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## GENETIC PARAMETER ESTIMATES FOR GROWTH TRAITS IN AN *Eucalyptus Urophylla* S.T. Blake PROGENY TEST IN TIMOR ISLAND

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GENETIC PARAMETER ESTIMATES FOR GROWTH TRAITS IN AN *EUCALYPTUS UROPHYLLA* S.T. BLAKE PROGENY TEST IN TIMOR ISLAND. Genetic parameters were estimated for growth traits of Ampupu (*Eucalyptus urophylla* S.T. Blake) progeny test grown in Southern Central Timor - East Nusa Tenggara Province, Timor Island. When the trial was one year old data were collected from 45 half-sib open pollinated families and assessed. There were genetic variations in height and diameter among families of *E. urophylla*. Growth traits had moderate heritability, both individually and in family, i.e. 0.28 and 0.55 for height and 0.41 and 0.66 for diameter, respectively. Genetic correlation between height and diameter was strong (0.96). However, the estimation of genetic parameter should be interpreted carefully since the trial was very young. Re-assessment of the trial should be carried out periodically to provide better understanding of the species regarding the dynamic of the genetic interaction between the species and its environment, effective age of selection and prediction of genetic gain.

Keywords: Ampupu, *Eucalyptus urophylla*, progeny test, heritability, genetic correlation

PENDUGAAN PARAMETER GENETIK SIFAT PERTUMBUHAN PADA UJI KETURUNAN *Eucalyptus urophylla* S.T. Blake DI PULAU TIMOR. Estimasi parameter genetik sifat pertumbuhan dilakukan terhadap uji keturunan Ampupu yang ditanam di Timor Tengah Selatan, Provinsi Nusa Tenggara Timur. Data berasal dari 45 famili half-sib pada usia satu tahun. Hasil evaluasi menunjukkan bahwa terdapat variasi genetik antara famili *E. urophylla* yang diuji untuk sifat tinggi dan diameter. Sifat pertumbuhan memiliki heritabilitas individu maupun heritabilitas famili yang tinggi, dimana untuk karakter tinggi masing-masing sebesar 0,28 dan 0,55, sedangkan untuk karakter diameter masing-masing 0,41 dan 0,66. Korelasi genetik antara sifat tinggi dan diameter tanaman sangat kuat (0,96). Namun demikian, hasil pendugaan parameter genetik ini harus ditafsirkan secara hati-hati mengingat umur tanaman yang masih sangat muda. Evaluasi parameter genetik terhadap uji keturunan ini perlu dilakukan secara berkala untuk mendapatkan pemahaman yang lebih baik mengenai dinamika interaksi antara genetik dengan lingkungan, umur yang efektif untuk melakukan seleksi dan prediksi perolehan genetik.

Kata kunci: Ampupu, *Eucalyptus urophylla*, uji keturunan, heritabilitas, korelasi genetik

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## I. INTRODUCTION

Ampupu (*Eucalyptus urophylla* S.T. Blake) is an endemic tree species in Indonesia with natural distribution in East Nusa Tenggara and Maluku, namely on the islands of Flores, Adonara, Alor, Lomblen, Pantar, Wetar and Timor at altitudes ranging from 70 m to 2,960 m above sea level and is spread across a distance of 500 km between longitudes 127°E and 122°E and between latitudes 7°30'S and 10°S (Eldridge, Davidson, Harwood, & Van Wyk, 1993). Since 1919 *E. urophylla* has been introduced to many countries and regions, including Australia, Papua New Guinea, China, Malaysia, Thailand, Vietnam, Argentina, Brazil and some African countries, which have humid and sub humid tropical climates with wet/dry seasons (Eldridge et al., 1993; Maid & Bhumibhamon, 2009). The popularity of planting this species either as a pure species or more commonly in hybrid with other eucalypt species, e.g. *E. grandis* and *E. camaldulensis* has increased significantly in Brazil, China, Vietnam and Thailand, particularly to provide raw material for pulp and paper production (Lan, 2011; Sein & Mitlohner, 2011; Manavakum, 2014; Kilulya, Msagati, Mamu, Ngila, & Bush, 2014; Ferraco et al., 2016). In these countries, this species has shown promising growth performance as reported by Maid and Bhumibhamon, (2009); and Sein and Mitlohner, (2011).

*E. urophylla* is not widely planted in Indonesia because it requires a specific site and several regions in Indonesia are outside its natural range. Trials in Sumatra and Kalimantan showed very poor growth, *E. pellita* could only grow well in the trial sites and operational plantations (Hardiyanto & Tridasa, 2000). Commercial plantations of this species in Indonesia to some extent is limited in comparison with *E. pellita* and *Acacia mangium* and the genetic material that is currently being deployed into such plantations at an early stage of development.. Further genetic improvement is anticipated to lead to yield gains for growers. Production of high quality seed source for many tree species is time consuming, because genetic growth parameters

need to be assessed in a progeny test that is well established and maintained. To initiate a genetic improvement programme, numerous provenance and progeny trials of the species had been established in small-scale experimental plantations at many locations in Indonesia. However, most of the tests failed due to various reasons such as livestock disturbances on the progeny test plots that had been developed in 1983 in West Nusa Tenggara. Since the earlier progeny trials were unsuccessful, the purposes of such trials of screening and re-selection for continued breeding and developing superior germplasma of *E. urophylla* had not been achieved. The dynamic of genetic interaction between a species and its environment based on genetic parameters therefore remains unknown. Established progeny test of *E. urophylla* is expected to be converted into a seedling seed orchard with individual and family tree selection. This will produce superior seeds that are expected to be adaptable to various plantation sites.

A progeny trial involving 45 families of *E. urophylla* had been established in Timor Island in October 2013. This paper evaluates the adaptability (survival) and genetic parameters for growth (height and diameter, heritability and genetic correlation) of families in the trial. The results will be used to form a basis for understanding the genotypic effects on growth of *E. urophylla* at an early age, and in the future it will be used to understand the relationships between early assessments of growth and survival and its impact at later ages. The data and information will then lead to a more effective targeting of the selection period to develop the best breeding strategies of *E. urophylla* in Indonesia.

## II. MATERIAL AND METHOD

### A. Material

The study was based on a progeny test, located at Bu'at (latitude 09°49'047" S, longitude 124°15'002" E, altitude 895 m a.s.l), South Mollo, Southern Central Timor District, East Nusa Tenggara Province, as shown in Figure 1

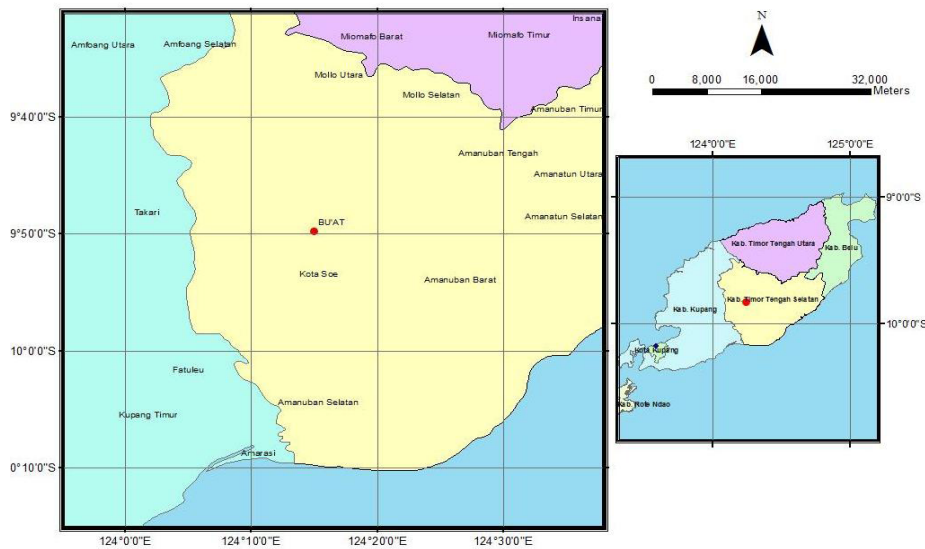


Figure 1. Map of the research site in Timor Island

and managed by the Forest Research Institute of Kupang. In 2014 the rainfall on the site was 1992.75 mm that occurred during 10 months with a total of 95 rainy days (BPS Kabupaten Timor Tengah Selatan, 2015). The progeny test was planted in November 2013, laid out as a randomised, resolvable Incomplete Block Design (IBD) that comprised of 45 families, 3 tree-plots and 5 complete block replications, at 4 × 4 m spacing. The row of each replication constitutes as incomplete blocks. The 45 families tested in the progeny trial were collected from the two largest population of *E. urophylla* in East Nusa Tenggara namely Fatumnasi (19 families) and Bu'at (26 families).

**B. Data Collection**

Assessments of growth parameters were undertaken in October 2014. The variables measured were survival rate to assess field adaptation, total height and diameter to assess growth performance. Plot survival rate (%) was the ratio of the number of surviving trees with those planted. All surviving trees in the trial were assessed for tree height and diameter. Survival data were summarised as percent plot-mean survival (i.e. the number of surviving trees in each plot divided by three). Total height (m) was measured from the ground to the top

of the tree, and stem diameter at 5 cm above the ground. The measurement of diameter at a height of 5 cm above the ground was done because the height of the plant had not yet reached a diameter at breast height (dbh).

**C. Data Analyses**

**1. Adaptability**

Analyses of variance were performed to determine statistical significance of survival rate among families. The analyses of variance are based on the following linear model (Williams, Matheson, & Harwood, 2002):

$$Y_{ijkl} = +B_i + P_j + F_{k(j)} + E_{ijkl} \tag{1}$$

where :

- $Y_{ijkl}$  : observation of tree in the  $l^{th}$  plot, the  $i^{th}$  replication,  $j^{th}$  provenance and the  $j^{th}$  family;
- $\mu$  : overall mean;
- $B_i$  : effect of the  $i^{th}$  replication;
- $P_j$  : the effect of the  $j^{th}$  provenance
- $F_{k(j)}$  : effect of the  $k^{th}$  family nested within the  $j^{th}$  provenance
- $E_{ijkl}$  : residual

## 2. Variation in Height and Diameter

Breeders want to select trees for higher production, better quality and higher survival ability. Growth performances (height and stem diameter) of tree species are important variables to be assessed in an improvement programme especially for pulp wood as they indicate the productivity of the species. Variations in individual data of tree growth performances were analysed using the restricted maximum likelihood (REML) procedure. The following linear model was used (Williams et al., 2002):

$$Y_{ijklmn} = \mu + B_i + P_j + R_k + CB_{k(i)} + F_{m(j)} + FB_{mi} + E_{ijklmn} \quad (2)$$

where :

- $Y_{ijklmn}$  : individual observation of tree in  $n^{th}$  plot, the  $i^{th}$  replication, the  $j^{th}$  provenance, the  $k^{th}$  row, the  $l^{th}$  column, and the  $m^{th}$  family;
- $\mu$  : overall mean;
- $B_i$  : fixed effect of the  $i^{th}$  replication;
- $P_j$  : fixed effect of the  $j^{th}$  provenance
- $R_k$  : random effect of the  $k^{th}$  row;
- $CB_{k(i)}$  : random interaction effect of the  $i^{th}$  column and the  $i^{th}$  replication;
- $F_{m(j)}$  : random effect of the  $m^{th}$  family nested within the  $j^{th}$  provenance
- $FB_{mi}$  : random interaction effect of the  $m^{th}$  family and the  $i^{th}$  replication (the plot effect)
- $E_{ijklmn}$  : random residual

## 3. Heritability

Genetic parameters including the narrow-sense heritability, “family heritability” and genetic correlation between traits were calculated using the REML variance component estimates. Heritability is the proportion of the genetic factors of interest that is inherited from parent to offspring (Zobel & Talbert, 1984). In other words, heritability is the statistical expression for the relative contribution of genotype and environment to the phenotype performance, and is useful in predicting gain from selection. The commonly applied coefficient of relationship for the first generation eucalypt progeny of

1/2.5 appears to be quite suitable for correcting variance component and heritability estimates (Bush, Kain, Matheson, & Kanowski, 2011). Individuals ( $h^2_i$ ) and a family ( $h^2_f$ ) heritabilities were calculated from the following equations (Zobel & Talbert, 1984):

$$h^2_i = \frac{2.5 \sigma^2_f}{\sigma^2_f + \sigma^2_{fb} + \sigma^2_e} \quad (3)$$

$$h^2_f = \frac{\sigma^2_f}{\sigma^2_f + \sigma^2_{fb} / b + \sigma^2_e / nb} \quad (4)$$

where :

- $\sigma^2_f$  : variance component of family
- $\sigma^2_{fb}$  : variance component of family and block interaction
- $\sigma^2_e$  : variance component of error
- $n$  : harmonic mean of trees per plot sum
- $b$  : harmonic mean of blocks sum

## 4. Genetic Correlation

Estimating genetic correlations between traits of interest and genotype by environment interaction ( $G \times E$ ) is also necessary in proposing the basis for setting up breeding populations and selecting environmentally stable genotypes. Genetic correlation can be used, for example, to determine the genetic relationships between the height and diameter traits of trees in a progeny test, and is a very important predictor of the effectiveness of the variable in the selection process (Gaspar, Louzada, Aguiar, & Almeida, 2008). Genetic correlations between growth traits (height and diameter) were calculated based on the following equation (Zobel & Talbert, 1984):

$$r_G = \frac{\sigma_{f(xy)}}{\sqrt{\sigma^2_f(x) * \sigma^2_f(y)}} \quad (5)$$

where :

- $r_G$  : genetic correlation
- $\sigma_{(xy)}$  : covariance component between variable ( $x$ ) and variable ( $y$ ) interaction
- $\sigma^2(x)$  : variance component of variable ( $x$ )
- $\sigma^2(y)$  : variance component of variable ( $y$ )

### III. RESULT AND DISCUSSION

#### A. Adaptability

At the end of the first year of growth, the survival rate across this progeny trial of *E. urophylla* was 83%. This was high and it might decrease with age. For example, in Chachoengsao Thailand, where *E. urophylla* was an introduced species that had to adapt to the new environment, the survival rate was 85%, 79%, 75% and 54% at the ages of 6, 8, 10 and 16 years, respectively (Maid & Bhumibhamon, 2009). The current progeny trial is within its natural distribution and natural altitudinal range. However survival rate also depends on adaptation to biotic and abiotic stress factors, and soil type and conditions (Maid & Bhumibhamon, 2009). The variable of survival rate could be used to select the best family, cause of this variable showed the adaptation of the family toward the extreme condition in their early establishment (Widiyatno, Naiem, Purnomo, & Jatmiko, 2014).

There were no significant differences in survival between the 2 provenances and the 45 families, although rates ranged between 33.33% and 100% per plot (families per replication) (Table 1). However, there were significant differences between replications, although silvicultural treatment and site preparation were similar. This might have been due to changes in soil properties and environmental gradients across the trial, but these were not measured. The progeny trial was located on a sloping area where there was a difference in the depth of the soil. Blocking was made across the slope so that replication at the lowest position has the deepest

soil depth (more than 60 cm), while replication at the top of the slope had a shallower soil depth (less than 30 cm). Thus all families of *E. urophylla* were considered well-adapted to this site. It has happened because the species was planted in a location that has climate characteristics similar to its natural distribution. *Eucalyptus urophylla* has the largest altitudinal range of any *Eucalyptus* species covering 70 m to 2,960 m above sea level. Annual rainfall for this species in Timor Island is 1300–2000 mm and the dry season lasts for 3-4 months (Sein & Mitlöhner, 2011).

#### B. Variations in Height and Diameter

Across the 45 families, mean total height was 53.3 cm, ranged between 17.0 cm and 145.0 cm, and mean diameter was 0.38 cm, ranged between 0.11 cm to 1.13 cm. These values were lower than that of in an *E. urophylla* plantation after the first year of growth in Vietnam (200 to 209 cm height and 1.9 to 2.3 cm diameter) (Kien, Jansson, Harwood, & Thinh, 2009; Sein & Mitlöhner, 2011) and in Brazil, where the mean annual height and diameter growth was 100.6 cm and 1.5 cm, respectively (Whitesell, DeBell, Schubert, Strand, & Crabb, 1992).

There were significant effect of the provenances and families on plant height and diameter as shown in the Table 2. Trees of the Fatumasi provenance show their superiority over Bu'at provenance as indicated by the mean of height and diameter of the trees. Mean of height and diameter of the trees of Fatumasi provenance was 56.07 cm and 0.40 mm respectively, while it was only 49.43 cm and 0.35 mm of the trees of Bu'at provenance. It is likely

Table 1. Analysis of variance of the survival rates of *E. urophylla* at the first year of growth

Source of variation	<i>df</i>	<i>s.s</i>	<i>m.s</i>	<i>F pr.</i>
Replication	4	10144	2535.877**	0.0002
Provenance	1	46.250	46.250 <sup>ns</sup>	0.7462
Family(Provenance)	43	18219	423.707 <sup>ns</sup>	0.5427
Residual	169	74362	440.011	

Note : *df*: degrees of freedom, *s.s*: sum of square, *m.s*: mean square, *F pr.*: *F* probability; \*\* : significantly different at  $p < 0.01$ , ns : not significantly different at  $p < 0.05$

Table 2. Analysis of variance of the height and diameter

Source of variation	<i>d.f</i>	<i>s.s</i>	<i>m.s</i>	<i>F pr.</i>
Height				
Replication	4	54861	13715**	<.0001
Provenance	1	5892	5891.623**	<.0001
Row	40	21438	535.950 <sup>ns</sup>	<.0056
Replication*Column	20	31035	1551.774**	<.0001
Family(P)	43	34093	792.862**	<.0001
Replication*family(P)	109	41291	378.815 <sup>ns</sup>	0.0929
Residual	323	100154	310.074	
Diameter				
Replication	4	1.112153	0.278**	<.0001
Provenance	1	0.353244	0.353**	<.0001
Row	40	0.706117	0.018*	0.0102
Replication*Column	20	0.955678	0.048**	<.0001
Family(P)	43	1.644769	0.038**	<.0001
Replication*family(P)	109	1.294985	0.012 <sup>ns</sup>	0.2391
Residual	323	3.450651	0.011	

Note : *d.f*: degrees of freedom, *s.s* : sum of square, *m.s* : mean square, *F pr.* : *F* probability; \*\*: significantly different at  $p < 0.01$ , ns : not significantly different at  $p < 0.05$ .

due to the fact that parent trees of Fatumnasi provenance which their progenies were included in this trial have a better performance in term of stem diameter to compare with those of Bu'at provenance. Significant differences among families within provenance tested on height and diameter traits indicated that there is high genetic variations families included in the trial. In other *Eucalyptus*, that among provenances and among families-within-provenances for both traits in *E. camaldulensis* were also shown a significant differences (Bush, Marcar, Arnold, & Crawford, 2013). As for survival, there were significant differences between replications, possibly for similar reasons to those stated above. This result reiterates the findings made by Maid and Bhumibhamon (2009) based on trial of up to six years old trees in Chachoengsao, Thailand. The differences among families of *E. urophylla* found in the present as well as other trials could be exploited to improve productivity of the plantations. Gains in genetic improvement programs could be achieved through family selection. The seed sources from the best performing families are the most appropriate choices for immediate future plantations in

areas having conditions similar to the trial site when genetically improved seed orchards have not yet been established.

### C. Heritability

The estimation of heritability and genetic correlations between traits shown in the Tabel 3 were based on the estimation of variance and covariance components (Bush et al., 2011).

Individual ( $b^2i$ ) and family ( $b^2f$ ) heritabilities for height and diameter were 0.28 and 0.41, and 0.55 and 0.66 respectively. These heritabilities can be considered moderate based on the Cotterill and Dean (1990) classification, that anticipates values of 0.1-0.3 and 0.4-0.6, respectively, for both variables. These heritabilities were quite high for these growth traits. It is most likely due to the seed were collected from closely together parent trees or there are more than usual inbred individuals or variability in inbreeding among families. Previous study by Borralho (1994) indicated that inbreeding depression and selfing among families could result overestimation of heritability calculation. Heritability for diameter in this study was found to be greater than heretability for height. This result is similar

Table 3. Estimated heritabilities and variance components of the height and diameter

Parameters	$h^2_i$	$h^2_f$	$\sigma^2_f$	$\sigma^2_{fb}$	$\sigma^2_e$
Height	0.28	0.55	0.0022	0.0007	0.0108
Diameter	0.41	0.66	42.32	26.12	310.53

Notes :  $h^2_i$ : individual heritability,  $h^2_f$ : family heritability,  $\sigma^2_f$  : variance component of family;  $\sigma^2_{fb}$  : variance component of family and block interaction,  $\sigma^2_e$  : variance component of error

with other studies of progeny trials of *E. urophylla* in Vietnam by Kien et al. (2009) and in China by Lan (2011) where heritabilities of both traits were relatively identical. It should be noted however that heritability is also expected to change with plant age. In general, heritability estimated for height and diameter had a tendency to increase with age. The trend of increasing heritability with age was also reported in the previous study of *E. urophylla* in China (Wei & Borralho, 1998) and in Vietnam (Kien et al., 2009). The later authors reported that heritabilities estimated for diameter and height increased about two fold in two progeny trials. Other eucalypt species (Gapare, Gwaze, & Musokonyi, 2003) and other tropical species such as *Araucaria cunninghamii* (Setiadi, 2010; Setiadi & Susanto, 2012) have also given similar trends. The change in heritability in long rotation crops such as forest trees is expected since genes involved in growth may change with age and these changes may be related to different growth phases (Missanjo, Kamanga-Thole, & Manda, 2013). Kien et al. (2009) stated that the increased heritability with age for growth traits could also result from competitive effects occurred at later ages in the stand, which may cause over estimation of heritability.

#### D. Genetic Correlation

The genetic correlation between height and diameter was 0.96. Thus the correlation was high and positive, which means that selecting for increase in diameter will be associated with an increase in height and vice versa. As for heritability, genetic correlation between tree height and diameter could be changed as trees get older. Genetic correlations estimated for diameter and height traits of two progeny

trials of *E. urophylla* from year 1 to year 8 and year 9 at two different sites in Vietnam showed that the genetic correlation changed without specific trends. Genetic correlations at similar ages between diameter and height were strong at all ages at both sites ( $r_g = 0.75$  to  $0.98$ ) (Kien et al., 2009).

If a genetic correlation in this trial remains relatively constant with tree age as reported in the previous study by Kien et al. (2009), selection was likely to be more efficient if using one variable only. In practice, selection within *E. urophylla* would be more easily conducted by selection based on diameter traits rather than height as it would greatly reduce the cost of measurement especially in later ages when trees were relatively tall. However, as diameter was measured at 5 cm height, it would first be necessary to assess this correlation at breast height.

#### IV. CONCLUSION

Evaluation of a one year old *E. urophylla* progeny test showed that the survival rate was relatively high (83%). There were also significant differences between families in both height and diameter. Individual and family heritabilities were 0.1-0.3 and 0.4-0.6 respectively, for both variables, and the diameter was greater than the height. The genetic correlation between height and diameter was strong and positive (0.96). However, these estimated genetic parameters should be interpreted carefully as the trial was very young. Assessment of the trial at later ages should be done periodically to provide better understanding of the species regarding the dynamic of genetic interaction between the species and its environment, effective age of selection and predicted genetic gain. The



progeny trial may be converted into seedling seed orchard through selection of superior individuals and families. This seedling seed orchard is expected to provide superior seeds for future plantation.

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