

GENETIC VARIABILITY IN RESISTANCE OF *Falcataria moluccana* (Miq.) Barneby & J. W. Grimes TO GALL RUST DISEASE

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ABSTRACT

During 2003-2009 large area plantations of *F. Moluccana*, in Java particularly, had been attacked severely by gall rust disease. In order to reduce the gall rust impacts, selection of gall-rust resistance of *F. moluccana* needs to be undertaken. Therefore, study of genetic variability on gall-rust resistance of *F. moluccana* was carried out at the 3 years old of gall rust disease resistance trial located at Kediri, East Java. The trial consists of 80 open pollinated families, including four seed sources from Kediri (East Java), Lombok (Nusa Tenggara), Papua and Candirototo (Central Java). In this study, several variables were assessed i.e. height, diameter, stem form, crown density, number of galls which existed on stems, branches, twigs, and crowns. The disease incidence and severity were calculated using the index scores of gall rust. Results revealed that there were significant differences in stem-form and stem-galls between families. Individual heritability (h^2_i) was relatively medium for stem-form, branch-galls and stem-galls, whereas h^2_i for diameter, crown density and twig-galls were low. Genetic correlations were strongly positive between diameter and the disease. Meanwhile, a correlation between stem-form and the diseases was strongly negative. Therefore, individual heritability for stem-form could be used to decrease disease incidence and severity. Index of disease incidence and severity varied significantly between 80 families in the trial. Grouping of the families based on the seed sources (provenances) showed that Papua seed sources exhibited the most resist or tolerate to the gall rust diseases. Therefore, further attention to the potential provenances involving the disease severity and incidence need to be paid.

Keywords: *Falcataria moluccana*, gall rust diseases, resistance, seed sources

I. INTRODUCTION

Falcataria moluccana (Miq.) Barneby & J. W. Grimes, is member of Fabaceae (Mimosoideae), formerly *Paraserianthes falcataria* (L.) Nielsen, in Indonesia commonly called sengon. *F. moluccana* has several synonyms i.e: *Albizia moluccana* Miq., *Albiziafalcata* Backer, *Albizia falcataria* (L.) Fosberg (Hidayat *et al.*, 2003). It also has several local names such as: sengon and jeunjing (Indonesia general); sengon laut (Java); sika (Moluccas); batai (Peninsular Malaysia and Sabah); kayu machis (Sarawak); white albizia (Papua New Guinea); molucansau, falcata (Philippines). It is categorised

as fast growing species which is indigenous to Maluku (Indonesia), Papua New Guinea, the Solomon Islands and the Bismarck Archipelago (Wagner *et al.*, 1999). Sengon is widely planted in tropical regions; within the area of natural distribution it is found at 0-1.200 m above sea level with mean annual temperature and precipitation of 22-29° C and 2000-4000 mm, respectively (Hidayat *et al.*, 2003). Sengon is the major forest resource especially in Java Indonesia with an over 1.200.000 ha plantation established in 2005 (RLPS, 2005). In addition to forest plantations, it is commonly planted in agro forestry systems and has shown potential in alley farming. The number of plantations increased every year since this species is one of the valuable multipurpose species replacing the tropical timber species as

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source of product of pulp and plywood industries. Sengon timber production in Ciamis West Java, increased dramatically from 2003 to 2006, the production was recorded 50.399.935 m³ in 2003 and 221.584.347m³ in 2006 (Siregar *et al.*, 2009).

Unfortunately, large area of sengon plantations in Java had been severely infected by gall rust disease, which caused the slump of sengon wood production; for example in Lumajang, East Java the production decreased from 755.710,71 m³ in 2009 to 671.408,66 m³ in 2010 (Dishut Lumajang 2011). During 2003-2009, *F. moluccana* has become widely infected by gall rust disease especially in Java Island (Rahayu, 2008). In East Java, the initial out break had been reported in 2003, unfortunately there was no serious attention to solve this problem by neither responsible department nor non-government institutions/industries (Rahayu 2010). In 2005 the gall rust disease had extended to the major area of sengon plantation including Banyuwangi, Bondowoso, Pasuruan, Malang, Probolinggo, Jember and Kediri. Based on the status, level of impact and condition of the worst-affected plantations (Rahayu 2008) suggested that the gall rust has been present for at least 3 years. Gall rust disease has also been found in Central Java (Temanggung, Wonosobo) in the early 2006; and in West Java (Ciamis) in 2008.

Uromycladium tepperianum (Sacc.) McAlp. has been identified as the cause of gall rust disease in *F. moluccana* plantations in Philipina, East Timor, Sabah Malaysia, Java and Bali Indonesia (Brown 1993 in Rahayu 2008; Braza 1997; Old and Cristovao 2003; PROSEA 2003; Rahayu *et al.*, 2005; Rahayu dan Lee 2008). Fifty seven species of acacias being recorded as hosts of *U. tepperianum* across Australia (McAlpine 1906; Warcup and Talbot 1981). The fungus also infects *Paraserianthes lophantha* ssp. *Lophantha* (Willd.) Nielson in Australia; *P. lophantha* ssp. *montana* (Junghuhn.) Nielsen in Java and on *Albizia* (*Paraserianthes*) spp. in Papua New Guinea (Shaw 1984; Lee 2003). Undetermined species of *Uromycladium* on *P. falcataria* have also been reported in Sabah (Anonymus 2001 in Old and Cristovao 2003) and the Philippines (de Guzman *et al.*, 1991). *Gall rust diseases causing formation of galls*

on foliage, branches and stem. The pathogen attacks all above-ground parts of susceptible host; however damage is most severe when shoot and stem are affected, as stems are girdled by the rust and then insects and saprophytes invade and live in the galls. As shoots are partially girdled and become under severe stress, massive defoliation occurs and, eventually, large trees can be killed; moreover, stems will also easily fall down when there is severe wind.

Silviculture technique and plantation management had been attempted to combat gall rust in Java, Indonesia (Anggraeni 2008; Rahayu 2008), even though it seems not powerful to eliminate this problem. Based on several studies in rust-tree combinations (Western gall rust of radiata pine caused by *Endocronartium barknessii* and phyllode rust of acacias caused by *Atelocaula digitata*) it was suggested that genes for the disease resistance exist in the host trees and their presence is a major factor in determining disease impact (Old *et al.*, 1986; Old *et al.*, 1999). Previous study on the responses of *F. moluccana* seedlings from 6 different seed sources revealed that seedlings from Wamena Papua exhibited the best seed source in relation to gall rust resistance (Rahayu *et al.*, 2009). This study therefore, reported the genetic variation of gall rust resistance on 3 years old of *F. moluccana* in a resistance trial, in order to select best families or provenances of *F. moluccana* showing better resistance to the gall rust diseases.

II. MATERIALS AND METHODS

A. Location and Experimental Design

Evaluation to disease severity and growth parameters were carried out at 3 years old of open pollinated *F. moluccana* resistance trial at Kediri, East Java (formerly established as progeny trial). The plantation (trial) planted in December 2007, by researcher of B2PBPTH Yogyakarta, in the area which damaged severely with gall rust. This trial was arranged in a randomised complete block design, comprises of 77 open pollinated families, 4 tree line plot and 6 block as replications. The 77 open pollinated families originating from 4 different seed sources are presented in Table 1. The evaluation was conducted on August 2010.

Table 1. List of seed sources of *F. moluccana* resistance trial at Kediri. East Java

| Seed source | Latitude | Longitude | Altitude | Family number |
|--------------------------------------|-----------------|-------------------|-----------|---------------|
| Candiroto, Java (Geographic race) | . 015' | 110°09' | 500 | 1 to 37 |
| Kediri, Java | 07°48' – 07°56' | 112°11'–112°15' | 121 – 618 | 38 to 57 |
| Lombok, Nusa Tenggara | 08°18' – 08°32' | 116°07' – 116°23' | 101 – 665 | 58 to 75 |
| Wamena, Papua | 1°1' – 4°5' | 123°47' – 139°15' | 25 – 1700 | 76 to 80 |

B. Assessments

Assessment of the traits was conducted on all individual trees in this plot. Height, diameter at breast height and number of gall in the stem were only three quantitative traits included in the assessment. Classification and scoring techniques were applied to characteristics which could not be measured quantitatively (Pinyopusarerk *et al.*, 2004). *Height (Ht)* referred to the total tree height, measured to the nearest 0.1 m. *Diameter (Dbh)* was defined as the stem diameter taken at 1.3 m from ground level and was measured to the nearest 0.1 cm. *Number of stem-galls (GStem)* referred to the total number of gall exist on the stem per tree (Rahayu 2010).

There are characteristics where quantitative measurements were not possible therefore were recorded by visual classification. Four qualitative characteristics were assessed, reflecting canopy form (*Cf*), stem form (*Sf*), number of branch-galls per tree (*Gbranch*) and number of twig-galls per tree (*Gtwig*). Those characters were scored based on Rahayu (2010) as follows:

Crown density (Cd) were simply scored into three classes base on density and thickness:

- 1 = very dense and thick
- 2 = medium dense
- 3 = sparse dense

Stem form (Cf) were simply scored into three classes:

- 1 = straight form
- 2 = medium bend form
- 3 = very bend form (double bend)

Number of branch-galls per tree (Gbranch) were simply scored into three classes:

- 1 = none gall exists on the branches
- 2 = gall ≤ 50% exists on the branches
- 3 = gall > 50% exists on the branches

Number of twig-galls per tree (Gtwig) were simply scored into three classes:

- 1 = none gall exists on the twigs
- 2 = gall ≤ 50% exists on the twigs
- 3 = gall > 50% exists on the twigs

Based on the data of stem-galls, branch-galls and twig-galls in the tree, the gall rust existence of individual trees then scored into six classes:

- 1 = health trees, none gall exist
- 2 = gall exist only in the twigs ≤ 50%
- 3 = gall exist on the twigs and branches ≤ 50%
- 4 = gall exist on the twigs and branches > 50%
- 5 = gall exists on the stem with or without existing on the twigs and branches
- 6 = died trees, due to gall rust attack

According to Rahayu (2008), the assesment of the amount of disease present by calculating the *disease incidence (DI)* and *disease severity (DS)* are fundamental data for describing and understanding the dynamics of diseases as well as for treatment evaluations. Therefore, gall rust DI and DS for each plot were calculated based on the index score using formulas as described by Rahayu *et al.*, (2009):

$$DI = \left(\frac{n}{N} \right) \times 100\%$$

$$DS = \left\{ \frac{(n1 \times z1) + (n2 \times z2) + \dots + (nx \times z6)}{(N \times Z)} \right\} \times 100\%$$

Where:

n = number of infected trees

n1, n2, n3, nx = number of trees with index score 1, 2, 3, ..., x

z1, z2, z3, zx = index score of gall rust presence 1, 2, 3, ..., x

N = total number of trees in one plot

Z = the highest score

Table 2. Gall rust disease incidence (DI) and severity (DS) status based on the value of DI and DS on *Falcataria moluccana* (Rahayu 2009)

| Value of Disease Incidence | Incidence Status | Value of Disease Severity | Severity Status |
|----------------------------|------------------|---------------------------|-----------------|
| <10% | Rare | 0% | Nil |
| 10 - <25% | Occasional | <25% | Low |
| 25 - <50% | Common | 25 - <50% | Medium |
| 50 - <75% | Very common | 50 - <75% | Severe |
| >75% | Widespread | 75 - 100% | Very severe |

C. Statistical Analysis

1. Analyses of variance

The individual trait related data were analyzed using the ANOVA procedure in Genstat Version 5.3.2 to check for homogeneity of variances (Payne *et al.*, 1987). Analyses on the data were based on the following linear model that has been described before to a randomized complete block design (RCBD) with family nested in provenance as follows:

$$Y_{ijk} = \mu + R_i + P_j + F_{k(j)} + e_{ijk}$$

where:

μ = the overall mean;

R_i = the effect of the i^{th} replicate

P_j = the effect of the j^{th} provenance

$F_{k(j)}$ = the effect of the k^{th} family within j^{th} provenance

e_{ijk} = the residual error with a mean of zero.

Y_{ijk} = the plot mean of the j^{th} provenance, k^{th} family within j^{th} provenance, the i^{th} replicate

2. Genetic parameters

The mean family variance components were used to estimate mean covering individual tree heritability (denoted as h_i^2) separately for each trait as follows:

$$h_i^2 = \sigma_f^2 / (\sigma_f^2 + \sigma_w^2) r \quad (\text{Cotterill 1987}), \text{ where:}$$

h_i^2 = individual tree heritability

r = coefficient of relationship among offsprings in the progeny

σ_f^2 = variance between families

σ_w^2 = phenotypic variance

$$= (\sigma_b^2 + \sigma_{fb}^2 + \sigma_c^2)$$

σ_b^2 = variance between block replications

σ_{fb}^2 = family x block interactions

σ_c^2 = residual error

The coefficient of relationship among offsprings in the progeny used in the computation of the individual tree heritability was assumed to 0.4 based on the assumption of many species of eucalyptus and other members of the family Myrtaceae (Moran 1992). The floral structure of *F. moluccana* which is in spike, with un-synchronous flowering within individual spike lead the assumption that open pollinated families of its species may carry a degree of inbreeding resulting from selfing and neighborhood mating (Baskorowati 2010; Hemsley and Ferguson 1985).

Genetic correlations (denoted r_g) were calculated according methodologies described by Williams and Matheson (1994) based on the following formula:

$$rg = \omega_{xy}(X,Y) / [\sigma_f^2(x) \cdot \sigma_f^2(y)]^{1/2}$$

where,

$\omega_{xy}(X,Y)$ = covariance of the two traits at the family level

$\sigma_f^2(x)$ = family- level variance components of trait (x)

$\sigma_f^2(y)$ = family- level variance components of trait (y)

rg = genetic correlations

III. RESULTS AND DISCUSSION

A. Genetic variation

Table 3 presents analysis of variance and estimated individual tree heritability in diameter, stem-form, crown-density, twig-gall, branch-gall and stem-gall in the progeny test of *F. moluccana* at Kediri trial at 3 years old after planting for a particular trait of every 4 trees.

Table 3. Significance of differences between replications and families and estimates of narrow-sense heritability of 2 years old of *Falcataria moluccana* plot resistance

| Traits | Statistical Significance (Mean square) | | Narrow sense Heritability |
|---------------|--|----------------------|---------------------------|
| | Between Replications | Between Families | |
| Diameter | 37.902 * | 14.022 ^{NS} | 0.02 |
| Stem-form | 4.943** | 0.744** | 0.14 |
| Crown-density | 1.158 ** | 0.254 ^{NS} | 0.07 |
| Twig-Galls | 3.000** | 0.189 ^{NS} | 0.02 |
| Branch-Galls | 6.230** | 1.519** | 0.24 |
| Stem-Galls | 0.584 ^{NS} | 0.496** | 0.16 |

** : significance at 1% level; * : significance at 5% level; ^{NS} : Non Significance

Table 4. Genetic and phenotypic correlation of diameter, stem-form, crown-density, twig-galls, branch-galls and stem-galls of 3 years old of open pollinated *F. moluccana* resistance trial at Kediri, East Java ($p < 0.001$). Genetic correlations were shown above the diagonal and phenotypic correlations were shown below the diagonal.

| Genetic \ Phenotypic | Diameter | Stem form | Crown form | Twig galls | Branch galls | Stem galls |
|----------------------|-------------|-------------|-------------|-------------|--------------|------------|
| Diameter | | -0.82 | 0.78 | 0.91 | 0.40 | 0.89 |
| Stem-form | 0.39 | | 0.71 | -0.15 | -0.53 | -0.78 |
| Crown form | 0.45 | 0.21 | | 0.05 | -0.04 | -0.67 |
| Twig galls | 0.07 | 0.01 | 0.10 | | 0.61 | 0.49 |
| Branch galls | 0.24 | 0.05 | 0.24 | 0.21 | | 0.66 |
| Stem galls | 0.20 | 0.02 | 0.09 | 0.11 | 0.17 | |

Differences in stem-form and stem gall were highly significant between families in the trial. Individual heritability was relatively medium for stem-form, branch-gall and stem-gall, whereas individual heritability for diameter, crown-density, and twig-gall were low. Genetic correlations between traits for two-year-old *F. moluccana* in the progeny test at Kediri trial are given in Table 4.

Diameter and stem-form, stem-form and branch-gall, stem-form and stem-gall, crown-density and stem-gall were, as expected, strongly negative associated with an r_g of - 0.82 for diameter and stem-form, r_g of - 0.53 for stem-form and branch-gall, r_g of - 0.78 for stem-form and stem-gall, and r_g of - 0.67 for crown-density and stem-gall. The strongly positive correlations were found between diameter and crown density, diameter and twig-gall, diameter and stem-gall, stem-form and crown- density, twig-gall and branch-gall, and between branch-gall and stem-

gall. It appears, however, that crown-density and twig gall; crown-density and branch-gall are weakly associated and selection for one is unlikely to affect the other, either adversely or favourably. The principal finding from the estimates of genetic correlations showed strongly adverse correlations between diameter and disease. However, the correlation between stem-form and disease was strongly benefited. The correlations were all calculated from a relatively enough number of families that they would be expected to have small standard errors, as heritability estimates. This suggests that it should be likely to improve stem-form simultaneously in the same breeding population. The results reported indicate that individual heritability for stem-form can be used to decrease disease in *F. moluccana* through selection and breeding. Such a program is now underway on East Java with one of the first activities being the thinning of the Kediri trial to convert it into a seedling seed orchard.

B. Disease incidence and disease severity

The index of disease incidence calculated based on plot mean varied significantly between 80 families in the trial ($ms = 1484.97$, $df = 79$, $Sign = < 0.0001$). The index of disease severity also varies significantly between 80 families in the trial ($ms = 156.5$, $df = 79$, $Sign = < 0.0001$). It is also evident, that great variation of disease incidence and disease severity exhibit among families, which several family numbers such as 76, 79 and 80 demonstrated lower disease incidence and disease severity compared to other families.

The variation of the index of disease incidence and disease severity between families probably due to the differences of seed originalities or seed sources planted in this trial. Different seed sources may vary in their susceptibility to certain disease, even if none of them is completely resistant. This partial resistance may influence disease development by decreasing the number of

successful infections by the pathogen, by increasing the latency period, by reducing the rate of lesion expansion or sporulation, or by any combination of these processes (Aljabes *et al.*, 1999).

Meanwhile, the regression analysis revealed that disease severity and disease incidence demonstrated strong positive correlation ($r=0.85$; see Figure 1). It means that families exhibited high disease severity increased the disease incidence. According to Aljabes *et al.*, (1999) that many components of the environment can indirectly influence the severity of disease through the host plant or by direct effect on the pathogen; for example: temperature, relative humidity, radiation and wind. Moreover, Rahayu (2010) mentioned that the development of gall rust in *F. moluccana* mainly influenced by the relative humidity, intensity of sun light, temperature, altitude, elevation and the presence of fog.

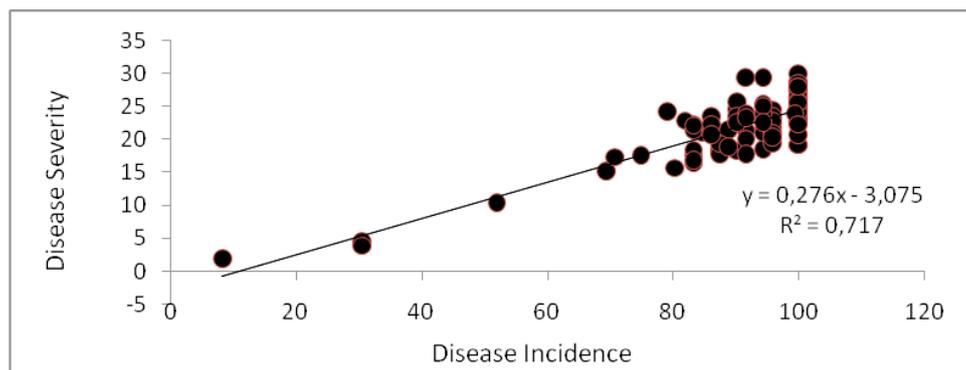


Figure 1. Correlation between disease and disease incidence (%) of 3 years old of open pollinated *F. moluccana* resistance trial at Kediri, East Java

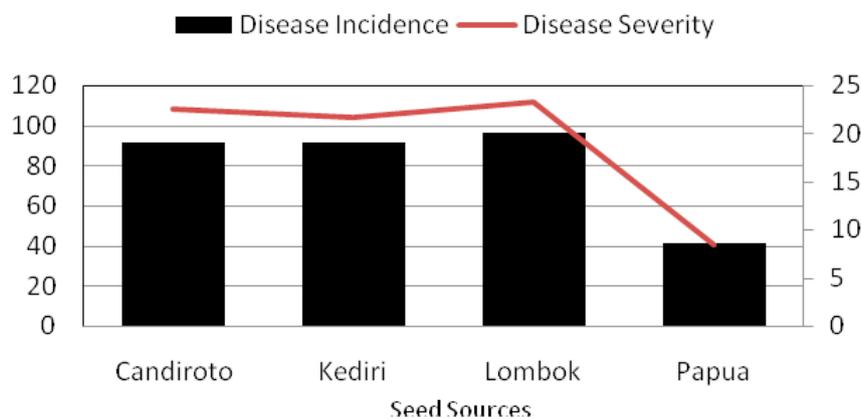


Figure 2. Gall rust disease incidence and disease severity of 3 years old of open pollinated *F. moluccana* resistance trial at Kediri, East Java, grouped based on seed sources

When the families grouped based on the seed sources (Figure 2), it shows that seed source from Papua (Papua Island) exhibited the lowest attack of gall rust, indicated by the low index of disease incidence and disease severity compared to seed sources from Candiroto (Java Island), Kediri (Java Island) and Lombok (Nusa Tenggara Island). Previous studies using isozyme analysis (Sheido and Widyatmoko 1993) revealed that genetic variation of *F. moluccana* at Java Island categorized at the low level; this study showed the value of heterozygosity of Javanese populations (0.098) were considerably lower than for a natural population from Irian Jaya (0.163). Other studies using isozyme analysis by Winarni (2003) and Wulan (2003) also revealed that in general population of *F. moluccana* in Java exhibited low genetic variation; $F_{ST} = 0.16$ and 0.18 respectively. Another study using RAPD showed the similarity of genetic relationship among Java populations, with the mean genetic distances within and between populations was similar (Suharyanto *et al.* 2002); even though another study using RAPD showed high genetic variation of its species at Java population (Widyastuti 2007).

Generally, low genetic diversity of a species leads to the chance of disease attack. It was also supported by recent studies of responses e.g. disease severity, infection rate and mortality of *F. moluccana* seedlings from several seed sources to inoculation with *U. tepperianum* which indicated that seedling from Wamena (Papua Island) was found to be the best seed source in relation to gall rust disease resistance (Rahayu *et al.* 2009).

This study indicated that low genetic variation of *F. moluccana* from Java Island caused the high risk of attack by gall rust, showed by the high disease severity and disease incident. Without high genetic variation, a population cannot develop in response to changing in environmental condition. For example, if a population is exposed to a new disease, selection will act on genes for resistance to the disease if they exist in the population. But if they do not exist - if the right genetic variation is not present - the population will not evolve and could be wiped out by the disease.

However, high risk of diseases also due to the influence of environmental conditions, such as relative humidity, intensity of sun light, temperature, altitude, elevation and the presence

of fog. Rahayu *et al.*, (2011) mentioned that fog is one of the most significant factors supporting gall rust disease development in *F. moluccana*. Therefore, population from Papua exploited from the village at about 2000 m above sea level, which is fogs are frequent making the seed source from Papua having adapted to foggy conditions. According to Rahayu *et al.*, (2011), the adaptation to foggy conditions may lead their adaptation of anatomical and morphological properties such as impermeable cuticles and intracellular modification are thus more resistance to gall rust fungus infections.

IV. CONCLUSION

Based on the assessments of the 3 years old of gall rust disease resistance trial of *Falcataria moluccana* at Kediri East Java, provenance Papua showed better performance than others with regards to gall rust resistance. Stem-form may be used as important character for selection.

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