in a modified Dean and Stark apparatus (Boland *et al.* 1991). Following distillation, pentane (1 ml) was added to maximise oil recovery, which was then dried over sodium sulphate and pentane evaporated at room temperature (Brophy & Doran 1996). A separate subsample (usually 3 g) of each collection was oven-dried at 70 °C for 12 hours for moisture content determination, so oil concentration could be expressed as a percentage of leaf dry weight (W/W% DW).

Determination of 1,8-cineole percentage (of total oils)

1,8-cineole content was determined by gas chromatography (GC) using a Hewlett-Packard 6890A gas chromatograph, with a 3390A integrator, an Alltech AT 35 column (60 m × 0.25 mm internal diameter), and flame ionisation detector operating at 300 °C. A 1 µl sample of oil in ethanol was injected at 200 °C. Hydrogen was used as the carrier gas (40–50 cm³/min) with an oven temperature program of 10 °C/min from 50 °C (1 min) to 250 °C (4 min). Major components were identified by comparing retention times against laboratory supplied Aldrich/Sigma pure components.

Samples were handled in two runs through the GC. In the first run, 1,8-cineole content was determined on most trees in replicate 1. This provided a preliminary estimate of within and between plot (family) variations in this trait. It was determined that by reducing the number of trees for chemical analysis from five trees per plot to the two trees of highest oil concentration per plot there still remained ample opportunity to identify trees of high 1,8-cineole per cent in combination with high oil concentration. The costs of GC analysis were also significantly reduced. In the second run, which covered samples from replicates 2 to 10, only oils from two trees of highest oil concentration per plot were analysed for percentage of 1,8-cineole.

Statistical analyses

Analyses of variance

The software package DataPlus Version 3, 2000 was used to pre-process the data and screen for outlying values. Plot means for all traits were calculated using DataPlus. Four replicates (2 and 8 to 10) had survived poorly in the first few months of planting and had required substantial in-filling with younger plants which had not grown well. It was decided to delete these replicates from the main analysis along with several data points that fell outside the normal range for various traits. Subsequent analyses were carried out on plot means for the trees remaining in six replicates of the trial, using GENSTAT Version 5.3.2, 1987. The plot counts were used to calculate plot mean survival percentages. Plot variances were then analysed using the ANOVA procedure in GENSTAT to check for homogeneity of variances. This revealed plot variances to be homogeneous and transformations of the original data were considered unnecessary. A further manipulation of the data was necessary, however, to improve the normality of the 1,8-cineole percentage data. The frequency distribution of this trait suggested a bimodal pattern because of the scattered occurrence of some low-cineole chemotypes. The low-cineole (< 22% 1,8-cineole) trees, which amounted to approximately 5% of trees remaining in the trial at the time of assessment, were deleted from the data set for analyses and estimation of genetic parameters.

Analyses of trial data were based on the following linear model:

$$Y_{ijklm} = \mu + R_i + X_{j(i)} + Y_{k(i)} + G_l + F_{m(l)} + e_{ijklm}$$

where

Y_{ijklm} = plot mean of the m th family within the l th provenance group in the k th column-within-replicate i and the j th row-within-replicate i in the i th replicate,

μ = overall mean,

R_i = effect of the i th replicate,

$X_{j(i)}$ = effect of the j th row within replicate i ,

$Y_{k(i)}$ = effect of the k th column within replicate i ,

G_l = effect of the l th provenance group,

$F_{m(l)}$ = effect of the m th family which is nested within the l th provenance group, and

e_{ijklm} = residual error with a mean of zero.

Computation of family means and analyses of variance (ANOVA) were carried out in two stages. The first stage involved mixed model analyses of the plot means employing the REML procedure in GENSTAT, for which families and replicates were treated as fixed effects while both rows-within-replicates and columns-within-replicates were treated as random. For the second stage, family means estimated from this first stage were then analysed, using the ANOVA procedure in GENSTAT, employing a nested treatment structure (families nested with provenance groups). The outputs from the two stages were then combined to produce composite analysis of variance tables for testing the significance of provenance group and family effects, following procedures described by Williams & Matheson (1994).

Genetic parameters

Appropriate variance components for genetic parameter computation were obtained by mixed model analyses of the individual family seedlots of *M. cajuputi*. Replicates and provenance groups were treated as fixed effects while columns-within-replicates, rows-within-replicates and families-within-provenance groups were assumed to be random effects (Williams & Matheson 1994). These analyses were conducted using the REML procedure in GENSTAT.

The mean family-within-provenance group variance components were used to estimate within provenance group individual tree heritabilities (denoted h^2) separately for each trait as follows (Williams & Matheson 1994):

$$h^2 = 1/r \times (\sigma_f^2 / \sigma_p^2) \quad (1)$$

where

- r = coefficient of relationship,
 σ_f^2 = variance between families-within-provenance groups,
 σ_p^2 = phenotypic variance
 = $(\sigma_f^2 + \sigma_m^2 + \sigma_i^2)$,
 σ_m^2 = variance between plots, and
 σ_i^2 = variance between trees within plots.

The coefficient of relationship used in computation of the individual tree heritabilities was assumed to be 0.4 rather than the value of 0.25 used for half-sib families. This was based on the assumption that, like many species of *Eucalyptus* and other members of the family Myrtaceae (Moran 1992), open-pollinated families from natural *M. cajuputi* subsp. *cajuputi* stands carry a degree of inbreeding resulting from selfing and neighbourhood mating.

Standard errors of the heritability estimates were calculated according to Becker (1984). Additive genetic correlations (denoted r_g) were calculated according to methodologies described by Williams & Matheson (1994) based on the following formula:

$$r_g = \frac{\text{Cov}_f(X, Y)}{[\sigma_f^2(x) \cdot \sigma_f^2(y)]^{1/2}} \quad (2)$$

where

- $\text{Cov}_f(X, Y)$ = covariance of the two traits at the family level,
 $\sigma_f^2(x)$ = family-level variance components of trait (x), and
 $\sigma_f^2(y)$ = family-level variance components of trait (y).

Phenotypic correlations (r_p) were estimated as simple correlation coefficients.

Expected genetic gains (denoted ΔG) from mass selection in the trial were estimated by the formula (Shelbourne 1991):

$$\Delta G = h^2 \times i \times \sigma_p \quad (3)$$

where

- i = selection intensity,
 σ_p = the phenotypic standard deviation for the trait of interest,
 and
 h^2 = individual tree heritability for the trait of interest.

It is important to note that estimates of genetic parameters here apply specifically to the particular environment and condition of individual trees at the Paliyan trial site at two years from planting and to the particular sampling strategy employed.

Results

Growth and oil characteristics

There was significant variation in most traits between provenance groups and between families-within-provenance groups (Table 2). The means estimated by the REML procedure for survival, height, diameter at 30 cm, oil concentration and 1,8-cineole per cent are given in Table 3.

Survival rate by family mean over all 10 replicates in the experiments averaged 71% (range 41 to 88%) (Table 3). Differences in survival between provenance groups were not significant but there were significant differences between families-within-provenance groups. The family with the poorest (41%) survival was from Pelita Jaya on Ceram Island (provenance group 2) and the best (88%) surviving family was family 8 from Waipirit, also on Ceram Island.

Differences in height and diameter growth were highly significant both between provenance groups and between families-within-provenance groups. The Gundih control was the fastest growing seedlot in the trial and ranked best for both height and diameter, although some individual families of *M. cajuputi* subsp. *cajuputi* came close to it in growth, e.g. families 1 and 25 from Buru Island and family 14 from Ceram Island. Although the provenance group means suggested that those from Ambon Island and Western Australia were the poorest in the trial, their means

Table 2 Mean squares for analysis of variance for survival, height, diameter at 30 cm, oil concentration and 1,8-cineole per cent of *Melaleuca cajuputi* subsp. *cajuputi* families represented in the progeny trial at Paliyan near Yogyakarta, Indonesia at two years of age

Trait	Source of variation ^a	Degrees of freedom	Mean squares and F-values
Survival percentage	Provenance group	6	1541.20 ***
	Family-within-provenance group	13	961.40 ***
	Residual	76	251.10
Height (m)	Provenance group	6	2.16 ***
	Family-within-provenance group	13	0.76 ***
	Residual	68	0.18
Diameter at 30 cm (cm)	Provenance group	5	2.67 ***
	Family-within-provenance group	13	2.09 ***
	Residual	56	0.37
Oil concentration (W/W% DW)	Provenance group	5	0.54 *
	Family-within-provenance group	13	0.94 ***
	Residual	56	0.18
1,8-cineole (% of total oils)	Provenance group	5	160.09 **
	Family-within-provenance group	13	220.15 ***
	Residual	42	33.96

* = significant at $p < 0.05$, ** = significant at $p < 0.01$, *** = significant at $p < 0.001$

^a: One family of *M. viridiflora* (family 15, provenance group 7) was included in the comparison of mean survival and growth.

were based on the performance of too few families (only one and three respectively) to provide an accurate representation of their potential. Among the individual families-within-provenance groups, family 9 (Ceram Island) and family 21 (Western Australia) performed poorly for growth. Similarly, *M. viridiflora* from Tanimbar (family 15) also showed the same lowest value for height growth in the trial.

Table 3 Mean survival, height, diameter at 30 cm, oil concentration and 1,8-cineole per cent of *Melaleuca cajuputi* subsp. *cajuputi* families represented in the progeny trial at Paliyan near Yogyakarta, Indonesia at two years of age

Provenance group no.	Provenance* group name	Family no.	Provenance name	Survival (%)	Height (m)	Diameter at 30 cm (cm)	Oil concentration (W/W % DW)	1,8-cineole (% of total oil)
1	Buru Island	1	Ratgelombang	78	3.0	3.5	1.9	42.7
		2	Masarete	74	2.6	2.8	1.8	60.2
		3	Masarete	77	2.6	2.8	1.7	50.9
		5	Masarete	70	2.4	2.7	1.5	53.4
		25	Masarete	82	3.0	3.3	1.4	51.3
	Group 1 means			76	2.7	3.0	1.6	51.7
2	Ceram Island	8	Waipirit	88	2.7	3.3	1.5	44.5
		9	Pelita Jaya	41	2.3	2.2	1.6	47.2
		10	Pelita Jaya	65	2.5	2.7	2.1	46.5
		11	Pelita Jaya	66	2.4	3.0	1.4	57.6
		12	Cotonea	69	2.4	2.7	1.4	53.9
		13	Cotonea	74	2.6	3.0	1.5	49.2
	Group 2 means			71	3.1	3.5	2.3	53.5
				68	2.6	3.0	1.7	50.5
3	Ambon Island	18	Suli	84	2.2	2.5	2.1	49.6
4	Northern Territory	19	Wangi	78	2.7	3.2	1.3	56.1
		20	Port Keats	72	2.5	2.8	1.8	55.3
		Group 4 means		75	2.6	3.0	1.5	55.7
5	Western Australia	21	Beagle Bay	47	1.9	1.5	2.1	45.5
		22	Beagle Bay	69	2.5	3.2	1.4	49.3
		23	Waterbank	60	2.4	2.8	1.7	47.4
		Group 5 means		58	2.3	2.6	1.7	47.6
6	Gundih	24	Gundih Plantation (Control)	84	3.2	3.8	1.4	51.3
<i>M. viridiflora</i>								
7	Tanimbar	15	Selaru Island	65	1.9	2.9		
	Overall means			71	2.6	3.0	1.7	51.0
	LSD (p = 0.05)			14.9	0.4	0.6	0.4	6.8

LSD = Least significant difference

* = One family of *M. viridiflora* (family 15, provenance group 7) was included in the comparison of mean survival and growth

Note: For the genetic analyses the four poorest surviving replicates (replicate 2 = 47%, replicate 8 = 33.5%, replicate 9 = 52.5% and replicate 10 = 45.5%) were excluded because of the extent of the missing values. This brought survival up to an average of 89% for the remaining six replicates.

Oil characteristics also varied significantly between provenance groups and between families-within-provenance groups (Table 2). Several of the provenance groups gave significantly higher oil concentrations than the Gundih control (provenance group 6), which had the second lowest ranking for this trait in the trial (Table 3). Families that performed significantly better than the control for oil concentration included 18 (Ambon Island), 1 (Buru Island), 10 and 14 (Ceram Island), and 21 (Western Australia). The Gundih control equalled the trial mean (51%) for the oil quality trait, 1,8-cineole per cent. Of the provenance groups, the Northern Territory families gave the best overall average for this trait (55.7%) while the Western Australian families were the poorest (47.6%), although both these groups were represented by too few families for accurate representation of their true average potential. Families that had the preferred level of 1,8-cineole in their oils (i.e. > 55%), and were significantly better than the control in this factor, were 2 (Buru Island), 11 (Ceram Island), 19 and 20 (Northern Territory) (Table 3).

Genetic parameters

Genetic and phenotypic correlations between growth and oil traits for two-year-old *M. cajuputi* subsp. *cajuputi* in the Paliyan trial are given in Table 4. Table 5 gives estimated individual tree heritabilities and standard errors along with the expected gains in growth and oil traits in the first generation, for a selection intensity of one in five trees.

Height and diameter were, as expected, strongly associated with an r_g of 0.95 and an r_p of 0.81. It appears, however, that growth and oil traits were weakly associated and selection for one is unlikely to affect the other, either adversely or favourably.

Heritabilities were moderately high for all traits studied (0.38 for height growth to 0.54 for 1,8-cineole per cent) as were their standard errors (Table 5). Expected gains in height, diameter, oil concentration and 1,8-cineole per cent following selection of the best tree in every five on the basis of the two-year data for *M. cajuputi* subsp. *cajuputi* from Paliyan were 15, 20, 21 and 10% respectively (Table 5).

Table 4 Genetic (r_g) and phenotypic correlations (r_p) between growth and oil traits of *Melaleuca cajuputi* subsp. *cajuputi* in the Paliyan trial at two years of age

Trait	Diameter		1,8-cineole (%)		Oil concentration (W/W % DW)	
	r_g	r_p	r_g	r_p	r_g	r_p
Height	0.95	0.81	-0.06	0.07	0.10	-0.18
Diameter at 30 cm			-0.07	0.01	0.01	-0.15
1,8-cineole per cent					-0.25	0.18

Table 5 Predicted individual heritability (h^2 using formula 1), standard error of heritability and genetic gain (ΔG using formula 3) over trial means of the four traits in the Paliyan trial of *Melaleuca cajuputi* subsp. *cajuputi* at two years of age

Trait	Trial mean	Intensity of selection*	$h^2 \pm SE$	Phenotypic variance	ΔG unit**	ΔG %
Height (m)	2.6	1.163	0.38 ± 0.57	0.74	0.38	15
Diameter at 30 cm (cm)	3.0	1.163	0.47 ± 0.18	1.14	0.58	20
1,8-cineole per cent	50.8	1.163	0.54 ± 0.50	71.61	5.3	10
Oil concentration (W/W% DW)	1.7	1.163	0.40 ± 0.31	0.59	0.35	21

* After Becker (1984)

**Gain estimates were based on the assumption that selection will be for single, independent traits

Discussion

Melaleuca cajuputi subsp. *cajuputi* survived reasonably well (overall 71%) and reached an average height of 2.6 m in two years of growth in the Paliyan trial. On average, the local control (Gundih) gave the best growth in the trial, presumably exhibiting the positive effects of selection for growth when seed was collected from planted trees at Gundih, Java. There were other families in the trial which were almost the equal of Gundih on family averages and there were individual trees within families that performed just as well as the best trees from Gundih.

The foliar oil concentration of *M. cajuputi* subsp. *cajuputi* families in the trial at two years of age averaged 1.7% (W/W% DW). This is considerably less than similarly determined oil concentrations of mature plantation trees of the same species at Gundih, which averaged 2.8% (Doran *et al.* 1998), and the mother trees of the progeny planted in the trial which also averaged 2.8% (Table 1). This is most likely a function of tree age, season of collection, growth environment and/or differences in the handling of leaves between Indonesia and Australia (Doran 1991, Murtagh 1999). Despite this anomaly, it is assumed that, as with several eucalypt species that have been closely studied, ranking for oil concentration can be reliably assessed after about 1.5 years of growth (Doran 1991).

The Gundih control sets the benchmark for oil characteristics as it was grown from seed currently used to establish commercial cajuput plantations near Gundih in Java. Individual families from the three Maluku islands of Ambon, Buru and Ceram and one Western Australian family were significantly better than the control in oil concentration. Some individual trees from nearly all families in the trial had oil concentrations higher than the control average, indicating plenty of scope for selecting superior trees.

Ideally, the content of 1,8-cineole in the oil should be as high as possible as this is the principal active ingredient in the oil (Doran 1999). The 51% of 1,8-cineole recorded for Gundih control is comparable with other estimates of this trait from

mature trees at Gundih (Doran *et al.* 1998) and in oils directly from the Gundih distillery (Doran 1999). The aim should be to select trees with oils containing greater than 55% 1,8-cineole. There were individual trees from nearly all families in the trial that averaged greater than 55% 1,8-cineole, again indicating plenty of scope for selecting better trees.

The principal finding from the estimates of genetic and phenotypic correlations was the apparent lack of any strongly adverse (or positive) correlations between growth and oil traits. However, as the correlations were calculated from a relatively limited number of families, they would be expected to have large standard errors. Nonetheless, the correlations obtained are consistent with several other studies of oil producing species such as *Eucalyptus camaldulensis* (Doran & Matheson 1994), *E. polybactea* (Grant 1997) and *M. alternifolia* (Doran *et al.* 1997). This suggests that it should be possible to improve growth and oil traits simultaneously in the same breeding population. It should be noted, however, that Butcher *et al.* (1996) found a negative genetic correlation between plant dry weight and oil concentration in *M. alternifolia*, so more study of this aspect in *M. cajuputi* is warranted.

The narrow-sense heritabilities estimated from the two-year measurements of *M. cajuputi* subsp. *cajuputi* at Paliyan (Table 5) are moderately high and at the top end of the range of heritabilities reported for growth traits in other oil-producing species, but equivalent to estimates from other studies for oil traits (see review in Doran 2002). However, the estimates obtained in this study are applicable to the Paliyan trial only. Due to the limited number of families (i.e. only 18) and large standard errors on the heritabilities, the estimates cannot be taken as representative of the species overall. The Paliyan heritabilities should be regarded as preliminary until more reliable estimates can be obtained from other larger trials.

Oil concentration and 1,8-cineole per cent is expected to improve by 21 and 10% respectively following selection at an early age (two years) and mating among the best 5% of trees (Table 5). The estimates of gains in 1,8-cineole per cent might be regarded as conservative because of the exclusion of the very low 1,8-cineole forms from these calculations. Simultaneously, height and diameter may be improved by 15 and 20% respectively (Table 5), although it should be noted that, in the case of the families in the Paliyan trial, this level of improvement is required to bring the selected population up to the growth performance of the Gundih control.

The results of this study indicate substantial gains in commercial oil traits could be achieved in *M. cajuputi* subsp. *cajuputi* through selection and breeding. Such a programme is now underway in Java with one of the first activities being the thinning of the Paliyan trial to convert it into a seedling seed orchard. The expected outcome from the use of improved *M. cajuputi* subsp. *cajuputi* seed is a more efficient cajuput oil industry in Indonesia with greater returns to the factories and, therefore, greater incentive to replace moribund plantations with improved germplasm. A significant flow-on of benefits from a more efficient cajuput industry to the many communities in Java relying on work in the industry for additional income is anticipated.

Acknowledgements

We wish to thank CSIRO, Collaboration on Science and Technology Australia/Indonesia (COSTAI) and Australian Centre for International Agricultural Research for funding support. CSIRO staff who assisted were B. Gunn and M. McDonald who collected the seedlots on which this study was based, H. Wu and C. Harwood who provided helpful comments on the text and K. Aken who prepared the map of collecting sites. Sukijan, Sumaryana and Surip measured the Paliyan trial and collected leaves for oil analysis. M. Russell of NSW Agriculture, Wollongbar undertook the oil analyses reported in this paper.

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