# AN OPTIMUM DESIGN FOR SEEDLING SEED ORCHARDS TO MAXIMIZE GENETIC GAIN: AN INVESTIGATION ON SEEDLING SEED ORCHARDS OF *Eucalyptus pellita* F. Muell

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### **ABSTRACT**

The optimum design of seedling seed orchards in order to maximize genetic gain was investigated. Variance components were estimated on the basis of heights of individual plants in the first-generation seed orchards of *Eucalyptus pellita* with ages of 1 to 5 years old. The seed orchards were established at two locations in Indonesia – South Kalimantan and South Sumatra. There were three types of variance components, comprising family variance, plot error variance, and within plot variance which were calculated and expressed as a percentage. A linear regression equation within plot variance was derived using family variance as an independent variable. The regression equation was statistically significant and could be used to determine relative size of the three variances under different levels of heritability. The optimum number of families of the intermediate heritability (= 0.20) were found to be around 40 to 50, with six to eight trees per plot for a seed orchard of 2 ha with 10 replicates. This result suggested that the design of the first-generation seedling seed orchards was close to the optimum to achieve maximum genetic gain through the two stages of selections, i.e. within plot selection and family selection.

Keywords: Eucalyptus pellita, genetic gain, optimum design, plot size, seedling seed orchard

### I. INTRODUCTION

Establishing a seedling seed orchard with open pollinated progenies of plus trees is widely applied as a first step towards forest tree improvement for fast growing species in Indonesia (Soeseno, 1988; Suhartono, 1991; Hashimoto *et al.*, 1996). At present, it might be the most reliable and practical way to meet an immediate demand for genetically improved seed to be used in industrial reforestation programs in Indonesia. This procedure is recommended as an alternative to clonal seed orchards for species with an early flowering habit and where vegetative propagation is difficult (Wright, 1976; Eldridge *et al.*, 2001).

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In a seedling seed orchard, genetic gains are captured in three stages i.e. plustree selection, half-sib family selection, and individual selection (Wright and Bull, 1963). The latter two gains generally depend on the design of the orchard, because this will determine intensity of family and individual selection. Meanwhile, the plus tree selection depend on the standard of selection used and will affect to the gain for the next generation of the breeding program. It is already known that there is an optimum allocation of selection intensities for any given set of heritability (Namkoong et al., 1988). The heritability values express the proportion of variation in the population that is attributable to genetic differences among individuals, where the amount of variation is measured and expressed as the variance (Zobel and Talbert, 1984; Falconer and Mackay, 1996). It is therefore an increasing number of families and number of trees per plot will increase variances of both, i.e. family and within plot. However, the optimum number of both variables should be found, to maximize the optimum number of genetic gain in a certain design of seedling seed orchard. Studies on the optimum allocation of materials to maximize genetic gains have been reported for seedling seed orchards (Namkoong, 1969; Kurinobu, 1993) as well as in clonal tests (Shaw and Hood, 1985).

In this study, the optimum design for seedling seed orchards originally proposed by Kurinobu (1993) was re-examined using real variance data estimated from trees with ages of 1 to 5 years in seed orchards of *Eucalyptus pellita* growing in South Kalimantan and South Sumatra. This study was to investigate the optimum number of families and number of trees per plot that maximize genetic gains under three levels of heritability: low (=0.1), intermediate (=0.2) and high (=0.3).

# II. MATERIALS AND METHODS

# A. Estimation of Variance Components

In this study, three types of variance components, i.e. family variance, plot error variance, and within plot variance, were estimated using height data from trees with ages of 1 to 5 years in the first-generation seedling seed orchards of *E. pellita* growing in South Kalimantan and South Sumatra. The seed orchard in South Kalimantan was established in January 1994, whilst those in South Sumatra were in January 1995. In South Kalimantan, the three provenances (South Kiriwo, North Kiriwo and Serisa Village in Papua New Guinea) were tested at a single site, whereas in South Sumatra they were tested separately at different sites. These seed orchards were arranged in design of randomized complete blocks (RCBD) with open-pollinated families allocated randomly within each replicate in the block. The numbers of families tested for the South Kiriwo (group A), North Kiriwo (group B) and Serisa Village (group C) provenances were 48, 39, and 34, respectively. The families were represented by an initial planting of five trees in a row plot with a spacing of 4 m × 1.5 m, with 10 replicates. The site characteristics in seedling seed orchards of *E. pellita* at the two locations are presented in Table 1.

Table 1. Site characteristics of seedling seed orchards of *E. pellita* at two locations

Information	Loc	ration
_	South Kalimantan	South Sumatra
Site	Pelaihari	Pendopo
Latitude (South)	3°58'	4°00'
Longitude (East)	114°38'	104°00'
Altitude (m asl.)	30	80
Rainfall (mm year <sup>-1</sup> )	2,730	2,781
Dry season	May-September	May-September
Soil type	Ferralsols	Acrisols
Temperature (min. – max.)	23° - 33° C	24° - 33° C
Wind	Intermediate	Low
Slope	0%	3%
Vegetation cover	Imperata cylindrica	Imperata cylindrica
Ground cultivation (plowing)	Twice	Twice
NPK fertilizer	Twice per year	Once per year

Notes: asl = above sea level; N = Nitrogen; K = Potassium; P = Phosphorus

Tree height was measured at both locations, when age of the trees reached 1 to 5 years old. Until the study period, within family-plot selections had been conducted twice, whereby the poorest two trees out of the five per plot were culled in the first step and the poorest individual of the remaining three was removed in the second step. The data as provided prior to this step were used in the analysis. The following linear model was used to estimate the variance components at each age and each orchard  $(Y_{iik})$ :

$$Y_{ijk} = \mu + R_i + F_j + RF_{ij} + \mathcal{E}_{ijk}$$
 (1)

where  $\mu$  is the overall mean,  $R_i$  is the *i*-th replicate effect,  $F_j$  is the *j*-th family effect,  $RF_{ij}$  is the plot error and  $\mathbf{E}_{ijk}$  is the within plot error.

To determine trends in the three types of variance components, under the different levels of heritability, they were expressed as percentages; and then a linear regression equation to estimate within plot variance was fitted. The regression coefficients were calculated with the least square approximation using family variance as an independent variable. In this study, the family variance  $(\sigma^2)$  was assumed to be forty percent of the additive genetic variance  $(\sigma^2)$ , which is the

main determinant factor of observable genetic properties of the population and of response of the population to selection (Falconer and Mackay, 1996), as shown by the following equation:

$$\sigma_A^2 = 2.5\sigma_f^2 \dots (2)$$

This is made because the out crossing rate in natural stands of eucalypt is around 0.70, and that 30% selfing offers an average coefficient of relationship among open-pollinated progeny of 1/2.5 (Squillace, 1974; Griffin and Cotterill, 1988).

### B. Prediction of Genetic Gain

A formula to predict genetic gain in seed orchards was first implemented by Namkoong *et al.* (1966). In this study, however, our primary concern was the gain obtained as a result of family and individual selection in the seed orchard. Therefore, the gain as a result of a plus tree selection and the terms with a genotype-environment interaction variance were omitted from the version used here. The modified formula to predict a local genetic gain (G) is as follows:

$$G = G_{2} + G_{3}$$

$$= i_{2} \cdot (1/4) \sigma_{A}^{2} / \sigma_{2} + i_{3} \cdot (3/4) \sigma_{A}^{2} / \sigma_{3}$$

$$= i_{2} \cdot \sigma_{2} \cdot (1/4) \sigma_{A}^{2} / \sigma_{2}^{2} + i_{3} \cdot \sigma_{3} \cdot (3/4) \sigma_{A}^{2} / \sigma_{3}^{2}$$

$$= i_{2} \cdot \sigma_{2} \cdot b_{f}^{2} + i_{3} \cdot \sigma_{3} \cdot b_{w}^{2} \dots (3)$$

where  $G_2$  and  $G_3$  are genetic gains for family and within plot selections, respectively, and  $\sigma_A^2$  is additive genetic variance.  $\sigma_2^2$  and  $\sigma_3^2$  are phenotypic variances for family mean and within plots, respectively and they are expressed as  $\sigma_2^2 = \sigma_w^2 / (n_p \cdot r) + \sigma_e^2 / r + \sigma_f^2$  and  $\sigma_3^2 = \sigma_w^2$ , where  $n_p \cdot r$ ,  $\sigma_e^2$  and  $\sigma_w^2$  are the number of trees planted per plot, the number of replicates, plot error variance, and within plot variance, respectively. Two types of heritability in Eq. (3),  $h_f^2$  and  $h_w^2$  are family heritability and within plot heritability, respectively. The first term on the right-hand side of the Eq. (3) is the gain due to selection of the best families and the second one corresponds to the selection of the best trees within each plot containing the best families. Thus  $h_g^2$  and  $h_g^2$  are the selection intensities for family selection and within plot selection.

In the case of seedling seed orchards, the proportion of selected trees is expressed by  $n_f/n_i$ , when the initial density per hectare of the seed orchard  $(n_i)$  and the final density  $(n_f)$  have been determined. When  $n_p$  is the number of trees planted per plot, then the proportion of within plot selection is  $1/n_p$ , and the proportion of family selection is  $n_f \cdot n_p/n_i$ , because the total proportion of the selected trees is the result of the proportion of within plot selection and that of family selection. Therefore, the ratios (P) for  $i_2$  and  $i_3$  are  $n_f \cdot n_p/n_i$  and  $1/n_p$  respectively. The intensities were calculated by an approximate formula (Lindgren, 1986):

$$i(P) = (1-P) [1.452 \sqrt{(\ln (1/P(1-P)))} - 0.45 + 1.122 (1-P)^{9.0} P^{0.84}] \dots (4)$$

where i(P) is an approximation for the selection intensity, and P is the proportion selected (for  $i_2$  and  $i_3$ ).

The change in genetic gain predicted by Eq. (3), based on the number of families, was examined under three levels of heritability i.e. low (=0.1), intermediate (=0.2) and high (=0.3). The size of seed orchard, number of replicates, and initial density were assumed to be consecutively 2.0 ha, 10 replicates and 1,666 trees per ha (4m  $\times$  1.5m spacing); these were the standard figures actually used during the first generation tree improvement program for *E. pellita* (Leksono and Kurinobu, 2005). The final density was assumed to be 150 trees per ha.

The heritability  $(h^2)$  is the ratio of additive genetic variance  $(\sigma^2_{A})$  to phenotypic variance  $(\sigma^2_{A})$ . It is of key importance in estimating genetic gains (G) which are determined by the heritability of the trait and the selection differential (S) that is used in selection program  $(G = h^2S)$ . Since the selection differential is dependent upon two factors comprising intensity of selection (i) and phenotypic standard deviation  $(\sigma_p)$ , then  $S = i\sigma_p$ , and the expected genetic gain becomes  $G = ih^2\sigma_p$  (Zobel and Talbert, 1984; Falconer and Mackay, 1996).

### III. RESULTS AND DISCUSSION

The relative variance components for height at the two locations are presented in Table 2. Proportions of family variances ranged from 1 to 15%, whereas the size of within plot variance and those of plot error were much larger than this, ranged from 10 to 85%. During the data collection period, within family-plot selections were undertaken twice consecutively at 2 and 3 years old; however, the effect of thinning was not obvious at both locations.

A regression fit of within plot variance against family variance is presented in Figure 1. The proportion of within plot variance (y) appears to decrease as the proportion of family variance (x) increases; this trend was statistically significant. Thus the regression equation obtained here (y = -1.527x + 0.737) was used in the subsequent analysis to determine the relative size of the three-variance components under different levels of heritability. The plot error variance was calculated as the residual of family variance and the estimate of within plot variance, both of which were calculated from the regression equation. The total of the relative size of the three variances ( $\sigma_x^2$ ) was unity (=1.0).

Table 2. Relative size of variance components for tree height in seedling seed orchards of *E.pellita* at the two locations

	Location												
Variance	So	uth Kalir	nantan (y	ears of a	ge)	South Sumatra (years of age)							
component -	1	2	3	4	5	1	2	3	4	5			
Group A													
Family	0.008	0.047	0.064	0.075	0.048	0.077	0.034	0.041	0.020	0.077			
Plot error	0.226	0.574	0.393	0.248	0.447	0.334	0.286	0.326	0.337	0.315			
Within plot	0.765	0.379	0.543	0.677	0.505	0.589	0.680	0.633	0.643	0.608			
Group B													
Family	0.053	0.035	0.135	0.123	0.138	0.068	0.066	0.037	0.032	0.113			
Plot error	0.107	0.108	0.342	0.284	0.266	0.224	0.145	0.213	0.119	0.229			
Within plot	0.841	0.857	0.523	0.594	0.596	0.708	0.789	0.750	0.850	0.658			
Group C													
Family	0.051	0.038	0.061	0.069	0.152	0.049	0.042	0.027	0.041	0.055			
Plot error	0.496	0.483	0.465	0.457	0.408	0.162	0.213	0.164	0.229	0.282			
Within plot	0.453	0.479	0.474	0.474	0.440	0.790	0.746	0.809	0.731	0.663			

Notes: Relative size of variance components was calculated assuming that the sum of family variance, plot error variance and within plot variance accounted for all the variability

With the variances determined by the above regression equation, genetic gains for each of the number of family, starting from 30 up to 100, were calculated by the Eq. (3) under three levels of heritability (Figure 2 with a detailed stepwise procedure was presented in Appendix 1). The size of the seed orchard and the number of replications (r) in this study were assumed to be 2.0 ha with 10 replications  $(n_i \cdot 2=3332 \text{ trees})$ , therefore the number of trees per plot  $(n_p)$  will decrease with the increase in the number of families tested in the orchard. The optimum number of families to obtain maximum gain was around 40 at intermediate and high heritability and 50 at low heritability, although the differences in the gain as a result of larger number of families were small. The optimum number seemed to increase slightly when the heritability was low. This is probably due to the fact that family selection tends to be more effective when the heritability is low (Falconer and Mackay, 1996).

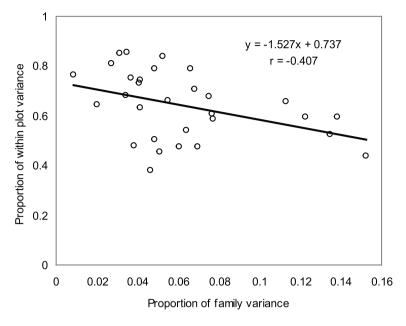


Figure 1. The regression fit (Y = -1.527x + 0.737) for within plot variance against family variance.

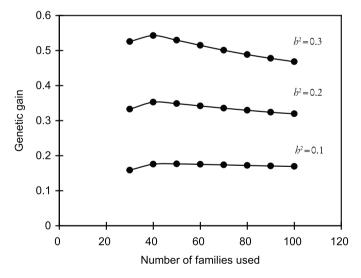


Figure 2. Changes in genetic gain with an increase in the number of families within the seed orchard and heritability  $(b^2)$ . The size of the seed orchard was assumed to be 2.0 ha with 10 replicates. A chart was drawn using the data presented in Appendix 1.

The optimum number of trees per plot, assuming the optimum number of families, was 6.7 for low heritability and 8.3 for intermediate to high heritability. This was slightly more than five trees per plot as proposed in the previous study (Kurinobu, 1993). This discrepancy in the number of trees per plot is due to the fact that the size of plot error variance assumed in this study was greater than the one used in the previous study; the proportion of plot error variance in this study was around 30%, compared to the one with less than 20% in the previous study. This larger size of plot variance inevitably reduces the gain by family selection; and it consequently emphasizes the gain by within plot selection, hence greater gain as a result of larger plot sizes.

The relative size of plot error variance used in this study, which was based on the results of multiple tests, is more realistic than the size used in the previous study, because that was based on a single test result (Kurinobu, 1993). Therefore the gain might be increased slightly if the number of trees per plot were around 6 to 8. Therefore, the design using five trees per plot as proposed in the previous study can result in almost the same amount of gain as long as the orchards were established appropriately.

# IV. CONCLUSION

The optimum number of families for intermediate heritability (= 0.20) was found to be around 40 to 50 with six to eight trees per plot in a seed orchard of 2 ha with 10 replicates. This result suggests that the design of the first-generation seedling seed orchards of *E. pellita* was close to the optimum to achieve maximum genetic gain through the two stages of selection i.e. within plot selection and family selection.

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Appendix 1. A procedure to calculate genetic gains under three levels of heritability

a.  $b^2 = 0.10$ 

Number Number per		er per	Selection rate		Selection intensity		Phenotypic variance		Heritability		Genetic gain		
of family	family		$P_2$	$P_3$	$i_{2}(P_{2})$	$i_{3}(P_{3})$	s <sup>2</sup> <sub>2</sub>	s <sup>2</sup> <sub>3</sub>	$b_{f}^{2}$	$b_{w}^{2}$	G <sub>2</sub>	G <sub>3</sub>	G
30	111	11.1	1.00	0.09	0.000	1.738	0.074	0.676	0.34	0.11	0.000	0.159	0.159
40	83	8.3	0.75	0.12	0.357	1.572	0.077	0.676	0.33	0.11	0.032	0.143	0.176
50	67	6.7	0.60	0.15	0.514	1.433	0.079	0.676	0.32	0.11	0.046	0.131	0.177
60	56	5.6	0.50	0.18	0.630	1.314	0.081	0.676	0.31	0.11	0.056	0.120	0.175
70	48	4.8	0.43	0.21	0.729	1.210	0.083	0.676	0.30	0.11	0.063	0.110	0.174
80	42	4.2	0.38	0.24	0.816	1.119	0.085	0.676	0.30	0.11	0.070	0.102	0.172
90	37	3.7	0.33	0.27	0.895	1.038	0.087	0.676	0.29	0.11	0.076	0.095	0.171
100	33	3.3	0.30	0.30	0.966	0.966	0.089	0.676	0.28	0.11	0.081	0.088	0.169

Note: Relative sizes for family, plot error and within plot variances were 0.040, 0.284 and 0.676, respectively

b.  $b^2 = 0.20$ 

Number Trees of per	Trees per	Selection rate		Selection intensity		Phenotypic variance		Heritability		Genetic gain			
family	family	$\operatorname{plot}_{(n_p)}$	$P_{2}$	$P_3$	$i_{2}(P_{2})$	i <sub>3</sub> (P <sub>3</sub> )	s <sup>2</sup> <sub>2</sub>	s <sup>2</sup> <sub>3</sub>	$b_{f}^{2}$	$h^2_{w}$	G 2	G <sub>3</sub>	G
30	111	11.1	1.00	0.09	0.000	1.738	0.116	0.615	0.43	0.24	0.000	0.333	0.333
40	83	8.3	0.75	0.12	0.357	1.572	0.118	0.615	0.42	0.24	0.052	0.301	0.353
50	67	6.7	0.60	0.15	0.514	1.433	0.120	0.615	0.42	0.24	0.074	0.274	0.348
60	56	5.6	0.50	0.18	0.630	1.314	0.122	0.615	0.41	0.24	0.090	0.251	0.342
70	48	4.8	0.43	0.21	0.729	1.210	0.123	0.615	0.41	0.24	0.104	0.232	0.335
80	42	4.2	0.38	0.24	0.816	1.119	0.125	0.615	0.40	0.24	0.115	0.214	0.329
90	37	3.7	0.33	0.27	0.895	1.038	0.127	0.615	0.39	0.24	0.125	0.199	0.324
100	33	3.3	0.30	0.30	0.966	0.966	0.129	0.615	0.39	0.24	0.135	0.185	0.319

Note: Relative sizes for family, plot error and within plot variances were 0.080, 0.305 and 0.615, respectively

c.  $b^2 = 0.30$ 

Number Trees of per	per	Selection rate		Selection intensity		Phenotypic variance		Heritability		Genetic gain			
family	family	$\operatorname{plot}_{(n_p)}$	$P_{_2}$	$P_3$	$i_{2}(P_{2})$	$i_{3}(P_{3})$	s <sup>2</sup> <sub>2</sub>	S 2 3	$b_{f}^{2}$	$h^2_{w}$	G <sub>2</sub>	G <sub>3</sub>	G
30	111	11.1	1.00	0.09	0.000	1.738	0.158	0.554	0.48	0.41	0.000	0.526	0.526
40	83	8.3	0.75	0.12	0.357	1.572	0.159	0.554	0.47	0.41	0.067	0.475	0.542
50	67	6.7	0.60	0.15	0.514	1.433	0.161	0.554	0.47	0.41	0.096	0.433	0.529
60	56	5.6	0.50	0.18	0.630	1.314	0.163	0.554	0.46	0.41	0.117	0.397	0.515
70	48	4.8	0.43	0.21	0.729	1.210	0.164	0.554	0.46	0.41	0.135	0.366	0.501
80	42	4.2	0.38	0.24	0.816	1.119	0.166	0.554	0.45	0.41	0.150	0.338	0.489
90	37	3.7	0.33	0.27	0.895	1.038	0.168	0.554	0.45	0.41	0.164	0.314	0.478
100	33	3.3	0.30	0.30	0.966	0.966	0.169	0.554	0.44	0.41	0.176	0.292	0.468

Note: Relative sizes for family, plot error and within plot variances were 0.120, 0.326 and 0.554, respectively

## Explanation of the calculation:

Selection rate and selection intensity: a rate of family selection  $(P_2)$  was calculated as given in the text  $(=n_j, n_p, n_i)$  and that within plot selection  $(P_3)$  is an inverse of the trees per plot  $(=1/n_p)$ . Selection intensity at each stage of selection  $(i_2 \text{ and } i_3)$  was calculated with Eq.(4) using the respective rate of selection.

Size of variances: in the gain prediction here, phenotypic variance for individual tree was assumed to be 1.0, hence the additive genetic variance is equal to the heritability. The family variance was calculated with Eq. (2), then within plot variance (y in relative rate) was estimated with the regression equation in Figure 1 (y = -1.527x + 0.737, where x is a rate of the family variance). The plot error variance was regarded as residuals of the phenotypic variance subtracted by the family variance and the within plot variance (= 1.0 - x - y).

Phenotypic variances and heritability: the phenotypic variance for family selection  $(\sigma^2_{j})$  was a family mean variance as given in the text  $(=\sigma^2_{f} + \sigma^2_{e}/r + \sigma^2_{w}/r \cdot n_p)$ , where the variances were relative rate) and that of within plot selection  $(\sigma^2_{j})$  was the estimate with Eq.(2). Heritability at the family selection  $(b^2_{j})$  was calculated as a rate of a quarter of additive genetic variance  $[(1/4)\sigma^2_{A}]$  to the family mean variance  $(\sigma^2_{j})$ , while the one for within plot selection  $(b^2_{w})$  was a rate of three quarter of additive genetic variance  $[(3/4)\sigma^2_{A}]$  to the within plot variance  $(\sigma^2_{j})$ .

Prediction of genetic gain: gains at each stage of selection were predicted with Eq.(3)