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Needle morphological variation within and among population of *Pinus merkusii* Jungh & De Vries in Central Aceh, Indonesia

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Abstract
This study has been conducted with the main objective of identifying the morphological variability of *P. merkusii* needle. The length of needles and fascicle sheath, shape and weight of the needle and its stomata density has been taken as the major traits to identify the variability. Altogether 1820 needles from 90 trees of 6 populations have been collected and analysed for morphological traits. Out of these, 60 needles from 6 populations were microscopically studied for stomata density and the correlation between length-weight was developed. The lengths and shape of needles are found significantly different according to population whereas the stomata density is found insignificant.

Keywords: *Pinus merkusii*, population, sites, fascicle, needle, stomata, stomata density

1. Introduction
Pinus merkusii, also called Sumatran Pine, is one of the truly tropical pines in the world, is a native to South East Asia which is extensively distributed in Thailand, Laos, Cambodia, Vietnam, Sumatra (Indonesia) and the Philippines islands of Luzon and Mindoro (Cooling, 1968). It has a large altitudinal range from a few meters above sea level to over 1800 m. (Cooling, 1968). It is found on a diversity of soils over many different parent materials and climatic variation**.**

P. merkusii is the medium to large size tree, reaching 25-45 meter tall, with a trunk diameter up to 1 meter, and pyramidal to conical crown on young age, and flatter and spreading on old age (Wageningen University, 2003). The needles are very slender, rigid, straight 15-25 cm long and less than 1 mm thick, green to yellow color and are found in a pair of two. The needles are persistent for 2 years and its dried leaves weigh around 60-90 milligram per fascicle (Wageningen University, 2003). It has usually a rough bark with deeply fissured predominantly in vertical direction and grayish in color.

2. Objectives
The main objective is to identify the variability in needle morphology within and among population of *P. merkusii*

- To assess the variability in the length and shape of needles,
- To assess stomata density and its variability
- To assess the correlation of needle length and weight

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3. Methodology

3.1 The populations and study sites

The four plantation and two natural populations of *P. merkusii* have been taken for the study. The plantations, very rarely mixed with natural old left over trees, are owned to Private concessionaires. Studied six different populations vary from each other in the habitat, elevation, aspects, plantation age, slope, spacing etc. The altitude varies from 610 m. (Blang Kuyu) to 1750 m. (Burni Telege) and from slightly sloped area to highly steep ground covering all distribution range. The ages of population vary from 10 years old plantations to 50 years. The plantations are generally spaced at 3m x 3m and in some places mixed with natural forest with irregular spacing. There is almost no silvicultural treatment being practiced.

These areas have longer rainy seasons with around 1600 mm. annual rainfall, and high temperature above 30 degree Celsius hence has very high relative humidity (District Agriculture Office, 2007).

3.2 Sampling design and needle collection

Six different populations of *P. merkusii* have been selected on the basis of physical distance. Each population lies more than 25 kilometer apart except Hakim Bade Dedalu and Burni Telege. These two sites are around 7 kilometer apart yet are separated with the vertex of hill so that there is no mutual follow of genetic resources. Altogether 90 trees, all above 10 cm diameter at breast height (dbh), 10 trees from three sites (Blang Kuyu, Burni Telong and Hakim Bade Dedalu) and 20 trees from three sites (Isaq, Burni Telege and Kecamantan Linge (Isaq) have been included into samples.

The dbh and height of the trees have been measured. The four fully developed twigs from all expositions namely North, South, East, West have been collected. One twig at each direction from the illuminating part has been collected and studied. The length of twigs has been measured from the apical bud to the lowest part where there is a live (green) needle available.

Around 1820 needles, five needles from each twig at its bottom, have been picked out and measured for needle and fascicle length and its shape. For the shape of needles, relative scaling from '**I' to 'V**' has been given where '**I**' has been marked for the straightest and the '**V**' is for the most curved. Ten needles from each population have been studied for the stomata density and weight/length relation. For the weight-length study, the needles used were around 2 months naturally dried in Silica Gel. Statistica 6.0 software has been used for the statistical analysis and interpretation of data.

3.3 The study of stomata

The epidermis from abaxial surface are taken out with crystal clear plastic tape and affixed into the clean microscope slide for the microscopic study. The microscope slides have been kept into the 40 times (object) and 10x (times) net ocular lens magnified microscope. The stomata inside the grid area of 240X240 micrometer (µm) have been counted. The numbers of stomata at five different places, along the middle part of needle, have been counted in each needle for the statistical analysis. The photo of cuticle structure has been taken with Axiocam MR3 for the analysis.

4. Results and Discussion

4.1 Length of twigs

Twigs are collected from the accessible height, mostly from the illuminating part of the tree. The length of twigs ranges from 2.8 cm (Isaq) to 106 cm (Burni Telege). The *P. merkusii* stand at Isaq

has been recently burnt and spaced densely. That might be the main cause of short twig lengths where as the Burni Telege is located at the highest elevation with high relative humidity, may be the reason for longer twig lengths. The effect of population on the variability of twig length has significant at p=0.05. The twigs from Burni Telege population are significantly different with all other population as well as it has highest variability within population.

There is no significant effect found on the size of twig lengths due to the aspects at p=0.05 however the twigs from North and from West are significantly different with each other. Twigs from East aspects have highest and from West have least variability within aspects.

4.2 Length of needles

The length of needles ranges from 7.6 cm (at Burni Telong) to 23.3 cm (at Hakim Bale Dedalu). The sites for Burni Telong is fairly flat (<5%slope) where as the Hakim Bale Dedalu is the steepest (>70% slope). The sampled trees in Burni Telong are the smallest (9.4m) where as the trees from Hakim Bale Dedalu is the tallest (21.4m) among the populations.

The absolute value for the average needle lengths ranges from 16.71cm (Burni Telong) to 18.53 cm (Kecamatan Linge) with merely 1.82 cm mean differences in comparison to pronounced ecological, environmental and probably the genetic variations however the ANOVA test shows that the needle lengths varies significantly among the population at p=0.05.

There is no significant effect of the aspects in determining the length of needle at p=.05. The mean length ranges from 17.50 cm (West) to 17.70 cm (South).

4.3 Length of Needle Fascicle Sheath

The Fascicle Sheath in *P. merkusii* needle is brownish and scaly, short and stout. Two needles are used to originate and attached with each other in each fascicle. Both of the needles are attached with each other in the beginning and at the later stage separates. The size of both needles is almost same with only 1 mm different with each other. The average length of fascicle is found 0.89 cm ranging from 0.1 cm to 1.5 cm irrespective of the length of needle. The mean fascicle length as per the population falls between 0.74 cm to 0.98 cm however the ANOVA and Post Hoc LSD tests (p=.05) show that the means are significantly different with each other except Burni Telong and Burni Telege (0.72) despite of large differences in their geographical differences.

The length of Fascicle is irrelevant to the length of needle. There is not significant linear relationship in between.

4.4 Weight of Needle

The diameters of needles are clearly observed different at different sites. Some needles are relatively stout and thicker whereas some are found relatively thinner. The length and thickness of the needles greatly influence the weight of needle. The average needle weight in Central Aceh is calculated as 0.0432 grams, ranging from 0.0310 grams (Blang Kuyu) to 0.0565 grams (Hakim Bale Dedalu) on averages according to population.

The weight of needles is significantly different to each other since the length of needle has been influenced by ecological, environmental variation of the populations studied.

4.5 Weight and Length Relationship

The weight of needle largely depends on its length. As the length grows, it gains the weight. The equation Weight of Needle (Y) =-0.017097+0.0036*x can predict the weight of needles at coefficient of determination of 0.43.

4.6 The shape of the needles

Generally the shape of *P. merkusii* needles is stout and straight. The relative scale from I to V have been given to the shape of needle. On the basis of pre evaluation to all the needles, the straightest needle has been scaled I and most curved needle as V. Most of the green needles at the time of collection are observed straight. Around 83.5% of the needles are found straight, and 13.9% are fairly straight contributing as it the needles is stout, rigid and straight with the total of 97.5%.

The applied Pearson's Chi Square test (at p=0.05) demonstrates, the shape of the needles is significantly influenced by the population. The locality factors as well as genetic characteristics might be the reasons behind the significantly influencing the needle shape.

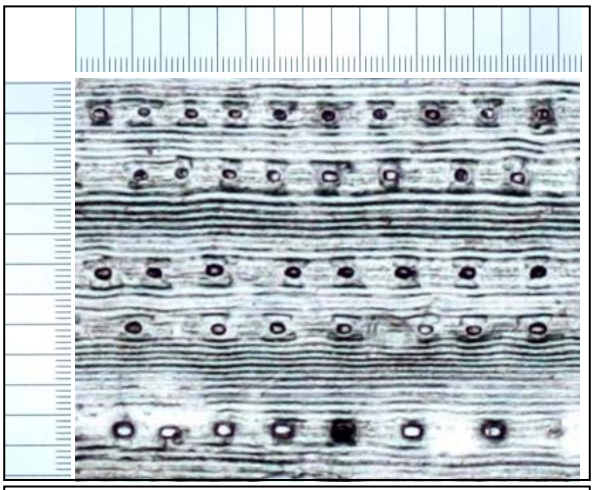
4.7 Exceptionally found three needles in a Fascicle

Some trees of *P. merkusii* had few fascicles with exceptional three needles despite it is two needle Pine. 10 fascicles from **Burni Telege** were found with 3 needles. This might be due to mutation or some other reason which may be the interesting subject for further research.

4.8 Stomata and its Density

The stomata in the Pine are arranged in rows, especially in grouped bands and are found at the regular interval in *P. merkusii*. Epidermal cells and stomata in conifer needles are all initiated at the base of the needle, developing henceforth in longitudinal files during needle growth **(Croxdale, 2000 cited in Kouwenberg L, 2003).**

The stomata studied were sunken due to dry sample. The epidermis from abaxial surface including stomata have been taken away as the imprint and fixed in microscope slide. The imprints were taken from the middle part of needle, and have been observed under the microscope at five places along the length for statistical analysis. Three to 9 stomata were seen under the Microscope inside the area of 0.0576 mm^2.



Stomata in regular row in *P. merkusii* needle under 200X magnification and the scale (in µm)

The stomata density is defined as the number of stomata available per squared millimeter of leaf area. The size and number of stomata varies from species to species and places to places. Because the number and size of stomata are highly influenced by its genetical characteristics and locality factors such as rainfall, relative humidity, temperature, and concentration of CO_2 etc.

The estimated mean stomata density per square mm of needle is 93.41 ranging from 52 to 142. Population wise, the lowest mean stomata density is found on the needles from Kecamatan Linge (Isaq) which is just 84.72 and the highest number of average stomata density is found on Isaq which is 99.02. Kecamatan Linge is the hottest area in the region due to curve valley type topography, hence might be less number of stomata due to adaptation.

Highest variability of stomata density has been found inside Burni Telege (CV=0.23) and the least within variability has been found inside Kecamatan Linge (CV=0.11). The ANOVA test identifies the estimated stomata density is not significantly influenced by population at p=0.05.

5. Summary

- The major morphological characteristics such as length of needle and fascicle, the shape of needles, the length of twigs etc are found significantly different among population. That might be due to the differences in the locality factor such as altitude, slope, temperature, RH, rainfall etc. The absolute differences in mean value is not profound however The ANOVA and LSD test clearly demonstrate the significant differences among populations on the major morphological traits that indicates there is less influence due to genetic variability however it is yet to be examined.
- The shape of needles is predominantly straight however they are significantly different among populations.
- There is not significant linear relationship between fascicle length and needle length but the weight and length of needles are significantly correlated.
- There is not significant difference in the stomata density among the populations.

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