

Genetic parameters for resin production of *Pinus merkusii* progeny test collected from three seed sources in Banyumas Barat Forest District, Indonesia

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Abstract. Nugrahanto G, Na'iem M, Indrioko S, Faridah F, Widiyatno, Abdillah E. 2022. Genetic parameters for resin production of *Pinus merkusii* progeny test collected from three seed sources in Banyumas Barat Forest District, Indonesia. *Biodiversitas* 23: 2010-2016. *Pinus merkusii* Jungh. et de Vriese is a native pine species in Indonesia used for the production of essential and economically valued products, such as wood and resin. The resin productivity can be increased significantly via breeding programs. The objective of this study is to analyze the genetic parameters of resin productivity based on the progeny performance in three seeds sources, namely, Sumedang seedling seed orchards (SSOs), Jember SSO, and East Java seed source. The drilling method was used for tapping trees, and then resin production was measured individually. Additional data on height, diameter, and branch-free height were also collected. Based on family variance component analysis data of resin production, it was noted that Sumedang SSO, Jember SSO, and East Java seed sources have relatively high genetic variations of 4.48%, 15.77%, and 3.80%, respectively. These values were then followed by a moderate family heritability value for all of them (0.451–0.551). A high value of individual heritability estimation was obtained for Jember SSO (0.658), and moderate values were found for Sumedang SSO (0.182) and East Java seed sources (0.158), respectively. The estimated genetic gains were calculated for resin production using selection intensity levels at 10%, 25%, and 50%. The result indicated that the Jember SSO seed source obtained the highest genetic gains at 17.55%, 12.73%, and 7.99%, for the above mentioned intensity levels, respectively while the lowest one was found from the Sumedang SSO seed source.

Keywords: Genetic variation, heritability, *Pinus merkusii*, resin yield

INTRODUCTION

Pinus merkusii Jungh. et de Vriese (pine) is one of Indonesia's native pine species that is crucial for improving marginal forest areas along with the economy of the community (Rodrigues-Corrêa et al. 2012) and developing plantation forests for timber and resin production. In Indonesia, it grows naturally on the island of Sumatra in three disjunct populations: Aceh, Tapanuli, and Kerinci (Imanuddin et al. 2020). At present, it has been planted extensively in Java Island covering a total area of more than 470,000 Ha (Rahayu and Karyanto 2015; Evayanti et al. 2019; Sukarno et al. 2020). *P. merkusii* is an essential forest tree species that has been producing excellent wood for furniture, sawn timber, boxes, pulp, paper, and high quality of oleoresin (Hadiyane et al. 2015; Imanuddin et al. 2020).

Products from processed pine resin, namely gondorukem (Kencanawati et al. 2017) and turpentine, currently have a higher price in international market and are also much needed in the domestic market. The method for increasing pine resin productivity is a very promising effort (Sukarno et al. 2020). One of the strategic efforts

being undertaken is through tree improvement programs aimed at increasing resin productivity and quality of resin products via combination of activities related to genetic, silvicultural, and forest management approach (White et al. 2014).

The resin productivity of pine plantations in Indonesia (0.85 tons/ha/years) is still lagging behind China (1.4 tons/ha/years) and Brazil (4.8 tons/ha/years) (Kencanawati et al. 2017; Lukmandaru et al. 2021; Verkerk et al. 2021). Prasetya (2008) reported that pine landrace plantation in Jember (East Java) have produced resin between 5 and 10 g/tree/day tapping. This value is not so much different than the result shown in Ponorogo (7.6 and 9.3 g/tree/day) (Wijayanto et al. 2019). However, resin production has been obtained from pine plantations in the Sumedang SSO, which has produced a very high resin production (33.8 g/tree/day) (Susilowati 2013).

The low productivity of *P. merkusii* resin is due to the fact that the planting seeds are obtained from unselected and unimproved stands. Therefore, one important strategy should be considered to increase resin productivity is by producing good quality of seeds through the establishment of a proper breeding program (Liu et al. 2013; Lai et al.

2017). The initial step of a breeding pine program is the establishment of progeny tests which then converted to seedling seed orchard (White et al. 2018). Thus, the tree improvement of pine in Indonesia has been conducted to select pine trees with resin yield of more than 50 g/tree/3 days tapping period. In total, 1,085 plus trees have been selected. From these selected trees, several progeny tests have been established. One of them was built in Compartment 37c, Lumbr Forest Sub-district, and Banyumas Barat Forest District at the end of 2007.

The breeding population of progeny test was developed by using subline method. Then, the progeny population was divided into three sub-populations based on the resource location of plus trees, i.e. Sumedang seedling seed orchard (SSO), Jember SSO, and East Java landrace.

In 2018, the progeny test plantation was already 11 years old. Hence, it was a good time to start evaluating the progeny test plantation, especially for resin yield traits to obtain the genetic parameter information. From this evaluation, a work to select families and individuals with high resin yields can be conducted. Additionally, the selected families and individual trees with high resin yields were very beneficial for the breeding programs of *P. merkusii* in the near future. These selected families and individual trees should be propagated vegetatively to accelerate the achievement of a high resin yield of pine plantation on the operational scale (Ruotsalainen 2014; Degen and Sebbenn 2016). The objective of this study is to analyze the genetic parameters of resin yield based on the phenotypes of families and individuals from each seed sources (Sumedang SSO, Jember SSO, and East Java landrace).

MATERIALS AND METHODS

Location and time

The research was conducted from December 2018 to June 2019 in the progeny test of *P. merkusii* in Compartment 37c, Samudra Forest Sub-sub-district, Lumbr Forest Sub-district, Banyumas Barat Forest District, Perhutani state-owned company of the Central Java regional division, Indonesia, which is geographically located at 07° 20'00" latitude and 108°55'00" longitude. The research location is situated at an altitude of 300–500 m above sea level. It has a daily average temperature of 26.3°C with minimum and maximum temperatures of 24.4°C and 30.9°C respectively, average rainfall of 3,500 mm/year, and is categorized as climate B according to Schmidt Fergusson. Additionally, it has a hilly topography with a slope of 30–40° and a Mediterranean soil type (Alfisol), which is derived from limestone and marl as the main material (Muslimin et al. 2013).

Materials and tools

The mother tree selection was conducted at Sumedang SSO, Jember SSO, and East Java landrace based on mass selection. The criteria for selecting the mother trees for resin yield are based on the tree's ability to produce resin and by direct detection through the tapping method. Pine trees are considered to be plus trees or mother trees if the selected trees have a high resin production (tapping production > 50 g/tree/3 days) (Figure 1). Besides the resin production criteria, the selected mother trees have to also fulfill other criteria such as good growth, relatively straight stem form, and free from pests and diseases (Lai et al. 2017). The seeds obtained from each parent tree are kept separately, and then used to establish a progeny test by using a subline approach based on the origin of the parent tree (Faculty of Forestry UGM, 2006). The planting design of each seed source used the incomplete block design, with different numbers of families, numbers of trees per plot, and blocks. The total individual trees used in our research was 3,580 (Table 1).

Research design

The resin production data were collected using a drilling system (closed system). Previously, the skin of tree was cleaned to make it easier to determine the drilling point. Drilling was performed with a modified drilling machine having a drill bit diameter of 3/8 inch (13 mm) at stem height of ± 30 cm above the ground and the slope of the borehole is 15–20° while leading/keeping the shaft upward. The drilling depth is kept ± 2 –3 cm from the outer wood surface so that the holes formed are still in the resin wood layer (adopted from Hadiyane et al. 2015). Harvesting of the resin is conducted on the seventh day after drilling, with the assumption that the resin will stop coming out due to resin coagulation in the area that closes the resin canals.



Figure 1. A. Drilling for tapping the resin. B. Collecting the resin in plastic bags

Table 1. Genetic material for the progeny test in the Banyumas Barat Forest District, Central Java, Indonesia

Seed source	Family	Block	Tree plot per family	Total number tree plot (individual tree)
Sumedang SSO	50	10	3	1,500
Jember SSO	40	5	4	800
East Java landrace	40	8	4	1,280

Drilling is conducted on the trunk in the opposite positions (right and left) on each tree and in the direction of the contour. One hole is slightly higher than the other. The resin that comes out is then collected in a plastic bag flowed through a stainless-steel pipe so that it is clean and free from dirt or rainwater. The resin obtained during resin collection period was then weighed using a digital scale to determine the weight of the resin on each of the observed individual trees.

The measurement of tree height, diameter, and branch-free height was also conducted to observe the growth parameters. Tree height is measured using a measuring pole starting from the soil surface to the apical point of the plant, while the stem diameter is measured using a diameter tape at the breast height/dbh (130 cm from the ground).

Data analysis

The results of observations and measurements in the field were analyzed using the analysis of variance method to determine the differences between the families. The following mathematical model was used:

$$Y_{ij} = \mu + B_i + BF_j + \varepsilon_{ij}$$

Where: Y_{ij} is the tree plot/individual tree observation, μ = the overall mean; B_i = the block effect; F_j = the effect of the j^{th} family; ε_{ij} = the residual error. The magnitude of effect of genetic factors on the appearance (phenotype) of a tree is estimated by the value of heritability. The calculated heritability is the heritability for half-sibs using the following formula (Zeng et al. 2013; Susilowati et al. 2013):

Family heritability (h^2_f):

$$h^2_f = \frac{\sigma^2_f}{\sigma^2_f + (\sigma^2_e/nr) + (\sigma^2_{fr}/r)}$$

$$h^2_f = \frac{\sigma^2_f}{\sigma^2_f + (\sigma^2_e/nr) + (\sigma^2_{fr}/r)}$$

Individual heritability (h^2_i)

$$h^2_i = \frac{4\sigma^2_f}{\sigma^2_f + (\sigma^2_e) + (\sigma^2_{fr})}$$

$$h^2_i = \frac{4\sigma^2_f}{\sigma^2_f + (\sigma^2_e) + (\sigma^2_{fr})}$$

Where: σ^2_f is the family component variance, σ^2_{fr} is the block family interaction component variance, σ^2_e is error component variance, r is harmonic average number of block, and n is harmonic average number of tree/plot.

Estimation of the magnitude of genetic gain in the hereditary tests commonly used by breeders to express the response to selection and recovery is using the following formulas (Cotteril & Dean, 1990; William & Matheson, 1994):

$$G = h^2 S = h^2 i \sigma_p$$

$$G = h^2 S = h^2_i \sigma_p$$

Where: σ_p is the standard deviation of the phenotype, i is the selection intensity for family selection. Here three values of i were taken as 1.755, 1.271, and 0.798 in corresponding to three different levels of selection intensity levels, i.e 10%, 25%, and 50% of the families, retained after rouging.

RESULTS AND DISCUSSION

Resin production

Based on the measurement results of 11-year-old *P. merkusii* progeny test from three seed sources at Banyumas Barat Forest District (Table 2), Sumedang SSO yielded the highest results, with an average resin yield of 17.43 ± 0.38 g/tree/3 days, an average height growth of 15.31 ± 0.04 m, an average diameter growth of 23.33 ± 0.12 cm, and a mean branch-free height of 6.57 ± 0.04 m. In general, the increasing resin production in the progeny test is in line with the increasing age of the plant (Sukarno et al. 2020). The average resin yield has increased between 23% and 83% from the measurement performed by Perhutani (2014) at the age of 7 years (8.38 – 10.25 g/tree/3 days). When compared with measurements at 4 years of age by Muslimin (2013), there was an increase of 182%–280% of the average resin yield of each seed source. The effect of tree age on resin yield has also been reported in *Pinus pinaster*, in which trees aged 30-40 years produced nearly two and three times than trees aged 20-30 and 10-30 years, respectively (Zas et al. 2020). Resin exudation also increased as plants aged in *Pinus elliottii* (De Oliveira Junkes et al. 2019).

In general, the average resin yield is still slightly lower than other *P. merkusii* plants with a slightly older age (12 years), as reported by Leksono (1994; Table 3). Apart from age and used plant materials as the probable cause, the environmental factors are also strongly suspected in affecting it. Different planting locations provide different growing environments related to soil fertility and climate (Moreira et al. 2015; Rodríguez-García et al. 2015; Neis et al. 2018; Zas et al. 2020). Silvicultural treatment (correct spacing, intra and inter tree relationships controlled by the management regime) also plays an important role in influencing plant growth and enhancing resin yield (Egloff, 2019; Baleko et al. 2021).

Differences in the resin yield also occurred among the pine species (Table 3) as reported in *P. taeda*, *P. palustris*, and *P. elliottii* (Roberds et al. 2003). Roberds and Strom (2006) generally have a lower resin yield than *P. merkusii* in this study. Even though at younger age, *P. massoniana* in China (8 yrs) has produced a higher resin yield (Liu et al. 2013) as compared to *P. merkusii* in our study. Likewise, it occurred in *P. elliottii* in China (Lai et al. 2017). This outcome clearly shows that the production of pine resin differs among species and also between the conditions in which it is grown.

Table 2. Average resin yield, growth of height, diameter, and branch-free height of 11-year-old *P. merkusii* progeny test in Banyumas Barat Forest District, Central Java, Indonesia

Seed source	Average			
	Resin yield (g/tree/3 day)	Height (m)	Diameter (cm)	Branch-free height (m)
Sumedang SSO	17.43±0.38	15.31±0.04	23.33±0.12	6.57±0.04
Jember SSO	15.36±0.51	14.83±0.07	22.57±0.16	6.12±0.07
East Java landrace	12.53±0.34	14.58±0.06	22.31±0.13	5.57±0.05

Table 3. Pine resin yield in the progeny test studies

Species	Age (years)	Resin yield (g/tree/3 day)	Drilling technique	Reference
<i>P. merkusii</i>	11	13.80	Borer	Nugrahanto (2020)
	7	8.94	Borer	Perhutani (2014)
	4	4.04	Borer	Muslimin (2012)
	12	20.58	Quare	Leksono (1994)
<i>P. elliottii</i>	27	116-124	Rill	Lai et al. (2017)
<i>P. massoniana</i>	8	26.07	Rill	Liu et al. (2013)
<i>P. taeda</i>	10	3.84-5.76	Quare	Roberds et al. (2003)
	20	6.21-10.08	Quare	Roberds and Strom (2006)
<i>P. palustris</i>	21	1.98	Quare	Roberds and Strom (2006)
	30	8.40	Quare	
	42	21.93	Quare	
<i>P. elliottii</i>	20	1.29	Quare	Roberds dan Strom (2006)

Genetic variations in the resin yield

In the progeny test of seed sources of Sumedang SSO and Jember SSO, block and family have given a very significant effect on the nature of resin yield ($P < 0.01$) (Table 4). The very significant differences between families in the research of Sumedang SSO and Jember SSO were also found in 4-years old pine plantation (Muslimin, 2013). It can be stated that at the seed source levels, the characteristics of resin production have still been affected by planting age from the family point of view (Lai et al. 2017). The control or management of this genetic factor provides an opportunity to conduct genetic enhancement in future generations via proper selection.

The interaction between block and family was also found to be very significant in the Jember SSO ($P < 0.01$), indicating that resin yield is not only the result of genetic performance, but also due to the interaction result between families and the environmental conditions where they are grown (Zeng et al. 2013; Lai et al. 2017). Therefore, different rankings of the best families in each block can be found.

In the East Java landrace progeny test, the family has given a very significant effect on resin yield ($P < 0.01$), but block has not ($P > 0.05$). It can be assumed here that in this landrace, genetic (family) factors still affect the characteristics of resin yield (Liu et al. 2013). The control of this genetic factor provides a crucial opportunity to conduct genetic enhancement in future generations through the selection activity (Susilowati et al. 2013).

Based on the estimation of the variance component of resin yield (Table 5), a 15.77% value of the family variance component (σ_f^2) was found for Jember SSO, whereas for Sumedang SSO, a value of 4.48% was obtained. A value of

3.80% was found for the East Java landrace. In general, the resin yield in all seed source was more affected by environmental factors. This result is not much different as compared to the result obtained by Leksono (1994) with the percentage of family variance component estimates (σ_f^2) were of 14.25% and 12.46%, respectively.

Table 4. Analysis of variance of the pine resin yield from the 11-year-old progeny test in the Banyumas Barat Forest District, Central Java, Indonesia

Source of variation	Mean squares of the landrace		
	Sumedang SSO	Jember SSO	East Java landrace
Block	2.59**	2.06**	1.22ns
Family	1.81**	0.77**	2.09**
BlockxFamily	0.82ns	0.62**	0.96ns
Error	0.77	0.05	1.15

Note: * $P \leq 0.05$; ** $P \leq 0.01$; ns: not significant

Table 5. Variance component estimation (vce) of resin yield from each variation source and their percentage of the total variance

Source of variation	Sumedang SSO		Jember SSO		East Java landrace	
	vce	(%)	vce	(%)	vce	(%)
Block	0.012	1.41	0.009	Block	0.012	1.41
Family	0.038	4.48	0.044	Family	0.038	4.48
Blockx	0.030	3.53	0.177	Blockx	0.030	3.53
Family				Family		
Error	0.769	90.58	0.049	17.56	1.39	96.12

Estimated heritability value

Heritability value is a way of estimating how a parameter (resin yield) is influenced by genetics and environment. The highest individual heritability value of resin yield was obtained by the Jember SSO (0.658), which belonged to a higher category. The individual heritability values of the resin yield in the Sumedang SSO and the East Java landrace were 0.182 and 0.158, respectively, which belonged to the moderate category (Table 6) (Cotteril and Dean 1990). The heritability of the resin yield family of the Sumedang SSO, the Jember SSO, and the East Java landrace yielded consecutive values of 0.551, 0.511, and 0.451, respectively, implying that they belong to the moderate category (Leksono 1994). For high resin yield heritability values, it means that resin yield character is controlled by gene. This indicates that tree improvement program for resin yield purposes through genetic selection would result in higher genetic gain (Susilowati et al. 2013).

The estimation of the heritability value of resin yield in the three seed source in this study yielded different results. This phenomenon can be related to the differences in the material sources of each seed source. Several studies on other coniferous species also showed differences in the heritability values: *P. taeda*; *P. elliotii* (Zhang et al. 2016; Lai et al. 2017); *P. massoniana* (Liu et al. 2013); and *P. caribaea* (dos Santos et al. 2016). The difference in heritability values may occur because it is affected by species, place, time, experimental patterns, different calculation procedures (Xu et al. 2017), plant age (Yang et al. 2013; Sukarno et al. 2020), environment and differences in the visualization of the drilling technique (Hadiyane et al. 2015; Rodríguez-García et al. 2016).

Expected genetic gain

Genetic gain was affected by selection activities. Genetic gain from seedling seed orchards could be

manipulated by selection method and selection intensity that is used to rogue the orchard shortly after crown closure (David et al. 2003). It was calculated based on the average population before and after selection was performed (Harjyanto et al. 2019). The selection of individuals, especially 10% of the proportion of selected individuals would increase the average genetic gain that ranged 85% and 169% of 25% and 50% selection intensity, respectively (Table 8). Estimates of genetic gain at the same level of selection intensity (*i*) also varied among the lseed source. The Jember SSO seed source had the highest individual heritability value (0.658) than all of seed sources (Table 6); hence, it received the highest estimate of genetic gain at the same selection intensity level.

Table 6. The estimated heritability of individual (h_i^2) and family (h_f^2) resin yield of the progeny test in Banyumas Barat Forest District, Central Java, Indonesia

Landrace	Heritability	
	Individual (h_i^2)	Family (h_f^2)
Sumedang SSO	0.182	0.551
Jember SSO	0.658	0.511
East Java seed source	0.158	0.451

Table 8. Estimated genetic gain of the expected resin yield of the *P. merkusii* progeny test at several selection intensity levels (*i*) in the Banyumas Barat Forest District, Central Java, Indonesia

Intensity of selection (<i>i</i>)	Expected genetic gain (%) of the seed source			Average
	Sumedang SSO	Jember SSO	East Java	
10%	8.67	17.55	10.44	12.22
25%	6.29	12.73	7.57	8.86
50%	3.95	7.99	4.74	5.55

Table 7. Estimated heritability pine resin yield in the progeny test studies

Species	Age (years)	Heritability		Reference
		Individual (h_i^2)	Family (h_f^2)	
<i>P. merkusii</i>				Perhutani (2014)
Sumedang SSO	7	0.49	0.57	
Jember SSO	7	0.20	0.19	
East Java landrace	7	0.57	0.56	
<i>P. merkusii</i>				Muslimin (2012)
Sumedang SSO	4	0.39	0.59	
Sulawesi SSO	4	0.42	0.49	
East Java Landrace	4	0.29	0.48	
<i>P. merkusii</i>				Leksono (1994)
Sumedang SSO	12	0.54	0.57	
Jember SSO	12	0.50	0.53	
<i>P. taeda</i>	10	0.44-0.59	-	Roberds et al. (2003)
<i>P. elliotii</i>	15	0.37	-	Pswarayi et al. (1996)
	27	0.41	0.11	Lai et al. (2017)
<i>P. massoniana</i>	8	0.47	0.38	Liu et al. (2013)
<i>P. caribaea</i>	27	0.25	0.62	dos Santos et al.(2016)

Muslimin (2013) conducted research on the estimation of the potential for genetic gain of resin production in the same progeny test (four seed sources of Banyumas Barat Forest District) at the age of four years and obtained the estimates that were not too different, namely obtaining a mean of 6.6, 10.5, and 14.5 at the selection intensity levels of 50%, 25%, and 10%, respectively. Leksono (1996) revealed the research results on the test plants of Sumedang SSO and Jember SSO, which had high individual heritability (0.54 and 0.50) when the plants were 12 years old. It was found that the increase in resin yield was estimated at 33.65% and 34.97% by selecting individuals leaving 4%–5% of the plant. Research on *P. elliotii* was conducted at a selection intensity level of 3% in 36 individual plants and resulted in an increase in genetic gain by 61.25% (Shimizu and Spir 1999). The results of the study by Dos Santos et al. (2016) on *P. caribaea* var. *hondurensis* in Brazil obtained an estimate of genetic yield of resin in a row of 14.5%, 15.81%, and 17.01% through an individual selection with intensity levels of 10%, 5.3%, and 2.6%, respectively.

Estimates of genetic gain in this study were obtained with the assumption of open pollination conditions. A significant increase in the estimated genetic yield will be obtained through the controlled pollination of selected families with resin yield more than 50 gr per 3 days of *P. merkusii* progeny test. The other research conducted by Mergen et al. (1955) on *P. elliotii* showed a resin yield production twice (100%) the average of untreated tree by conducting controlled pollination on high resin yield trees. Our result research suggests that there are genetic variations in the production of resin between the populations in most of the seed source with an estimated value of the variance component of 4.48%, 15.77%, and 3.80% for the seed sources of Sumedang SSO, Jember SSO, and East Java landrace. The estimated individual heritability value (h_i^2) was categorized as moderate for Sumedang SSO and East Java seed source landrace (0.182 and 0.158) and high for Jember SSO (0.658), whereas the estimated family heritability (h_f^2) value was moderate for all seed source: 0.551, 0.511, and 0.451 for Sumedang SSO, Jember SSO and East Java landrace, respectively. The expected genetic gains of resin yield at 10% selection intensity were 8.67, 17.55, and 10.44 for Sumedang SSO, Jember SSO and East Java landrace, respectively. At the selection intensity levels of 25% and 50%, the expected genetic gains were 6.29 and 3.95 for Sumedang SSO; 12.73 and 7.99 for Jember SSO; and 7.57 and 4.74 for East Java landrace, respectively. Thus, the best family selection based on the selection intensity of *P. merkusii* in each seed source would be continued with rouging activity to convert the progeny test to SSO with high resin yield production for *P. merkusii* reforestation program. On the other hand, we could establish the hedge orchard of *P. merkusii* using the best families to develop large scale forest plantation for sustainable forest management in the future.

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