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Research paper

Genetic parameters of growth and biomass in *Leucaena leucocephala* for wood energy

Parámetros genéticos de crecimiento y biomasa en Leucaena leucocephala *para dendroenergía*

RINA LAKSMI HENDRATI[1], NUR HIDAYATI[1], EKO BHAKTI HARDIYANTO[2], MUJI SUSANTO[1], LILIANA BASKOROWATI[1], MASHUDI[1] AND SUGENG PUDJIONO[1]

[1]*Center for Forest Biotechnology and Tree Improvement Research and Development, Indonesian Ministry of Environment and Forestry, Yogyakarta, Indonesia.* biotifor.or.id
[2]*Faculty of Forestry, Gadjah Mada University, Yogyakarta, Indonesia.* ugm.ac.id/en

Abstract

Leucaena leucocephala is a potential species for wood-energy production in Indonesia. A study of genetic improvement was initiated with a progeny test of 80 lines from 10 seed sources. Plant height and stem diameter were measured at 6 and 18 months, growth index (GI) calculated at 18 months and wood biomass production measured at 30 months. Differences between seed sources for height and diameter were observed at 6 months but not at 18 months. Significant differences between lines within seed source were observed for height and diameter at 6 months and these differences remained at 18 months. Differences between lines for GI and biomass were significant at 18 and 30 months respectively. At 18 months, line mean heritability for height, diameter and GI were estimated to be moderate, namely 0.62, 0.61 and 0.62 respectively. At 30 months line mean heritability for biomass was moderately low (0.39). Genetic correlations between height and diameter were moderately high at 6 months (0.74) and increased at 18 months (0.82), while correlation between diameters at 6 and 18 months was high (0.93). The expected genetic gain from selecting the 25 and 10 best lines with a high line value for GI was 33.7% and 48.8% respectively, with lines from the Indonesian local seed sources found to be the best performers. Three lines from the newly introduced Tarramba cultivar also had good performance. These results are discussed in relation to the future improvement program of this species for wood energy production.

Keywords: Genetic variation, heritability, multi-purpose species, line value, progeny test.

Resumen

Leucaena leucocephala es una especie potencial para la producción de dendroenergía en Indonesia. Se inició un estudio de mejoramiento genético con una prueba de progenie de 80 líneas de 10 fuentes de semillas. La altura de la planta y el diámetro del tallo se midieron a los 6 y 18 meses, el índice de crecimiento (IG) se calculó a los 18 meses y la producción de biomasa de madera se midió a los 30 meses. Se observaron diferencias entre las fuentes de semillas para la altura y el diámetro a los 6 meses, pero no a los 18 meses. Se observaron diferencias significativas entre las líneas dentro de la fuente de semillas para la altura y el diámetro a los 6 meses y estas diferencias se mantuvieron a los 18 meses. Las diferencias entre líneas para IG y biomasa fueron significativas a los 18 y 30 meses respectivamente. A los 18 meses, se estimó que la heredabilidad media de la línea para la altura, el diámetro y el IG era moderada: 0.62, 0.61 y 0.62, respectivamente. A los 30 meses, la heredabilidad media de línea para la biomasa fue moderadamente baja (0.39). Las correlaciones genéticas entre la altura y el diámetro fueron moderadamente altas a los 6 meses (0.74) y aumentaron a los 18 meses (0.82), mientras que la correlación entre los diámetros a los 6 y 18 meses fue alta (0.93). La ganancia genética esperada de seleccionar las 25 y 10 mejores líneas con un alto valor de línea para IG fue del 33.7% y 48.8% respectivamente, y se encontró que

Correspondence: Liliana Baskorowati, Center for Forest Biotechnology and Tree Improvement Research and Development, Yogyakarta 55582, Indonesia. E-mail: liliana.baskorowati@gmail.com

las líneas de las fuentes de semillas locales de Indonesia son las de mejor desempeño. Tres líneas del cultivar Tarramba recientemente introducido también tuvieron un buen desempeño. Estos resultados se discuten en relación con el futuro programa de mejoramiento de esta especie para la producción de energía de la madera.

Palabras clave: Especie multipropósito, heredabilidad, prueba de progenie, valor de línea, variación genética.

Introduction

Leucaena leucocephala, a multipurpose legume tree, has been used as fuel wood for decades in Indonesia, due to its high wood energy quality reaching 4,700 cal/g, with a wood density of 0.67 g/cm and lignin content of 31.6% (Toruan-Mathius et al. 1994). It has a combustion index higher than coal (Shrestha et al. 2015). It also has potential for producing charcoal, briquette and wood pellets (Acda and Devera 2014), producing abundant fast regrowth after cutting the main stem, so that annual wood energy can be harvested without replanting for up to 15–20 years (Pagad 2010). Under an annual precipitation of 1200–1300 mm, it was reported to produce 38.8 t/ha of wood in the first year with a calorific value of 4,700 kcal/kg and 5 t/ha of branches and 6.9 t/ha of leaves when harvested at 50 cm above ground level (Rengsirikul et al. 2011). This species thus provides a profitable option for supplying high quality energy from productive wood biomass.

Natural forest is not able to efficiently capture carbon due to reaching growth maturity. However, fast growing *L. leucocephala* potentially absorbs much more carbon due to its continuing active growth under routine annual harvests. *L. leucocephala* can act as a green manure, producing nitrogen from the rhizobia in its roots and through leaf drop (Ceccon et al. 2015). It grows well in arid environments, producing roots up to 5 m depth for water access (Brewbaker et al. 1972). Under an annual rainfall of ~900 mm, it produced a litter fall of more than 10 t/ha/yr which is easily decomposed (Ceccon et al. 2015). The species has the capacity to fix nitrogen amounting to 250 kg/ha/yr (Casanova-Lugo et al. 2014).

Increasing interest in wood biomass as a renewable energy source is due to several advantages such as lower moisture content, higher lignin content, easy handling and storage, and lower ash and nitrogen content compared to other types of biomass (Sims et al. 2006). Compared to other legume species commonly used for fuel wood, *L. leucocephala* has a higher lignin content (31–33%) than *Senna siamea* (21–22%) and *Gliricidia sepium* (26–27%) (Mainoo and Appiah 1996). Higher lignin content boosts the heating value (Günther et al. 2012) making it more efficient in combustion than coal (Demirbas and Demirbas 2009).

The increased interest in growing *L. leucocephala* for wood energy has led to the initiation of a genetic improvement program of the species in Indonesia, with the objective to identify the best wood biomass alternative for more sustainable energy. Information on the genetic parameters of *L. leucocephala* is very limited in Indonesia. Research is needed on assessing the genetic variation of populations and lines and identifying best lines, followed by producing improved seed by converting the better lines from the progeny test to a seed orchard. Genetic parameters such as heritability, line value and genetic gain should be determined and used to formulate efficient selection strategies within the breeding process. Unlike other *Leucaena* species which are self-incompatible and highly cross-pollinated, *L. leucocephala* is predominantly self-pollinated (Brewbaker and Styles 1982). This paper reports variation and genetic growth parameters, following progeny testing at the ages of 6 and 18 months and biomass yield measurements at 30 months, which can be used for selecting the better lines for biomass production before individual selection on wood quality for energy is undertaken.

Material and Methods

Plant material

Seeds were collected from 80 parent trees located in 10 regions in Indonesia where *L. leucocephala* is grown (Table 1). Except for Fatuleu, East Nusa Tenggara, the original accession of all seed sources and the number of original trees that contributed seed to these seed sources were unknown, but likely to be cultivars of K8 (Peru) and K28 (Cunningham), which were introduced to Indonesia in the early 1970s (Toruan-Mathius et al. 1994). The Fatuleu seed source was established using Tarramba, a cultivar bred in Hawaii from accession K636 collected from Mexico (Nulik et al. 2013), with the seeds introduced from Australia. Parent trees were selected phenotypically from the populations based on their growth performance: height, diameter and health.

Trial Establishment

Seeds of each line were soaked separately in hot water (90 ºC) for 5 minutes, drained and replaced with cold

Table 1. Details of seed sources of *Leucaena leucocephala* populations for establishing the progeny test.

Seed source (population)	No. of parent trees	Rainfall (mm/yr)	Altitude (masl)	Latitude	Longitude
Subang, West Java	13	3,049	108–143	06° 34' 42" S	107° 45' 56" E
Majalengka, West Java	14	2,871	40–66	06° 41' 62" S	108° 17' 80" E
Baros, Central Java	10	1,961	23–500	06◦ 59' 22" S	108° 52' 50" E
Kulon Progo, Yogyakarta	6	1,908	19–79	07° 52' 23" S	110° 07' 35" E
Bantul, Yogyakarta	4	1,961	65–94	07° 50' 31" S	110° 20' 33" E
Sleman, Yogyakarta	6	2,345	170–318	07° 44' 65" S	110° 20' 95" E
Pemogan, Bali	5	1,741	0–3	08° 43' 29" S	115° 11' 39" E
Manado, North Sulawesi	8	2,780	17–122	1° 32' 37" N	124° 55' 03" E
Fatuleu, E. Nusa Tenggara	8	800–900	495–500	09° 52' 22" S	123° 43'47" E
Madura, East Java	6	900	102–142	07° 01' 27" S	112° 55'33" E

(Source: Hendrati and Nurrohmah 2019).

water and left for 12 hours. The seeds were then sown in a separate germination box for each line. Two to three weeks after sowing, germinated seeds were transplanted into separate 15x10 cm plastic bags previously filled with media containing a 1:2 mixture of topsoil and compost. After about 4 months, seedlings were large enough to be transplanted into the field.

The progeny test was established in January 2018 in Brebes, Central Java, Indonesia (245 masl). The mean annual rainfall was 1960 mm. The average daily temperature was 29 ºC with a mean minimum of 24 ºC and maximum of 35 ºC. The soil was a Grumosol or Vertisol (Soil Survey Staff 1992). The trial was laid out in a randomized complete block design, consisting of the 80 lines in four-noncontiguous plots, replicated 4 times. Tree planting spacing was 2 x 2 m within plots.

At 6 and 18 months, height and stem diameter (at 20 cm above ground at 6 months and at 130 cm above ground at 18 months) were recorded. Thinning by cutting the whole tree at 5 cm above the ground was conducted after the second measurement at age 18 months by removing the two poorest trees of every line in each block. Thinning was intended to provide wider spacing for the remaining trees to promote early flowering and seed production as the progeny is progressively converted to a seed orchard (Wheeler 1991).

At 30 months, another tree in each plot (leaving one best individual per line to grow in the field) was cut at 100 cm above the ground and separated into stem, branches and twigs and then the components weighed. Leaf was excluded in this study because it has no potential for energy production. Samples of the woody biomass were taken and dried at 105 ºC to constant weight to determine total dry weight of biomass. Growth index (GI) was estimated at 18 months using a formula for multipurpose tree species as GI = basal diameter^2 × height (Mullen and Gutteridge 2002).

Data Analyses

Individual tree data were analyzed using the General Linear Model procedure with the following linear model:

$$Y_{ijk} = \mu + B_i + Sj + L(S)_{jk} + B_iF(S)_{jk} + \varepsilon_{ijk}$$

where:

Y_{ijk} is individual tree observation;
μ general means;
B_i effect of ith block;
S*j* effect of jth seed source;
$L(S)_{jk}$ effect of *jk*line within *j*th seed source;
$B_iF(S)_{jk}$ interaction effect of *i*th block and *jk* line; and
ε_{ijk} the residual error.

Block was considered fixed while seed source and line within seed source were considered to be random. Variance components were estimated for the random effect by using the Restricted Maximum Likelihood (REML) procedure (Williams et al. 2002). Line means were predicted with the Best Linear Unbiased Prediction (BULP). The use of mixed model procedures (REML and BLUP) was appropriate where data were unbalanced such as found in the progeny test reported here. Data analyses were performed with the software R ver. 4.02.

As *L. leucocephala* is a self-pollinated species where genetic variation within lines is considered homogeneous, similar to a clone, broad-sense heritability is more relevant to quantify the genetic control of particular traits in the species. The line mean heritability was estimated using the following formula:

$$H^2_{mean} = \sigma^2_l / (\sigma^2_l + \sigma^2_{bl}/b + \sigma^2_e/nb)$$

where:

σ^2_l is component of variance due to line;
σ^2_{bl} is the component of variance due to block × line interactions;
σ^2_e residual error; b the harmonic mean number of blocks per line; and
n the harmonic mean number of trees per line.

The variance component of block (σ^2_b) is not included in the denominator of the formulae of heritability, implying that the estimated heritabilities are appropriate to selection on block adjusted data. The Genetic Coefficient of Variation (GCV) of measured traits was calculated as follows:

$$GCV\ (\%) = \sqrt{\sigma^2_l}) / \bar{X} \times 100\%$$

where $\bar{X}$ is the general mean.

Genetic correlation (r_g) between traits, as well as age-age genetic correlation of the same trait were calculated according to Williams et al. (2002):

$$r_g = \{\sigma l_{(x,y)}\} / \{\sigma^2_{l\ (x)} \times \sigma^2_{l\ (y)}\}^{1/2}$$

where:

$\sigma l_{(x,y)}$ = covariance component at line level of two different traits or the same trait of different ages,

$\sigma_f^2(x)$ = variance component of trait x at line level,

$\sigma_l^2(y)$ = variance component of trait y at line level.

The covariance component was calculated using the following method (Williams et al. 2002):

$$\sigma_l(x,y) = ½\ (\sigma^2_{l(x+y)} - \sigma^2_{l(x)} - \sigma^2_{l(y)})$$

where $\sigma^2_{l(x+y)}$ is the variance component for the sum of the traits. All of the variance components were estimated as previously described.

Genetic gain (G) for line selection was predicted by using the following formula:

$$G = H^2_{mean} \times i \times \sigma_p$$

where:

H^2_{mean} = line mean heritability,

i = selection intensity,

σ_p = phenotypic standard deviation.

Results

At 6 months, differences between seed sources (populations) were highly significant for height (p=0.016) and stem diameter (p=0.002). The seed source mean for height ranged from 1.57 to 2.09 m, while that for stem diameter ranged from 1.13 to 1.56 cm. However, at 18 months the differences between seed sources were not significant for height (p=0.37) and stem diameter (p=0.061). The seed source mean for height ranged from 5.17 to 5.72 m and stem diameter ranged from 4.30 to 5.01 cm. At 18 months, the growth index (GI) between seed sources also did not differ significantly (p=0.103) (Table 2).

At 6 months, differences between lines within seed source were significant for height (p <0.0001) and diameter (p=0.027). Differences between lines within seed source also remained significant for height (p <0.0001) and diameter (p <0.0001) at 18 months. GI between lines also differed significantly (p<0.0001). GCV increased with increasing age, for example GCV for height and diameter at 6 months were 6.5 and 4.8% respectively and the corresponding figures at age 18 months were 7.1 and 8.2% respectively. The estimates of line mean heritability at 18 months for height (0.62) and diameter (0.61) and GI (0.62) were all moderate, while that of biomass at 30 months was low (0.39) (Table 3).

At 6 months the genetic correlation (rG) between height and diameter was moderately high and positive (0.74), and it increased at 18 months to 0.82. The genetic correlation between 6 and 18 months for height was estimated to be low but positive (0.38); it was very strong and positive (0.93) for diameter. Genetic gain expected from selecting the best 25 lines with high GI was 33.7 %, while selecting the best 10 lines for GI resulted in an expected genetic improvement of 48.8%. The best selected lines based on their line value for growth index (GI) are listed in Table 4.

Table 2. Analysis of variance of *L. leucocephala* progeny test for growth.

Source of variation	Height		Diameter		Growth Index (GI)	
	Mean square	*p value*	Mean square	*p value*	Mean square	*p value*
Age 6 months						
Replication	25.246	<0.0001	12.333	<0.0001		
Source	1.604	0.016	1.312	0.002		
Line (Source)	0.650	<0.0001	0.401	0.027		
Replication × Line (Source)	0.337	0.975	0.288	0.907		
Error	0.403		0.325			
Age 18 months						
Replication	114.895	<0.001	69.664	<0.0001	392855.7	<0.0001
Source	3.903	0.368	5.887	0.061	26309.5	0.103
Line (Source)	3.521	<0.0001	3.049	<0.0001	15405.5	<0.0001
Replication × Line (Source)	1.203	0.986	1.245	0.917	6973.9	0.039
Error	1.475		1.416			

Table 3. Growth and estimates of genetic parameters of *L. leucocephala*.

Age and trait	Mean	Genetic parameter				
		σ^2_l	σ^2_{bl}	σ^2_e	GCV (%)	H^2_{mean}
6 months						
Height (m)	1.85	0.014	0	0.388	6.5	0.37
Diameter (cm)	1.27	0.004	0	0.325	4.8	0.16
18 months						
Height (m)	5.46	0.151	0	1.391	7.1	0.62
Diameter (cm)	4.69	0.147	0	1.362	8.2	0.61
Growth index (GI)	1.77	0.098	0.049	0.587	17.7	0.62
30 months						
Wood biomass* (kg/tree)	17.0	8.764	0	42.311	10.8	0.39

* = dry weight.

Table 4. The best 25 lines based on predicted line value for GI.

Rank	Line Code	Line value	Origin of population
1	27	2.52	Majalengka, West Java
2	40	2.44	Pemogan, Bali
3	55	2.32	Fatuleu, E. Nusa Tenggara
4	54	2.30	Fatuleu, E. Nusa Tenggara
5	37	2.29	Brebes, Central Java
6	51	2.28	Majalengka, West Java
7	16	2.12	Subang, West Java
8	1	2.11	Subang, West Java
9	45	2.08	Manado, North Sulawesi
10	4	2.08	Subang, West Java
11	21	2.06	Majalengka, West Java
12	65	2.04	Sleman, Yogyakarta
13	2	2.00	Subang, West Java
14	35	1.98	Brebes, Central Java
15	79	1.96	Kulon Progo, Yogyakarta
16	70	1.95	Sleman, Yogyakarta
17	10	1.95	Subang, West Java
18	5	1.93	Subang, West Java
19	52	1.92	Fatuleu, E. Nusa Tenggara
20	3	1.92	Subang, West Java
21	68	1.92	Sleman, Yogyakarta
22	67	1.91	Sleman, Yogyakarta
23	34	1.90	Brebes, Central Java
24	75	1.90	Kulon Progo, Yogyakarta
25	9	1.90	Subang, West Java

Discussion

Results of the current study revealed that the best seed source based on GI was Majalengka (West Java), followed by Pamogan (Bali) and Fatuleu (East Nusa Tenggara) (Table 4). In a previous study, comparison of 9 Indonesian seed sources with cv Tarramba collected from Fatuleu East Nusa Tenggara, indicated that Tarramba performed better in the nursery (Hendrati and Hidayati 2018). After 6 months in the field, the best seed source for height and diameter was found to be the Tarramba cultivar (Hendrati and Nurrohmah 2019). Tarramba is a cultivar released in Australia but selected at the University of Hawaii. It is outstanding for leaf biomass and was introduced to Kupang (Nulik et al. 2013) for cattle fattening in Amarasi, East Nusa Tenggara. This cultivar is said to be tolerant to the psyllid insect (CABI 2017), although not resistant, and better in producing wood for energy compared to the previous outstanding cultivars Peru and Cunningham (Rengsirikul et al. 2011). However, after 18 months in the field, differences among seed sources disappeared for all traits. Nevertheless, the best three seed sources were identified to be from Majalengka (West Java) followed by Pamogan (Bali) and Fatuleu (E. Nusa Tenggara) in terms of GI. Except for the Fatuleu seed source where the genetic material is *L. leucocephala* cv. Tarramba, the introduction of *L. leucocephala* ssp. *glabrata* to Indonesia was poorly documented and earlier introductions presumably originated from K8 and K28 varieties (Toruan-Mathius et al. 1994). It is possible that the genetic material of the remaining 9 seed sources were interrelated and the genetic differentiation between seed sources or populations has not yet occurred. Therefore, selection to identify best seed sources for growth and biomass is not yet feasible.

At the line level, however, variation existed at 6 months and remained significant after 18 months in the field and generated moderate broad-sense heritability for growth (Table 3), suggesting that selection to identify the best lines is possible. Similar results were reported from a trial of *L. leucocephala* at 9, 12 and 15 months after planting in Tamil Nadu, India which found that broad-sense heritability estimates for growth were moderate: 0.55, 0.46, and 0.60 respectively for height, diameter and GI (Sangram and Keerthika 2013). In self-pollinated species, broad-sense heritability based on line mean is mostly used for estimating genetic gain from selection, while broad-sense heritability on an individual tree can be used in marker-assisted selection (Xu et al. 2009).

Genetic correlations between height and diameter were moderately high and positive at 6 months (0.74) and even increased at 18 months (0.82), suggesting that selection on height or diameter will not have a substantial effect on the other trait. The very high positive genetic relationship between 6 and 18 months for diameter (0.93) demonstrates that diameter growth at early ages is a good indicator for future performance.

The expected genetic gain from selecting the 10 best lines is substantial. However, the genetic variation in growth of selected lines will obviously decline as number of lines is reduced compared with the initial number of lines. It is worth mentioning that the genetic parameters reported in this study might be overestimated if the line-site interactions of the measured traits were of significant importance as the progeny test was only conducted at one site. For seed production, the reduction in genetic variation should be acceptable considering the species is predominantly self-pollinated. Seed will be produced by selfing in each line without causing inbreeding depression. For future genetic improvement programs, however, selection within pure lines cannot be expected to yield rapid genetic advance. The best selected lines may be used for controlled crosses to produce F_1 progenies and then to self them to generate suitable F_2 genetic variation for the next cycle of selection. This is one of the possible breeding strategies that may be adopted and which is usually carried out in self-pollinated species and is quite common in agricultural crops (Brown et al. 2014). Controlled crosses by introducing genetic material of *L. leucocephala* from new accessions in addition to crossing the best selected lines may also be used. Developing *Leucaena* hybrids having hybrid vigor for a specific trait of interest has been done previously (Dalzell 2019).

It is interesting to note that lines from the local seed sources of West Java and Bali had high line (genetic) value for GI. The best lines (Table 4) are all *L. leucocephala* ssp. *glabrata* and three lines of Tarramba cultivar (rank 3, 4 and 19) collected from Fatuleu (East Nusa Tenggara) seed source. This inferred that in terms of wood biomass, lines from local seed sources found in Indonesia are very promising, showing good performance and worth further examination for their wood-energy quality.

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