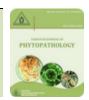


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HERITABILITY OF EARLY GROWTH TRAITS AND THEIR GENETIC CORRELATIONS FOR GALL RUST DISEASE OF FALCATARIA MOLUCCANA

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ABSTRACT

A study to understand the degree of genetic factor influence on gall rust disease caused by Uromycladium falcatarium sp. nov, of eleven Falcataria moluccana seed sources from Indonesia and Malaysia, has these objectives, i.e.: (1) determine the heritability of gall rust disease severity particularly on stem and height of *F. moluccana* seedlings, (2) estimate genetic correlation between gall rust disease severity and height of *F. moluccana* seedlings. The preliminary study was conducted at the Laboratory of Forest Health and Protection of Faculty Forestry, Universitas Gadjah Mada, Yogyakarta, Indonesia, while preparing seedlings and artificial inoculation assessment which conducted at Sabah Softwood's Nursery and F.moluccana plantation, Tawau Malaysia. Randomized Complete Block Design (RCBD) with 3 blocks, 7 replications in each block and 4 seedlings as plot in each replication were set up for gall rust inoculation and the control for 11 seed sources (7 from Indonesia and 4 from Brumas, Tawau Malaysia) of *F. moluccana* seedlings. Gall rust disease severity was calculated using modified Chester's formula. Broad-sense heritability for gall rust disease severity and height were calculated using Baker formula, and covariance analysis of genetic correlations was computed by the formula given by Zobel and Talbert. Linear additive model was employed to estimate the components of variance for seed sources. The broad sense heritability of gall rust disease was moderate (0.42 - 0.47)for inoculated seedlings and 0.40 - 0.41 for control, but moderate to high for inoculated seedling (0.5 - 0.84) and for control (0.44 - 0.79). The genetic correlation between gall rust disease severity and height of seedling was unstable. high (0.85) and positive only at 27 days after inoculation. This finding indicates that the contribution of genetic and environmental factor to f gall rust disease severity on 11 seed sources F.moluccana seedlings were almost the same. Hence, it is also difficult to select the best seed source using height and gall rust disease severity characters concurrently.

Keywords: heritability, genetic correlation, Uromycladium falcatarium, Falcataria moluccana.

INTRODUCTION

Gall rust disease caused by *Uromycladium falcatarium* sp. nov., previously named *Uromycladium tepperianum* is one of the most significant diseases of *Falcataria moluccana*, particularly in some tropical and sub-tropical planted forests (Lee, 2004; Rahayu *et al.*, 2010; Doungsa-Ard *et al.*, 2015). The disease affects both

Submitted: February 02, 2020 Revised: May 05, 2020 Accepted for Publication: June 03, 2020 * Corresponding Author: Email: tatarahayu@yahoo.com © 2017 Pak. J. Phytopathol. All rights reserved. seedlings in the nursery and mature trees in the field. In matured trees, the fungus infects and causes development of chocolate brown, cauliflower-like or whip-like galls on stem, branch, petiole, shoot and pod (Rahayu *et al.*, 2010). Trees infected by gall rust become almost leafless as the galls on the branches absorb all the sap nutrients and block the movement of sap beyond the galls (Morris, 1997), have sparse crowns and produce fewer flowers and pods. On seedlings, the galls are commonly found near the base of stems, leaf stalk or shoot (Rahayu *et al.*, 2010). Infected seedlings usually lose their leaves and become stunted which eventually dying, and disease incidence of 90 to 100% have been reported in nurseries (De Guzman *et al.*, 1991). Furthermore, 20 days' infection of gall rust fungus on the stem, shoot and leaf stalk separately on the two months' age of seedlings were caused 82%, 70% and 73% mortality of the seedlings, respectively (Rahayu *et al.*, 2006). Generally, the rust fungus is more easily infects young tissue such as seedlings cells than matured or old cells of *F. moluccana* trees. The infected seedlings may be able to survive in the nursery; however, it will pass the rust inoculum in the field and develop the symptom quickly.

Heritability in the sense of breeding value is one of the most important and mostly used in quantitative genetics to determine the contribution of genetic factors of a certain trait. According to Zobel and Talbert (1984), heritability values express the proportion of variation in the population that is attributable to genetic differences among individuals. Most of the reported studies on heritability of gall rust disease on other tree species was showed moderate. For example, heritability estimates for infection by Cronartium quercuum f.sp. fusiforme on an individual seedling from 6 sources of slash pine (Pinus elliottii var. Elliottii and var. densa) ranged from 0.25 to 0.45 within P.e densa and from 0.20 to 0.59 within P.e. elliottii (Nelson et al., 1996). Another experiment on field susceptibility of Populus trichocarpa seedling and its hybrids against Melampsora occidentalis leaf rust showed that narrow-sense heritability increased within years (1988-1990) from moderate to high, with heritability value of 0.43, 0.53 and 0.79, respectively (Hsiang et al., 1993). In the other side, genetic correlations among traits plays a role in determining the degree to which indirect selection, or selection for one trait in the hopes of improving another trait, will be successful. For example, genetic correlation between crown destruction caused by leaf blight disease with height and diameter of a 8-year-old progeny trial of Eucalyptus urophylla in East Kalimantan Island were -5.3 and -0.20, respectively (Rahayu, 2001). It's indicates that crown destruction by leaf blight disease could cause greater reduction in tree height rather than in diameter. Other study reported that the genetic correlation between Blister-rust resistance and growth traits of 17 year-old Pinus strobes x P. peuce hybrid was found to be low and insignificant indicating no genetic relationship between these two characteristics (Blada, 2000).

In this article, we present results from a retrospective of gall rust disease test of *F. moluccana* seedlings from 11 seed sources. According to Sniezko and Koch (2017),

long period of time was needed in order to calculate the heritability value of a particular trait of the trees, such as *F. moluccana*, due to their biology aspect as a perennial plant. However, in this case we deal with rust fungus as bio-tropic agents, which persistently attacked until the host dead, so heritability evaluation could be calculated since the plant still young or seedling. In addition, the evaluation were conducted since the *F.moluccana* still on its seedling stage due to the rust fungus' ability to kill plants since the seedling period. The objectives of this research were to (1) determine the heritability of gall rust disease severity particularly on stem and height of *F. moluccana* seedlings, (2) estimate genetic correlation between gall rust disease severity and height of *F. moluccana* seedlings.

MATERIALS AND METHODS

Preliminary study in order to reproduce and purify the gall rust spore, also to ascertain the optimal spore density for artificial inoculation were conducted at the Laboratory of Forest health and protection of Faculty Forestry, Universitas Gadjah Mada, Yogyakarta, Indonesia. While artificial inoculation of gall rust disease on seedling from eleven F. moluccana seed sources were maintenance under 6-year-old F. moluccana at Sabah Softwood Bhd in Tawau, Sabah Malaysia.plantation as shade trees which were favourable for supporting gall rust disease development. The incidence and severity of gall rust disease of F. moluccana trees were 20% and 30% respectively. The site has 165 m a.s.l. with undulating topography with a gradient of 5° – 10° . The relative humidity up to 70% in the day and up to 95% at night; temperature ranged from 22 °C to 32°C; sunlight intensity ranged from 50 to 400 flux in the day, and wind speed was about 15 - 40 km/hour. Total research was conducted from March to November 2006.

Seedlings material: *F. moluccana* seedlings originated from 11 seed sources from Indonesia and Tawau, Sabah, Malaysia were evaluated. Detailed information of each seed source is presented in Table 1. The seed were initially treated with boiling water of 90° C for five minutes, followed by soaking them in tap water for 12 hours before they have sown. The seeds were sown directly to 8 x 8 x 12 cm polyethylene bags containing sand and topsoil in ratio of 3:7 (v/v). Seedlings were maintained in the nursery for 8 weeks. At 8 weeks seedlings with height ranged from 7 to 11 cm then used for artificial inoculation. **Fungal material:** Since the rust fungus *U. falcatarium* is an obligate parasite, spore multiplication have to be done using live plant material as host, in this case on *F.moluccana* seedlings. Mature teliospores of *U. falcatarium* from 4 years old of *F.moluccana* trees in SSB plantation were collected, then used for artificial inoculation on *F.moluccana* seedlings for multiplication. Thus the gall with mature teliospore appear from the inoculated seedlings were considered pure, having similar characteristic and supposed come from one race. Crude scrapings of galls from inoculated seedling were made to collect teliospores, then the teliospores were suspended in distilled water containing Tween 20 (0.2 ml/L). In order to maintain the amount of active teliospores on the seedlings, inoculation was repeated three times, once a day. Inoculum density of the first, second and third inoculation were 23.4×10^6 , 13.7×10^6 and 14×10^6 teliospores/mL, respectively. At each inoculation, a total of 5 ml inoculum was applied onto each seedling, with 3 ml dripped on the stems from the shoot tip using a measured pipette, and 2 ml sprayed for the entire seedling using spray bottle. Control seedlings were treated using the same procedure using distilled water as inoculum.

Table 1. List of F. moluccana seed sources used for artificial inoculation using gall rust fungus, Uromycladium

	falcatariun	n				
No	Seed source	Origin	Altitude (m a.s.l)	Latitude	Longitude	Seed lot number
1	Wamena	Papua	1500	4º01'S	138º31'E	A01 A02 A05 A09 A11 C03
2	Walang Gintang	East Flores	280	4º01'S	138º31'E	508 A 509 A 502 A 550 480 481 483 484 485 486
3	RO5	Brumas, Sabah	-	-	-	Mixed from Solomon Island and Sri Lanka
4	RO2	Brumas, Sabah	-	-	-	Imported from Indonesia
5	R2001	Brumas, Sabah	-	-	-	Mixed from the Philippines, Sri Lanka, Solomon Island and Indonesia
6	Morotai	North Moluccas	50	2º22'S	128º25'E	530 533 539 540 541 546 548 555 556 559
7	Kediri	East Java	200	7°49'S	112º01'E	429 430 432 438 439 440 441 443 445 446
8	Jasinga	West ava	325	6º29'S	106°27'E	039 040 041A 042 047 051 051A 057 058 061 063
9	East Timor	Timor	900	8°43'S	125°34'E	510 511 516 517 519 524 525 527 528 529
10	Ampel	Central Java	590	7º32'S	110º35'E	244 245 249 250 253 254 258 262 263 264
11	2S/75	Brumas, Sabah	-	-	-	Imported from Sri Lanka

Experimental Design: Randomized Complete Block Design (RCBD) with 3 blocks, 7 replications in each block and 4 seedlings as plot in each replication were set up for gall rust inoculation and the control separately. In order to prevent contamination of control seedlings by teliospores from the inoculated seedlings, the distance between the inoculated ones and control plots was 100 m.

Height of seedlings: The height of *F. moluccana* seedlings was recorded for three times, i.e. 7, 27 and 47 days after inoculation (DAI). Height of the seedling was measured using a ruler.

Evaluation of gall rust disease Severity: Gall rust disease symptom on the seedlings were vary, and

could appear on the shoot, leaf, leaf stalk and stem. However, according to Rahayu *et al* (2006), gall rust symptom in the stem were significantly correlated with the seedlings' reduced growth due stem is the most susceptible part for gall rust fungus infection. Therefore, the estimations made in this research were based on the scores in the stem. Index score were grouped into four categories as modified from Rahayu *et al* (2009) as shown in Table 2. According to Rahayu *et al* (2010), gall rust disease symptom on seedlings will appear at 6 to 7 days after inoculation (DAI), therefore the gall rust symptom in this research was recorded every 10 days, started from 7 DAI until 37 DAI.

Table 2. Index score for	gall rust symptoms o	n <i>F. mollucana</i> :	seedlings under shade trees
Tuble 21 mach beore for	gain rabe by impedinib o	n i i i intontacanta i	beeamigs anaer shaae trees

Index Score	Gall rust symptom on the stem
0	no gall rust symptoms
1	small necrotic spot, small pustule to light defect with malformation, size range from 1 mm to 5 cm
2	strong defect with malformation or galls, size ranged from 5 cm to above
3	Strong defect with malformation or galls, seedling started to dry up to dead due to gall rust disease

Based on the index score of gall rust symptom, gall rust disease severity (DS) was calculated using modified Chester's formula (1959) as follows:

Disease Severity (DS) = {[$(n_0 \times z_0) + (n_1 \times z_1) + ... + (n_3 \times z_3)$] / (N×Z)} × 100% ...[1]

where:

DS = Disease Severity

 n_0 , n_1 , n_2 , n_3 = number of seedlings with index score 0, 1, 2, 3

z₀, z₁, z₂, z₃ = index score 0, 1, 2, 3

N = Total number of seedlings in one plot (4), Z = the highest score (3)

The resistance category were grouped into five with respect to gall rust disease severity as shown in Table 3.

Table 3. Resistance category of *F. moluccana* seedlings to gall rust disease caused by *Uromycladium tepperianum* based on gall rust severity

Gall rust disease severity (%)	Category
> 0 to ≤ 10	highly resistant (HR)
$> 10 \text{ to } \le 30$	resistant (R)
$> 30 \text{ to } \le 70$	moderate (M)
$> 70 \text{ to } \le 90$	susceptible (S)
> 90 to \leq 100	highly susceptible (HS)

Genetic and statistical considerations with respect to calculated heritability and genetic correlation in growth and gall rust disease severity assessment

Broad-sense heritability (h_{B}^{2}) for gall rust disease severity and height of seedling were calculated with the following formula as proposed by Baker (1984):

$h^2B = 0$	$\sigma^2 G / \sigma^2 P$		[2]
where:			
σ^2 G =	$(\sigma^2_e + r \sigma^2_{BxSS} + r \sigma^2_{SS}) - (\sigma^2_e + r \sigma^2_{BxSS})$	s)/r	[3]
$\sigma^2 P =$	$(\sigma^2 G + \sigma^2 e)/r$		[4]
$h^2{}_B$	= Broad-sense Heritability,	σ^2 G	= Genotypic variance
$\sigma^{2}P$	= Phenotypic variance,	σ^{2}_{e}	= Environmental variance
$\sigma^{2}{}_{B}$	= Block variance,	σ^2 ss	= Seed Source variance
σ^2_{BXSS}	= Block X Seed Source Variance,	r	= no. of Replications

Expected genetic gain was strongly influenced by genetic correlation. Data from inoculated seedlings were used. So that actual response to gall rust fungus could be appropriately estimated. Covariance analysis of genetic correlations between gall rust disease score and height of seedling was computed by the formula given by Zobel and Talbert (1984).

$r_{G} (X, Y) = (\sigma^{2} SS(XY)) / (\sigma^{2} SS(X) + \sigma^{2} SS(Y))$	[5]
$\sigma^{2} ss(xy) = 0.5 (\sigma^{2} ss(x + y) - \sigma^{2} ss(x) - \sigma^{2} ss(y))$	[6]
$\sigma{}^{_2}{}_{SS(XY)}$ = covariance component of height and gall rust disease score	
σ^2 ss (x) = variance component of seed sources for gall rust disease severity	

 $\sigma^{2}_{SS(Y)}$ = variance component of seed source for character of height

 $\sigma^2 ss_{(X+Y)}$ = variance component of height and gall rust disease severity

STATISTICAL ANALYSIS

The following linear additive model was employed to estimate the components of variance for seed sources:

 $Y_{ij} = \mu + \tau_i + \beta_j + \tau \beta_{ij} + \epsilon_{ij}$ where: [7]

Y_{ij} = Disease severity or height mean of 4 seedling of seed sources *i* in block *j*.

- μ = The population means
- τ_i = The random variable effect for block *j*, *j* = 1, 2, 3
- β_i = The fixed effect for seed source, *i* = 1 s/d eleven i.e. Wamena, Walang Gintang, R05/95, R02/95, R02/2001, Morotai, Kediri, Jasinga, East Timor, Boyolali and 2S/75 seed sources
- $\tau\beta_{ij}$ = The random interaction seed sources by block

 ε_{ij} = The error term ~NID (0, σ^2_e ; where i = 1,2,3 block; j = 1,2,3, ..., eleven seed sources)

Assumptions:
$$\tau_i \sim \text{NI}(0,\sigma_t^2)$$
, $\beta_j \sim \text{NI}(0,\sigma_b^2)$, $\epsilon_{ij} \sim \text{NI}(0,\sigma^2)$

RESULTS

Based on artificial inoculation results, seedlings from all eleven seed sources had moderate resistance up to 27 days after inoculation (DAI) with disease severity ranged from 40% to 60%. After that, the disease severity of ten seed sources were continued to increase until ranged between 73% to 94% (susceptible and highly susceptible) at 37 DAI and 47 DAI, while seedlings from Wamena seed source was maintained on moderate resistance with disease severity ranged on 50% to 55%. It was shown that control seedlings were also got very small infection with < 4% disease severity (Figure 1). The disease severity either from inoculation and control result then will be used for calculating heritability and genetic correlation, as well as height of seedling performance in Figure 2.

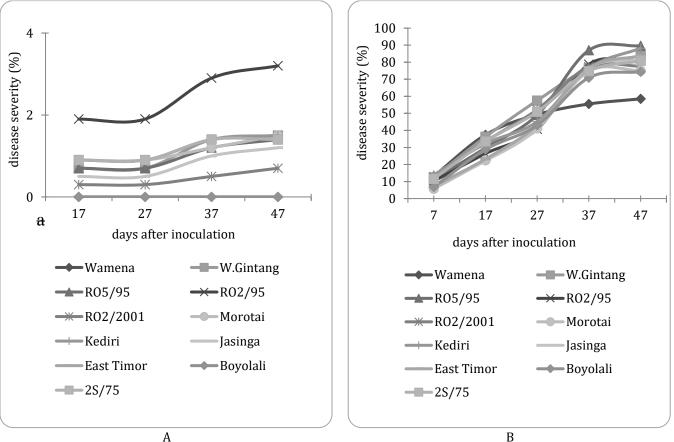


Figure 1. Disease Severity of *F. moluccana* seedlings from eleven seed sources (a) with inoculation (b) control at 7 to 47 days after inoculation

Generally, infection of rust disease will significantly reduce growth of the trees. However, at the seedling stage, the reduction was slight and may not significantly different such as observed at 7 until 47 DAI. However the height development of control seedling (b) were showed better than inoculated seedlings (a).

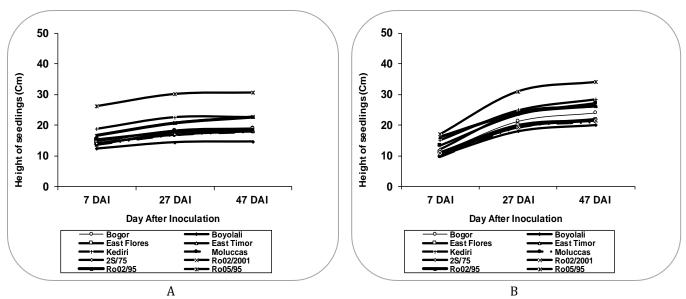


Figure 2. Height of *F. moluccana* seedlings from eleven seed sources (a) with inoculation and (b) control at 7 to 47 days after inoculation

Broad-sense heritability: Broad-sense heritability estimates for gall rust severity either on inoculated or control seedlings were almost the same and found moderately, ranging from 0.41 to 0.47 for the inoculated one and 0.40 to 0.41 for control seedlings

respectively (Table 3). Generally, broad-sense heritability estimated on inoculated seedlings was greater than those on the control, due to the rust infection will induce the expression of host genetic responses.

Table 3. Genotypic variances (σ^{2}_{G}), phenotypic variances (σ^{2}_{P}), and broad-sense heritability estimates (h^{2}_{B}) for gall rust severity of *F. moluccana* seedling from eleven seed sources

Day After		Inoculated			Control	
Inoculation	σ^{2} G	$\sigma^{2}P$	$h^{2}B$	σ^{2} G	$\sigma^{2}P$	$h^{2}B$
7	86.208	185.420	0.46	1.379	3.444	0.40
17	408.401	866.684	0.47	3.535	8.582	0.41
27	562.068	1323.734	0.42	3.746	9.179	0.41
37	703.517	1485.021	0.47	9.383	22.727	0.41
47	645.623	1396.135	0.46	11.483	28.631	0.40

Broad-sense heritability estimated for height of inoculated and the control seedlings ranged from moderate to high. The heritability on inoculated and control seedling at 7 days before inoculation was high, and become moderate after inoculation (Table 4). However, at 47 DAI, the increment value of genotypic and phenotypic variance of Table 4. Genotypic variances (σ ²_G), phenotypic variances inoculated seedlings were less than control. This condition expressed due to the stressed inoculated seedling as a result of the rust infection. Finally the broad-sense heritability estimated on control seedlings (0.44) were lower than those on inoculated seedlings (0.5), although those are still in the same moderate level.

Table 4.	Genotypic variances (σ^{2}_{G}),	phenotypic variances (σ 2 P), and	broad-sense ([h ² ^B] heritability	estimated for
	height of <i>F. moluccana</i> see	dlings from eleven seed	l sources			

Day of		Inoculated			Control	
Observation	σ^{2} G	$\sigma^{2}P$	$h^2{}_B$	σ^2 G	$\sigma^{2}{}_{P}$	h^2 B
7 DBI	12.383	14.653	0.84	9.343	11.763	0.79
7 DAI	19.545	27.507	0.71	18.473	28.303	0.65
27 DAI	21.497	31.287	0.69	23.207	35.097	0.66
47 DAI	31.460	62.84	0.50	121.660	279.790	0.44

Notes: DBI = day before inoculation, DAI= day after inoculation

Genetic correlations between gall rust disease severity and height of seedlings at 7 DAI and 47 DAI were negative and

ranged from low to moderate. However, genetic correlations at 27 DAI were positive and high as shown in Table 5.

Day after inoculation	Component Variants		Component covariance severity- height	Genetic Correlation
(DAI)	σ ² severity	σ ² height		(r _G)
7 DAI	1.436	15.040	-8.044	-0.37
27 DAI	-0.296	17.715	-4.479	0.85
47 DAI	43.960	37.220	-41.124	-0.02

Table 5. Genetic correlation between gall rust disease severities caused by *Uromycladium falcatarium* and height of seedlings from eleven *F. moluccana* seed sources

DISCUSSION

According Falconer (1989), broad-sense heritability includes all genetic influences on variation, including additive, dominance, epistasis, and genetic–environmental interaction effects. The moderate heritability estimates of gall rust disease severity indicated that the effect of genetic contribution (0.42 - 0.47) was almost the same with environmental contribution (0.53 - 0.58) to gall rust disease severity trait. The gall rust infection was only able to increase from 1 to 5% of the genetic expression to gall rust disease when compared to the control or un-inoculated seedling. This means that gall rust fungus infection on seedlings only able to induce small expression on gall rust resistant.

Other studies on other rust diseases show that the broadsense heritability estimates were also commonly moderate. According to Nelson *et al.* (1996), heritability estimates for infection of *Cronartium quercuum* f.sp. *fusiforme* on an individual seedling from 6 sources of slash pine (*Pinus elliottii* var. *Elliottii* and var. *densa*) ranged from 0 to 0.45 within *P.e densa* and from 0.20 to 0.59 within *P.e. elliottii*.

The same principle works on the characteristics of gall rust disease severity, gall rust infection of *F. moluccana* seedlings was only able to increase 3 to 6% of the genetic expression of height characteristic. However, the broad-sense heritability for height characteristic ranged from moderate to high (50% to 84%). This means that the contribution of genetic factor of the seed sources to height character was about 50% to 84%, while 16 to 50% were contributed by environmental factors. Thus, superior seedlings will only perform better in the suitable environmental conditions, including good soil fertility, moderate relative humidity (70% \ge RH \le 80%), low altitude, etc (Rahayu *et al.*, 2018). According to Rahayu *et al.* (2009), Wamena seed sources, was found to be the best seed source in relation to gall rust disease resistance, based on artificial inoculation under field conditions.

Genetic correlations between gall rust disease severity and height of seedlings in 7 DAI and 47 DAI were found to be negative and low, i.e. 37% and 2%, respectively. However, genetic correlation in 27 DAI was found to be positive and high (85%). This finding indicates that most genetic correlation between characters of height and response to gall rust fungus has occurred in 27 DAI. Seedlings from RO5 showed highest both on height and gall rust disease severity. Genetic correlations among traits are useful and of interest to tree breeders due to they indicate the degree in which a particular trait will change as a result of a change in another trait (Zobel and Talbert, 1984). In addition, those traits also play a role in determining the degree to which indirect selection, or selection for that particular, will be successful. For example, genetic correlation between crown destruction caused by leaf blight disease with height and diameter of 8year-old progeny trial of Eucalyptus urophylla in East Kalimantan Island were - 5.3 and -0.20, respectively (Rahayu, 2001). This indicated that crown destruction by leaf blight disease could cause reduction in tree height rather than in diameter. Similar result reported that the genetic correlation between Blister-rust resistance and growth traits of 17 years old Pinus strobes x P.peuce hybrid at age 17 was low and insignificant. The study strongly suggested that there were no genetic relationship between disease and growth traits. The lower or no correlation indicated difficulty in improving these blister rust resistance and growth simultaneously (Blada, 2000).

This study discovers that taller seedlings normally could have higher gall rust severity suggesting that subsequent concurrent improvement in height and gall rust disease resistance will be difficult to conduct. However, due to the most genetic correlation between characters of height and response to gall rust fungus has occurred in 27 DAI, consequently, under infected nursery, selections the highquality of seedlings have to conduct since 27 days after seedlings placed in the nursery. It is also indicated that the correlation genetic between character of height and responds to gall rust disease were not stable, and cannot use for selection for two those characters.

CONCLUSIONS

The severity of seedlings from eleven *F. moluccana* seed sources against gall rust disease were approximately contributed by 41- 46% of genetic factor and 54 – 59% environmental factors respectively. However, the height character of seedling was approximately accounted by 50-

84% of genetic factor and 16-50% of environmental factors. The genetic correlation between gall rust disease severity and height of seedling was high (85%) and positive only at 27 days after inoculation. Cconsequently, under infected nursery, selections the high-quality of seedlings have to conduct since 27 days old seedlings.

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