OLEORESIN PRODUCTION, TURPENTINE YIELD AND COMPONENTS OF *PINUS MERKUSII* FROM VARIOUS INDONESIAN PROVENANCES

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SUKARNO A, HARDIYANTO EB, MARSOEM SN & NA'IEM M. 2015. Oleoresin production, turpentine yield and components of *Pinus merkusii* from various Indonesian provenances. Oleoresin production and turpentine yield of *Pinus merkusii* plantation of three subpopulations from Aceh provenance (Jantho, Takengon and Blangkejeren) and Java land race were examined. The plantation is located in Jember District (600 m above sea level), east Java, Indonesia. Significant differences in oleoresin and turpentine yield were found between the three subpopulations. Oleoresin yield declined with increasing altitude of subpopulation origin, while turpentine yield increased with increasing altitude of subpopulation origin. The oleoresin yields were 12.2, 14.5, 18.0 and 21.1 g hole^{-1} tree^{-1} day^{-1} for Takengon, Java land race, Blangkejeren and Jantho respectively. Repeatability estimates for oleoresin yield were moderate to high, ranging from 0.57 to 0.74. Turpentine yields were 13.6, 15.3, 16.0 and 19.6% for Java land race, Jantho, Blangkejeren and Takengon subpopulations respectively. The greatest turpentine components in all subpopulations were α-pinene (73.3–87.2%) and δ-3 carene (7.3–19.3%). Other minor components (< 3%) were β-pinene, camphene, myrcene, sabinene and limonene. Sabinene was not found in Java land race and Blangkejeren, while limonene was not found in Takengon.

Keywords: Aceh provenance, resin tapping, borehole method, genetic variation, repeatability

INTRODUCTION

Pinus merkusii is planted extensively in Java, Indonesia. It is native to northern Sumatra and occurs as three disjunct populations, namely, Aceh, Tapanuli and Kerinci (Cooling 1968). The species was introduced to Java in 1924 from an unknown subpopulation in Aceh provenance and since then, has been planted in Java by Perum Perhutani—a state owned enterprise—covering approximately half a million hectare. It has also been grown in smaller scales in south Sulawesi and west Sumatra (Hardiyanto et al. 2000).

The wood of *P. merkusii* is excellent for furniture, sawn timber and paper. The tree is also being tapped for oleoresin which is processed to high quality gum rosin and turpentine. In recent years, Perum Perhutani has intensified oleoresin tapping in response to higher price of gum rosin and turpentine in the international market. Gum rosin and turpentine industries employ many local communities and contribute around 30% of the company's revenues. Worldwide, Indonesia ranks third in the production of gum rosin and turpentine after China and Brazil, contributing to about 8% of the world gum rosin market (Fachrodji et al. 2009).

It is likely that the genetic base of the first introduction of *P. merkusii* to Java was narrow. Later genetic base of the species was widened by introducing new genetic materials from natural populations in Aceh. These introduced germplasms were established in experimental plantings in Jember (east Java). Little information is available regarding oleoresin production, turpentine yield and composition of the subpopulations of Aceh provenance. This paper presents results of the first assessment of the yields of oleoresin and turpentine and the chemical composition of turpentine in *P. merkusii* from three subpopulations of Aceh provenance.

MATERIALS AND METHODS

Seeds were collected in April 1996 from natural populations in Aceh (northern Sumatra). Details of the locations are presented in Table 1. The experimental plantations (tree spacing

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Table 1 Location of *Pinus merkusii* seed collection

Subpopulation	Longitude (E)	Latitude (N)	Altitude (m)
Jantho	95° 41' 89"	5° 10' 23"	500–700
Takengon	96° 57' 72"	4° 28' 01"	700–1500
Blangkajeren	97° 26' 16"	3° 53' 42"	700–1000
Java land race	113° 52' 42"	7° 67' 63"(S)	700

was 4 m × 4 m) of each subpopulation were established in February 1997 in Jember (7° 67' S, 113° 52' E) at an elevation of 600 m above sea level. Climate of the site is tropical warm humid with mean annual rainfall of 2400 mm, rainy season from October till April followed by marked dry season from May till September. The terrain of the site is flat. The soil is Inceptisol derived from volcanic parent material (Hardiyanto 1996).

Oleoresin and turpentine yield

All three subpopulations were 13 years old when they were tapped for oleoresin. In addition, a stand of local land race of *P. merkusii* (Java land race), also aged 13 years and located nearby, was also tapped. All trees were tapped for oleoresin for the first time. Resin was tapped by drilling holes into the sapwood. Using a borer, each tree was drilled with two holes, each of diameter 16 mm at slope of approximately 45° and depth of 6 cm. The first hole was at 20 cm above the ground and the second, at a slightly higher position on the opposite side of the stem. Each subpopulation and Java land race were represented by 160 trees per subpopulation which were expected to provide sufficient quantities of oleoresin to be processed into gum rosin and turpentine. The exuding oleoresin was directed into oil-resistant plastic bag using PVC pipe tightly inserted into the hole. The oleoresin was collected 24 hours after tapping and weighed using a field digital balance. The use of plastic bag for oleoresin collection prevented contamination from rain and debris.

Oleoresin was converted into its primary fraction of gum rosin (diterpenes) and gum turpentine (mono- and sesquiterpine) by steam distillation. The laboratory had a processing capacity of 1.5 kg of oleoresin in 2 hours with electric heating at 160–170 °C. Turpentine collected was weighed and its chemical composition determined using gas chromatography–mass spectrometry. The remaining distillation residue, i.e gum rosin was also collected and weighed.

Statistical analysis

Analysis of variance followed by Duncan's multiple range test was used to test significant differences between subpopulations. Relative contribution of variability in phenotypic variation between individual trees in oleoresin yield was estimated with repeatability parameter. Repeatability is intra-class correlation (r) between two independent measurements performed on the same individual tree and can be expressed as ratio of variation between individual trees to the total phenotypic variation. This method requires that variance components be estimated from measurement of between and within individual trees in a subpopulation. Repeatability was then estimated using ratio of variance estimates (Falconer & Mackay 1996, Roberds & Strom 2006):

$$r = \frac{\sigma^2_a}{\sigma^2_a + \sigma^2_w} \quad (1)$$

where r = ratio or repeatability σ^2_a = variance component between individual trees and σ^2_w = variance component within individual trees. Denominator in the ratio denotes an estimate of phenotypic variance $\sigma^2_p = \sigma^2_a + \sigma^2_w$. Residual maximum likelihood in the SPSS software was used to estimate variance components. Standard error (SE) for repeatability was calculated using equation 2 (Becker 1992):

$$SE(r) = \sqrt{\frac{2\,(1-r)^2\,[1+(k-1)r]^2}{k(k-1)(N-1)}} \qquad (2)$$

where k = coefficient of variance component for the subpopulation, N = total number of individuals and r = repeatability.

RESULTS AND DISCUSSION

Oleoresin and turpentine yields

Mean oleoresin yields were 12.2, 14.5, 18.0 and 21.1 g $hole^{-1}$ $tree^{-1}$ day^{-1} for subpopulations of Takengon, Java land race, Blangkejeren and Jantho respectively (Table 2). Differences between subpopulations in oleoresin yield were significant. Natural distributions of the subpopulations of Jantho, Takengon and Blangkejeren are disconnected and separated by 35–50 km of broad leaf forest and, thus, gene flow between these subpopulations is unlikely to occur (Cooling 1968).

Yield of oleoresin in the Jantho subpopulation was 17–73% higher than the rest of the Aceh subpopulations and Java land race, indicating that Jantho subpopulation had good potential for developing plantation for high oleoresin production. Oleoresin yield was negatively correlated with elevation (r = -0.95). Yield decreased with increasing elevation of the subpopulation origin. Oleoresin yield in Java land race (14.5 g $hole^{-1}$ day^{-1}) was significantly lower than Blangkejeren (18.0 g $hole^{-1}$ day^{-1}) and Jantho subpopulation (21.1 g $hole^{-1}day^{-1}$), but not significantly different from Takengon subpopulation (12.2 g $hole^{-1}$ day^{-1}) (Table 2).

Mean gum rosin yields were 72.5, 76.7, 77.4 and 78.3% for Jantho, Blangkejeren, Takengon and Java land race subpopulations respectively. The gum rosin yield from Jantho was significantly lower than other subpopulations (Table 2). Mean turpentine yields for Java land race, Jantho, Blangkejeren and Takengon subpopulations were 13.6, 15.3, 16.0 and 19.6% respectively (Table 2). Turpentine yield was positively correlated with elevation (r = 0.87), i.e. increasing with higher elevation of the subpopulation origin. Differences in turpentine yield between populations have also been reported for several other pine species (Nerg et al. 1994, Barnola & Cedeno 2000).

Turpentine yield in this study was higher than that produced by the local Perum Perhutani factory which ranged from 12.0 to 13.4% (Anonymous 2012). The oleoresin processed in the factory was tapped using bark chipping method and collected in open container. Consequently, it is mixed with impurities such as needles, twigs, barks, insects and soil. The amount of impurites ranged from 2 to 5% of the oleoresin collected in container (Anonymous 2012). The impurities had to be screened and removed before the oleoresin is processed into gum rosin and turpentine. In contrast, the oleoresin tapped by borehole method and collected in closed plastic bag is free from impurities. So borehole method could increase turpentine yield with lower processing cost. However, it requires higher cost in terms of tapping equipment (bore) and collection apparatus (plastic pipe and bag which can only be used once in oleoresin collection) compared with bark chipping method.

Repeatability estimates of oleoresin yields were moderate to high. Repeatability value is categorised as low (< 0.3), moderate (0.3–0.5) or high (> 0.5 high) (Rice et al. 1970, Falconer & Mackay 1996) (Table 3). Repeatability values for the subpopulations Takengon, Blangkejeren and Java land race were similar, i.e., 0.57, 0.59 and 0.58 respectively, while that of the subpopulation of Jantho was higher (0.74). Variation between individual trees contributed substantially to the phenotypic variation in oleoresin yield. Repeatability estimates of oleoresin yield in this study were within the range (0.43–0.77) reported for *Pinus elliottii*, *Pinus taeda* and *Pinus palustris* (Roberds & Strom 2006).

The degree of the genetic control on tree characteristics is expressed as narrow-sense heritability which is ratio between additive genetic (σ^2_a) and phenotypic (σ^2_P) variance. The heritability, within the ranges 0 and 1, is estimated from measured attributes in progeny test. The closer the heritability value is to 1, the greater the genetic control (Zobel & Talbert 1984, White et al. 2007). Repeatability value of oleoresin yield reported in the present study may be interpreted as the upper limit of the broad-sense heritability (σ^2_G / σ^2_P) where $\sigma^2_G = \sigma^2_a + \sigma^2_d$ and σ^2_d = non-addivitive genetic variance. When σ^2_d is small then repeatability is not different from heritability. The magnitude of σ^2_d for oleoresin

Table 2 Yields (mean ± standard error) of oleoresin and turpentine in *Pinus merkusii*

Subpopulation	Mean diameter (cm)	Oleoresin (g hole^{-1} tree^{-1} day^{-1})	Gum rosin yield (%)	Turpentine yield (%)
Jantho	31.1	21.1 ±1.4 a	72.5 ± 2.3 a	15.3 ± 1.1 a
Takengon	31.7	12.2 ± 0.8 b	77.4 ± 1.1 b	19.6 ± 0.1 b
Blangkejeren	32.8	18.0 ± 1.5 a	76.7 ± 1.1 b	16.0 ± 0.4 a
Java land race	29.3	14.5 ± 1.1 b	78.3 ± 0.7 b	13.6 ± 0.9 a

Numbers accompanied by the same letter are not significantly different according to Duncan's multiple range test at p = 0.05

Table 3 Repeatability estimates of oleoresin yield of *Pinus merkusii*

Subpopulation	Phenotypic variance	Proportion to phenotypic variance (%)		Repeatability
	σ^2_p	σ^2_a	σ^2_w	
Jantho	3.12	73.9	26.1	0.74 ± 0.02
Takengon	1.46	56.8	43.2	0.57 ± 0.03
Blangkejeren	2.59	59.0	41.0	0.59 ± 0.03
Java land race	2.28	57.9	42.1	0.58 ± 0.03

σ^2_a = genetic variance, σ^2_w = variance component within individual trees

yield in *P. merkusii* is not known; however, it is likely to be small. For instance, narrow-sense heritability estimate of oleoresin yield (0.52) reported from the progeny test of Java land race population (Leksonono & Hardiyanto 1996) was similar to the repeatability estimate of Java land race subpopulation found in the present study (0.58). Repeatability studies are of importance to provide information about genetic variation between individual trees within populations. The repeatabity value can be readily estimated from measurements of traits such as oleoresin yield in the existing population when progeny test of the population to provide precise heritability estimates is not readily available (Falconer & Mackay 1996, Roberds & Strom 2006).

It has been reported that individual tree heritability of oleoresin yield in open-pollinated progeny tests of *P. merkusii* established at two sites in Java was 0.52 (Leksono & Hardiyanto 1996), indicating that oleoresin yield in *P. merkusii* was strongly under genetic control. A study of oleoresin yield of *P. merkusii* revealed that the individual tree heritability of oleoresin yield in open-pollinated progeny tests in central Java involving six sublines ranged from 0.07 to 0.42 (Muslimin 2011). Genetic studies in other pine species also showed that oleoresin yield had high individual heritability. Individual heritability of oleoresin yield in *Pinus elliottii* was 0.85 (Franklin et al. 1970). Oleoresin yield in *Pinus taeda* was also strongly under genetic control with estimated individual heritability ranging from 0.44 to 0.59 (Roberds et al. 2003).

Considerable differences between subpopulations and moderate to high genetic control in oleoresin yield indicate that substantial genetic gain in oleoresin yield can be obtained by selecting parent trees having high oleoresin yield. Parent trees can be selected from each subpopulation.

Turpentine composition

The main component found in the turpentine of *P. merkusii* was α-pinene, amounting to 73.3, 73.9, 81.7 and 87.2% (Table 4) for Jantho, Takengon, Blangkejeren and Java land race respectively. This was followed by δ-3 carene, i.e. to 19.3 , 19.1, 11.9 and 7.3% (Table 4) for Jantho, Takengon, Blangkejeren and Java land race respectively. The major components of turpentine from Aceh provenance and Java land race in the chromatogram are presented in Figure 1.

Table 4 Chemical constituent of turpentine of subpopulations of *Pinus merkusii*

Compound	Chemical concentration (%) in subpopulation			
	Jantho	Takengon	Blangkejeren	Java land race
α-Pinene	73.3	73.9	81.7	87.2
δ-3-Carene	19.3	19.1	11.9	7.3
β-Pinene	1.7	1.5	1.8	2.2
Camphene	0.9	1.3	1.5	0.8
β-Myrcene	1.1	1.1	0.7	0.5
α-Thujune	0.7	0.7	0.8	0.3
Sabinene	0.3	0.3	–	–
Limonene	1.8	–	2.1	1.0
α-Terpinolene	0.7	–	–	–
Unidentified	–	1.3	–	–

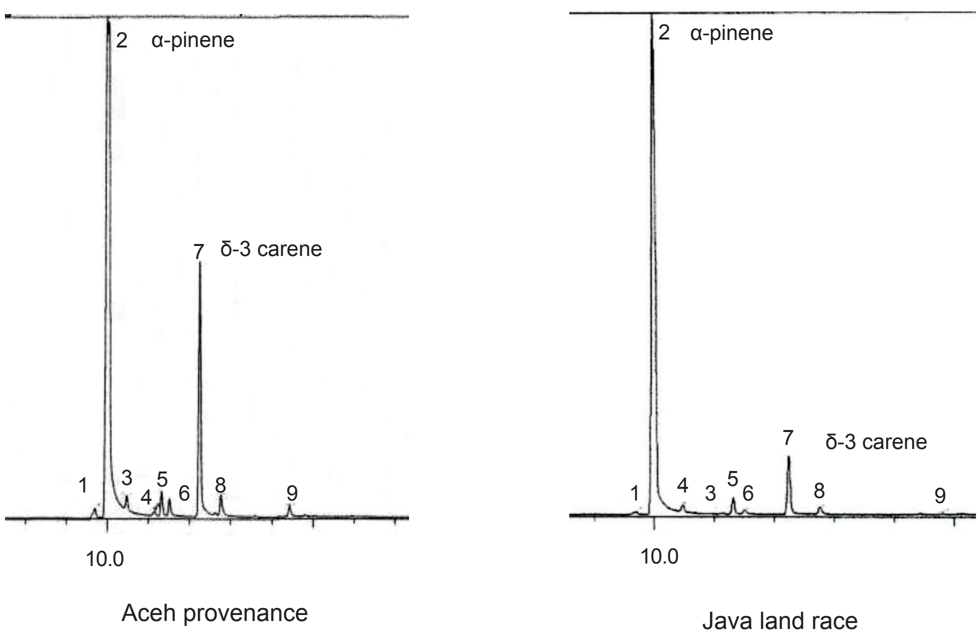

Figure 1 Major components of turpentine in *Pinus merkusii;* 1= α-thujune, 3 = camphene, 4= sabinene, 5 = β-pinene, 6 = β-myrcene, 8 = limonene, 9 = α-terpinolene test

Turpentine also contained small amounts (< 3%) of β-pinene, β-myrcene, limonene and camphene. The content (about 2%) of β-pinene found in Java land race was similar to that of limonene in the subpopulations of Blangkejeren and Jantho. Sabinene was found in Takengon and Jantho, but not in Blangkejeren and Java land race (Table 4). Wiyono et al. (2006) reported that turpentine produced in gum rosin obtained from factories in Java and North Sumatra had 82–86% α-pinene, 8–12% δ-3 carene and 2.2–2.4% β-pinene. Composition of turpentine from the subpopulation Blangkejeren was similar to that of the Java land race. This supports the hypothesis that *P. merkusii* was introduced to Java was from Blangkejeren subpopulation (Hardiyanto et al. 2000).

CONCLUSIONS

Oleoresin and turpentine yields of *P. merkusii* differed significantly between the three subpopulations of Aceh provenances. Unlike turpentine, oleoresin yield decreased with increasing elevation of the subpopulation origin. Oleoresin yield was moderately to highly under genetic control.

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