Impact of within family variation on growth superiority of improved seeds of *Eucalyptus pellita* observed in genetic gain trial

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Abstract. Progenies from two seedling seed orchards (SSO) consisting of the same families could perform different growths. Concerning this fact, improved seeds of *Eucalyptus pellita* collected from two SSOs based primarily on the same families and provenances were tested together in a genetic gain trial. The study aims to observe the impact of within family seed trees in two SSOs on growth superiority of the improved seeds. The trial was established in Wonogiri, Central Java and laid out in randomized complete block design with three seed sources: SSO-A, SSO-B and one seed stand (as control), 100 trees/plot, 4 replications and 4x1.5 m spacing. Measurements were conducted at seven periodical times (16 months up to 66 months of ages). Improved seeds from the two SSOs displayed better growths than that from seed stand at all measurements. The growth superiority of SSO over the control varied among the two SSO. SSO-A showed consistently higher superiority relatively than SSO-B, on average of 110%, 236%, 122% and 165% in height, dbh, stem volume, and stand volume, respectively. The results demonstrated the importance of within family variation of *E. pellita* in establishing seed orchard for seed production and further breeding.

1. Introduction

*Eucalyptus pellita* F. Muell. is one of the main species for Industrial Plantation Forest (HTI) to supply raw materials for pulp and paper industry. Several performance advantages of *E. pellita* trees are fast growth, good form traits and wood properties suited for industries, and easy to clonally propagate[1]. *E. pellitawas* recognized to grow well in the tropics and tropical humidias and it is also relatively resistant to pests and diseases [2] [3] [4] [5] [6].

In relation to increasing plantation productivity of *E. pellita*, intensive tree improvement programs have been carried out to produce genetically improved seeds. Following the breeding strategy, genetically improved stocks have been produced either through seed collected from seedling seed orchards (SSO) or clonal development. The SSOs are then commonly established in some regions to ensure the quantity and sustainability of seed production. Concerning to the realized genetic gain from the improved seeds collected from the SSO, further verification of the productivity in operational scale is necessary. However, there are some issues regarding the discrepancy of genetic gain predicted from the SSO to the gain realized in operational plantation. One of the issues is the impact of within family variation in SSO on the different quality of seed. This is because progenies from two seedling seed orchards (SSO) consisting of the same families could perform different growth.
The Center for Biotechnology and Tree Improvement (CFBTI) under technical cooperation with Japan International Cooperation Agency (JICA) has initiated tree improvement program of *E. pellitathrough establishing first-generation SSOin several sites in 1994 and 1995 [7][8][9]. Genetic materials of *E. pellitafrom CFBTI collection were distributed to some sites for establishing the progeny trial that was followed by successive selection procedures for conversion into SSOas seed sources. Seeds produced from the SSO were then used for establishing genetic gain trial to verify the realized genetic gain [10]. Realized genetic gain to evaluate the achievement of breeding program could be assessed through genetic gain trial where the improved seeds are compared to unimproved seeds in the plots with the same silvicultural treatment [11][12].

In this study, growth of *E. pellita trees derived from improved seed collected from two different SSOs but consisting of the same genetic background of family structures were observed in genetic gain trial. The study aims to identify the impact of within family variation in the two SSO on growth superiority of the improved seeds. The results of study are expected to provide alternative strategy in optimizing the increase of productivity from improved seeds produced in the SSO.

2. Material and method

2.1. Genetic gain trial

The observation was conducted in genetic gain trial of *E. pellita established in Wonogiri, Central Java in 2006. The genetic gain trial is located at 07° 32’S and 110° 41’E, an altitude of 141 m above sea level with annual rainfall of 1645 mm and climate type C according to Schmidt and Ferguson [13]. The soil type in the trials Vertisols and the previous vegetation was dominated by *Dalbergia latifolia.*

The improved seed collected from two SSOs consisting the same genetic background of family structures were planted together with unimproved seed as control in the gain trial. Thus, the treatment in the trial consisted of improved seeds collected from two SSOs: Ep 06A (hereinafter referred to as SSO-A) and Ep 06B (hereinafter referred to as SSO-B), and unimproved seed through bulking of seed collected from natural distribution in South and North of Kiriwo, Papua New Guinea (Table 1). The two SSOs were converted from first-generation progeny trial consisting of the same families originated from provenances of Australia, Indonesia and Papua New Guinea. The experimental design of trial was a randomized complete block design (RCBD) with a 4 x 1.5 m spacing, three seed sources as treatment, 100 squared-tree plot, and 4 replications.

Table 1. Seed information in the genetic gain trials *E. pellita* in Wonogiri, Central Java

| No | Seed source | Location | Provenance | Remarks          |
|----|-------------|----------|------------|-----------------|
| 1  | Ep 06A      | SSO-A    | SSO site 1, Perawang, Riau | Australia, Ind, PNG | Single population SSO |
| 2  | Ep 06B      | SSO-B    | SSO site 1, Perawang, Riau | Australia, Ind, PNG | Single population SSO |
| 3  | Natural stand (control) | South of Kiriwo and North of Kiriwo, PNG | South of Kiriwo, PNG | Bulking seed |

Ep= *Eucalyptus pellita; SSO= Seedling Seed Orchard; PNG=Papua New Guinea; Ind= Indonesia*

2.2. Data collection

Field measurements were conducted for survival rates and growth traits involving total tree height and diameter at breast height (dbh) at seven periodical times: 16 months, 22 months, 28 months, 36 months, 48 months, 53 months and 66 months after planting. To get accurate data, measurements were only made on 64 inner trees (8 x 8 trees) in each plot. Stem volume was calculated for each individual tree using formula as follows [18]:

\[
V = EXP(-10.8706) \times H^{1.2596} \times D^{1.9316} 
\]
The stand volume in each plot was calculated by summing the stem volume from living trees per plot, which was then used to determine the productivity of stand volume per hectare (m³/ha) using the following equation [17]:

\[
\text{Stand volume per hectare} = \frac{\sum_{\text{living trees per plot}} \text{volume}}{\text{Area per plot in m}^2} \times 1000
\]

2.3. Data analysis
In this purpose of study, the analysis data was made using analysis of variance (ANOVA) to observe the differences among the three seed sources tested. The analysis was calculated using the linear model as follows [14]:

\[
Y_{ij} = \mu + B_i + S_j + E_{ij}
\]

where:
- \(Y_{ij}\): average seed sources i-th and j-th repetition;
- \(\mu\): general population average;
- \(B_i\): effect of the i-th repetition;
- \(S_j\): effect of the j-th seed sources; and
- \(E_{ij}\): error.

If the results of the analysis show significant differences, an analysis with Duncan’s Multiple Range Test (DMRT) was then proceeded to distinguish seed sources based on the average parameters observed.

2.4. Genetic gain
To observe the growth superiority, realized genetic gain of improved seeds from two SSO (SSO-A and SSO-B) over the control (natural stand) was calculated using the following formula [15]:

\[
\Delta G = \frac{\bar{x}_{\text{improved}} - \bar{x}_{\text{unimproved}}}{\bar{x}_{\text{unimproved}}} \times 100\%
\]

The differences in growth superiority between the two SSOs over the control were then used to observe the impact of within family variation on the improved seed quality produced from the two SSOs.

3. Result and discussion
3.1. Survival rate
In the genetic gain trial, it is necessary to calculate the survival rate which shows the number of survived trees to adapt to the environment of planting. In this study the survival rate was used to determine the adaptability and the level of success in planting the improved seed from the two SSOs in Perawang, Riau to different environments of Wonogiri, Central Java. The ability to survive in a new environment as a form of adaptation is a mechanism of individuals in dealing with environmental changes that affect physiological processes in themselves [16].

The survival rates of *E. pellitain* genetic gain trial at seven periodical measurements are presented in Table 2. The average survival rate of *E. pellitavarieties among the three seed sources. The two SSOs showed consistently high survival rate over the control along all measurements. At the final age of observation (66 months) the survival rate was 75% for SSO-A, followed by SSO-B at around 67%, and control at around 61%. It indicated that improved seed of *E. pellitacollected from SSO of different sites could perform better adaptation than unimproved seed in the new environment of planting. This result also indicated that the two SSOs consisting the same families structure produced improved seed with different qualities for site adaptation. Nirsatmanto et al. [17] reported that high survival rate provides an advantage in estimating the effect of seed sources on increasing stand productivity.
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| Table 2. Average survival rates of *E. pellita* in genetic gain trial plot in Wonogiri, Central Java |
|---|---|---|---|---|---|---|---|
| No | SS | 16 months | 28 months | 36 months | 48 months | 53 months | 66 months |
| N | SR | N | SR | N | SR | N | SR | N | SR | N | SR |
| 1 | SSO-A | 58 | 91.67 | 58 | 91.15 | 58 | 91.67 | 57 | 90.10 | 54 | 84.38 | 51 | 79.69 | 48 | 75.00 |
| 2 | SSO-B | 53 | 83.33 | 54 | 84.90 | 53 | 83.33 | 51 | 80.21 | 48 | 75.00 | 45 | 70.83 | 42 | 66.67 |
| 3 | C | 51 | 79.69 | 52 | 81.77 | 50 | 78.65 | 50 | 78.13 | 45 | 70.83 | 44 | 69.27 | 39 | 60.94 |

SS=seed sources; N=average number of plants per plot; SR=survival rate (%); C=control

3.2. Growth trend

The growth trend of *E. pellita* in the gain trial across the six periodical measurements are presented in Table 3. Although the analysis of variance showed an insignificant difference in all growth traits, the highest growth traits were found in SSO-A, followed gradually by SSO-B and control.

| Table 3. Growth of *E. pellita* in genetic gain trial in Wonogiri, Central Java |
|---|---|---|---|---|---|---|---|
| No | Ages (months) | Seed Sources | SSO-A | SSO-B | C | |
| h | dbh | vol | h | dbh | vol | h | dbh | vol |
| 1 | 16 | 3.24 (14.2) | 2.82 (9.6) | 0.91 (36.9) | 3.10 (9.2) | 2.64 (3.4) | 0.80 (21.6) | 2.83 | 2.55 | 0.64 |
| 2 | 22 | 4.10 (13.3) | 4.04 (15.5) | 2.44 (53.0) | 3.81 (5.0) | 3.63 (3.0) | 1.89 (15.9) | 3.61 | 3.52 | 1.57 |
| 3 | 28 | 7.63 (21.0) | 6.48 (6.6) | 11.84 (48.5) | 7.07 (11.7) | 6.24 (2.2) | 11.06 (37.4) | 6.30 | 6.13 | 8.08 |
| 4 | 36 | 8.26 (13.4) | 7.38 (12.5) | 16.42 (44.8) | 8.00 (9.4) | 6.83 (3.7) | 14.01 (22.1) | 7.27 | 6.57 | 11.31 |
| 5 | 48 | 11.77 (18.6) | 10.00 (8.5) | 41.75 (38.5) | 10.56 (6.6) | 9.41 (2.2) | 33.65 (12.2) | 9.88 | 9.21 | 29.29 |
| 6 | 53 | 13.69 (11.9) | 11.26 (9.2) | 61.52 (28.3) | 12.30 (0.6) | 10.80 (4.4) | 50.19 (4.3) | 12.23 | 10.34 | 48.11 |
| 7 | 66 | 16.55 (11.0) | 13.16 (5.0) | 104.13 (18.0) | 15.84 (6.8) | 12.71 (1.2) | 94.37 (7.3) | 14.93 | 12.56 | 88.96 |

h=height (m); dbh=diameter at breast height (cm); vol=stem volume (x 10^{-3} m^3)

In general, the growth of *E. pellita* in the genetic gain trial in Wonogiri, Central Java was lower when compared to *E. pellita* in the other location. In Pelihari, Riau height and diameter growth were 2-3 times faster [18]. This is due to differences in soil fertility and climatological conditions between Wonogiri and the other location. The planting site of the genetic gain trial in Wonogiri is categorized as marginal land with relatively low rainfall, which is < 2000 mm/year [13].

The improved seed from two SSO showed consistently better growth than the control, and SSO-A performed better than SSO-B along all measurements. In detail, this result also showed that within family variation in two SSOs affected not only survival rates, but also the growth traits including height, dbh and stem volume. From the two parameters of survival rates and growth traits, it is clear that SSO-A has better seed source than SSO-B although the two SSOs consist of the same families.

3.3. Stand volume productivity

Genetic improvement is commonly represented as the value of productivity increase in stand volume per unit area. This is because individual stem volume cannot always be used as a good indicator in estimating the potential increase of total volume productivity [17]. The general aim of tree breeding is to increase stand volume at harvest, through indirect selection of stem size at the beginning of rotation [19]. In this study, the results of analysis of variance showed that seed sources did not significantly affect stand volume productivity of *E. pellita* up to the age of 48 months. The significant differences in
stand volume were observed starting from the age of 53 months. It indicated that the genetic impact on stand volume becomes evident only as the trees getting older [17] [20].

Figure 1. Stand volume productivity

The trend of stand volume among the seed sources observed in genetic gain trial at seven periodical time of measurements are presented in Figure 1. The two SSO showed better stand volume than the control. As observed in growth traits (Table 3), SSO-A was consistently showing better stand volume than SSO-B, and it was significantly different at 66 months of age. Tree density, closely related to plant survival rate, is an important factor of stand volume. However, the effect of differences in survival rate between the two SSOs (Table 2) provided less impact on stand volume as compared to the effect of genetic.

Table 4. Average stand volume (m³/ha) and realized genetic gain (%) in genetic gain trial in Wonogiri, Central Java

| No | SS    | 16 months | 22 months | 28 months | 36 months | 48 months | 53 months | 66 months |
|----|-------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|
|    | Vol G | Vol G     | Vol G     | Vol G     | Vol G     | Vol G     | Vol G     | Vol G     |
| 1  | SSO-A | 0.89g     | 57.53     | 2.40a     | 70.53     | 11.59a    | 73.14     | 15.87a    |
|    |       | 67.02     | 64.98     | 52.89a    | 47.62     | 84.50a    | 45.29     |
| 2  | SSO-B | 0.72a     | 27.18     | 1.69a     | 20.28     | 9.71a     | 44.99     | 11.92a    |
|    |       | 25.40     | 18.85     | 38.22ab   | 6.68      | 68.24b    | 17.33     |
| 3  | C     | 0.56a     | 1.41a     | 6.70a     | 9.50a     | 22.84a    | 35.83b    | 58.16c    |

SS=seed source; C=control; Vol=stand volume; G=realized genetic gain
Values followed by the same letter on the same line are not significantly different at test level 5%

3.4. Realized genetic gain
Realized genetic gain calculated as increase percentage of improved seed from each SSO over the control was used to identify the differences in growth superiority in this study. Along with the age of measurements, SSO-A showed consistently higher genetic gain than SSO-B for all measured traits. The average of genetic gain across the measurements for SSO-A were 14.8% for height, 9.6% for dbh, 38.3% for stem volume, corresponding for the SSO-B were 7.0%, 2.9%, 17.3% for height, dbh, stem volume, respectively (Table 2). A remarkable higher genetic gain was observed in stand volume averaging at around 60.9% for SSO-A and 23.0% for SSO-B (Table 4). In general, following the growth pattern, the magnitude of genetic gain tended to decrease as the ages increase.

The results in this study showed that SSO-A produced higher genetic gain than SSO-B for all growth parameters in all ages. Although the two SSOs consisted of the same genetic background in family structures, the differences in the productivity of seed produced was evident. Observing the realized genetic gain would be important to identify the superiority value among the tested seed sources. This is because assessing the magnitude of potential increase or loss in productivity would
become easy. Imposing the stand volume as one of growth parameters is also important as it is a good parameter to access the economic value of productivity in plantation as final target of improving productivity from tree improvement program.

Genetic variation tends to be higher in populations that have greater growth and more suitable environmental conditions[21][22]. Superior growth in SSO-A over SSO-B indicates a higher genetic variation in SSO-A, and because the two SSOs consist of the same families, thus additional genetic variation should occurs within the family. The increase in growth and genetic parameters of SSO-A would affect the magnitude of genetic gain.

Table 5. Average of realized genetic gain across seven measurement ages for two SSOs in genetic gain trial of E. pellita in Wonogiri, Central Java

|       | Seed Source |       |       |       |       |       | Stand Vol |
|-------|-------------|-------|-------|-------|-------|-------|-----------|
|       | SSO-A       |       |       |       |       |       |           |
| h     | dbh         | Stem Vol | Stand Vol | h     | dbh         | Stem Vol | Stand Vol |
| 14,78 | 9,57        | 38,31   | 60,87  | 7,03  | 2,85        | 17,26    | 22,96     |
| (110,17) | (235,80)     | (122,00) | (165,12) |       |             |           |           |

Numbers in parentheses are the increased genetic gain (%) of SSO-A over SSO-B

4. Conclusion

The results of study revealed that the improved seed produced from the two SSOs of E. pellita showed better growth and survival rate than unimproved seed in genetic gain trial. The realized genetic improvement clearly varied between the two SSOs, although they consisted of the same genetic background of family structures. This indicated that the impact of within family variation evidently affected the genetic quality of seed produced from the SSO. The results of study imply that information from genetic gain trial is necessary to assess total genetic quality in SSO in order that the quality of seed produced could be optimized to provide maximum productivity. The verified SSO in producing high quality seed could be further multiplied and deployed through establishing a clonal seed orchard to ensure the same genetic quality of seed production.

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References

[1] Sulichantini E D 2016 Pertumbuhan tanaman Eucalyptus pellita F. Muell dilapang dan dengan metode kultur jaringan, stekpucuk, dan biji(Ziraa’ah 412) pp 269–275
[2] Harwood C E 1998 Eucalyptus pellita an Annotated Bibliography (Victoria, Australia: CSIRO Publishing)
[3] Lukmandaru G, Zumaini U F, Soeprijadi D, Nugroho W D and Susanto M 2016 Chemical properties and fiber dimensions of Eucalyptus pellita from the 2nd generation of progeny tests in Pelaihari, South Borneo, Indonesia (Journal of Korean Wood Science Technology 44) pp 571–588 https://doi.org/https://www.cabdirect.org/cabdirect/abstract/20163271766
[4] Ramadan A, Indrioko S and Hardiyanto E B 2018 Parameter genetiksifat pertumbuhan dan kinerapatanayuklon Eucalyptus pellita F. Muell. diduatapanyang berbeda Kalimantan Timur (Jurnal Pemuliaan Tanaman Hutan 122) pp 115–125 https://doi.org/https://doi.org/10.20886/jpht.2018.12.2.115-126
[5] Setyaji T, Sunarti S and Nirsatmanto A 2016 Early growth and stand volume productivity of selected clones of Eucalyptus pellita (Indonesian Journal of Forestry Research 31) pp 27–32 https://doi.org/10.20886/ijfr.2016.3.1.27-32
[6] Sunarti S 2012 VariasipertumbuhanjaringanpadaujiklonEucalyptus pellita F. Muell. di Wonogiri, Jawa Tengah (Jurnal Pemuliaan Tanaman Hutan61) pp 57–63 https://doi.org/10.20886/jpth.2012.6.1.57-63

[7] Kurinobu S, Nirsatmanto A and Susanto M 1994 General Information of Seed Source Establishment of Acacia mangium, Eucalyptus pellita and Eucalyptus urophylla in South Kalimantan (Fiscal Year 1993/1994) Report

[8] Leksono B and Kurinobu S 1996 General Information of Seed Source Establishment of Eucalyptus pellita in Riau Fiscal Year 1995/1996 Report

[9] Nirsatmanto A and Hashimoto K 1995 General Information of Seed Source Establishment of Acacia mangium and Eucalyptus pellita Wonogiri in Central Java Fiscal Year 1994/1995 Report

[10] Leksono B, Nirsatmanto A, Setyaji T and Surip S 2006 General Information of Gain Trial of F-1 Eucalyptus pellita Establishment in Java, Kalimantan and Sumatra (collaboration with BTR Banjarbaru and BTR Palembang) Fiscal Year 2004-2006 Report

[11] St. Clair J 1993 Evaluating realized genetic gains from tree improvement In H E Burkhart, T G Gregoire and J Smith (Eds) Modeling stand response to silvicultural practices: Proceeding of the IUFRO S401 Conference (27 September – 1 October 1993) pp 145–157 (Blacksburg, Virginia, USA)

[12] Weng Y H, Tosh K, Adam G and Fullarton M S 2008 Realized genetic gains observed in a first generation seedling seed orchard for jack pine in New Brunswick, Canada (New Forests36) pp 285–298 https://doi.org/10.1080/00382167.2008.9629888

[13] Sunarti S, Na’iem M, Hardiyanto E B and Indrioko S 2013 Breeding strategy of Acacia Hybrid (Acacia mangium × A. auriculiformis) to increase forest plantation productivity in Indonesia (Jurnal Manajemen Hutan Tropika/Journal of Tropical Forest ManagementXIX2) pp 128–137 https://doi.org/10.7226/jtfm.19.2.128

[14] Hai P H, Harwood C, Kha L D, Pinyopusarerk K and Thinh H H 2008 Genetic gain from breeding Acacia auriculiformis in Vietnam (Journal of Tropical Forest Science204) pp 313–327

[15] Wright J A, Osario L F and Dvorak W S 1996 Realized and Predicted Genetic Gain in The Pinus patula Breeding Program of Smurfit Carton de Colombia (South African Forestry Journal175) pp 19–22 https://doi.org/10.1080/00382167.1996.9629888

[16] Lekevicius E and Loreau M 2012 Adaptability and functional stability in forest ecosystems: a hierarchical conceptual framework (Ekologia584) pp 391–404

[17] Nirsatmanto A, Setyaji T and Wahyuningsyos R S 2014 Realized genetic gain and seed source x site interaction on stand volume productivity of Acacia mangium (Indonesian Journal of Forestry Research11) pp 21–32 https://doi.org/10.20886/ijfr.2014.1.1.21-32

[18] Mangkuwibowo F, Indrioko S and Nirsatmanto A 2018 Interaksifamilii × lokasipadaujikjeturunan generasiikeduaEucalyptus pellita(Jurnal Pemuliaan Tanaman Hutan121) pp 25–39 https://doi.org/10.20886/jpth.2018.12.1.25-39

[19] Callister A N, England N and Collins S 2013 Predicted genetic gain and realised gain in stand volume of Eucalyptus globulus (Tree Genetics & Genomes9) pp 361–375 https://doi.org/10.1007/s11295-012-0558-8

[20] Zobel B J, and Talbert J 1984 Applied forest tree improvement (New York: John Wiley&Sons) p 505

[21] Carson S D 1991 Genotype x environment interaction and optimal number of progeny test sites for improving Pinus radiata pine in New Zealand (New Zealand Journal of Forestry Science211) pp 32–49

[22] Cotterill P P and Dean C A 1988 Changes in the genetic control of growth of radiata pine to 16 years and efficiencies of early selection (Silvae Genetica37–4) pp 138–146 Retrieved from https://www.thuenen.de/media/institute/fg/PDF/Silvae_Genetica/1988/Vol_37_Heft_3-4/37_3-4_138.pdf