Appendix B. – Full correlation matrix of yearly cumulative cone yield (CCY), with standard errors in parentheses from the pair-wise analysis. Genetic and phenotypic correlations are given in the upper and lower diagonals, respectively.

Age	11	12	13	15	16	17	18	19	20	21	22	23	24	25	30
11		1.00	0.99	0.98	0.99	0.98	1.00	0.95	0.97	0.95	0.92	0.91	0.88	0.81	0.81
		(0.00)	(0.06)	(0.07)	(0.00)	(0.00)	(0.09)	(0.06)	(0.09)	(0.09)	(0.10)	(0.11)	(0.12)	(0.14)	(0.14)
12	0.80		0.98	0.90	0.93	0.89	0.87	0.84	0.86	0.83	0.79	0.77	0.74	0.70	0.68
	(0.01)		(0.02)	(0.05)	(0.05)	(0.04)	(0.07)	(0.08)	(0.07)	(0.08)	(0.10)	(0.10)	(0.11)	(0.12)	(0.13)
13	0.54	0.86		0.94	0.93	0.93	0.93	0.91	0.91	0.87	0.84	0.81	0.76	0.73	0.70
	(0.03)	(0.01)		(0.02)	(0.03)	(0.03)	(0.03)	(0.04)	(0.04)	(0.05)	(0.03)	(0.08)	(0.09)	(0.10)	(0.11)
15	0.50	0.74	0.91		0.98	0.99	0.98	0.98	0.99	0.96	0.96	0.94	0.93	0.90	0.89
	(0.03)	(0.02)	(0.01)		(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.02)	(0.03)	(0.02)	(0.04)	(0.05)	(0.05)
16	0.42	0.71	0.88	0.97		0.98	0.97	0.96	0.98	0.97	0.96	0.95	0.94	0.93	0.92
	(0.04	(0.03	(0.02	(0.00)		(0.01)	(0.01)	(0.02)	(0.01)	(0.02)	(0.02)	(0.03)	(0.00)	(0.04)	(0.04)
17	0.35	0.65	0.88	0.96	0.98		0.99	0.99	0.99	0.97	0.96	0.95	0.94	0.92	0.91
	(0.05)	(0.04)	(0.02)	(0.01)	(0.00)		(0.00)	(0.01)	(0.01)	(0.02)	(0.02)	(0.03)	(0.03)	(0.02)	(0.04)
18	0.34	0.63	0.87	0.95	0.97	1.00		1.00	1.00	0.98	0.97	0.96	0.96	0.93	0.94
	(0.05	(0.04	(0.02	(0.01)	(0.01)	(0.00)		(0.00)	(0.01)	(0.02)	(0.02)	(0.03)	(0.03)	(0.04)	(0.03)
19	0.35	0.60	0.83	0.93	0.95	0.98	0.99		1.00	0.98	0.98	0.96	0.95	0.94	0.92
	(0.05)	(0.04)	(0.02)	(0.01)	(0.01)	(0.00)	(0.00)		(0.00)	(0.01)	(0.01)	(0.02)	(0.02)	(0.03)	(0.03)
20	0.37	0.61	0.82	0.92	0.95	0.97	0.98	1.00		0.99	0.98	0.97	0.96	0.95	0.93
	(0.05)	(0.04)	(0.03)	(0.01)	(0.01)	(0.01)	(0.00)	(0.00)		(0.01)	(0.01)	(0.01)	(0.02)	(0.02)	(0.03)
21	0.36	0.58	0.79	0.88	0.93	0.95	0.96	0.98	0.99		0.99	0.99	0.99	0.98	0.97
	(0.05)	(0.05)	(0.03)	(0.02)	(0.01)	(0.01)	(0.01)	(0.00)	(0.00		(0.00)	(0.01)	(0.01)	(0.01)	(0.02)
22	0.34	0.55	0.75	0.86	0.91	0.93	0.94	0.97	0.97	0.99		1.00	1.00	0.99	0.99
	(0.05)	(0.05)	(0.04)	(0.02)	(0.02)	(0.01)	(0.01)	(0.01)	(0.01	(0.00)		(0.00)	(0.00)	(0.00)	(0.01)
23	0.34	0.55	0.73	0.83	0.90	0.91	0.95	0.95	0.96	0.98	1.00		1.00	1.00	0.99
	(0.05)	(0.05)	(0.04)	(0.02)	(0.02)	(0.02)	(0.01)	(0.01)	(0.01)	(0.00	(0.00		(0.00)	(0.00)	(0.00)
24	0.33	0.53	0.71	0.82	0.94	0.89	0.90	0.95	0.95	0.98	0.99	1.00		1.00	0.99
	(0.06)	(0.06)	(0.05)	(0.03)	(0.02)	(0.02)	(0.02)	(0.01)	(0.01)	(0.00)	(0.00	(0.00)		(0.00)	(0.00)
25	0.31	0.51	0.68	0.80	0.86	0.87	0.88	0.92	0.93	0.96	0.98	0.99	1.00		1.00
	(0.06)	(0.06)	(0.05)	(0.03)	(0.03)	(0.03)	(0.02)	(0.02)	(0.02)	(0.01)	(0.00)	(0.00)	(0.00)		(0.00)
30	0.29	0.46	0.64	0.76	0.83	0.85	0.86	0.90	0.91	0.95	0.97	0.98	0.99	0.99	
	(0.06)	(0.06)	(0.05)	(0.04)	(0.03)	(0.03)	(0.03)	(0.02)	(0.02)	(0.01)	(0.01)	(0.00)	(0.00)	(0.00)	

Responses of *Falcataria moluccana* seedlings of Different Seed Sources to Inoculation With *Uromycladium tepperianum*

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Abstract

Falcataria moluccana (batai) is one of the valuable multipurpose tree species for forest plantations, particularly in Malaysia and Indonesia. Gall rust disease caused by *Uromycladium tepperianum* (Sacc.) is one of the most destructive diseases in Batai plantations. The disease causes severe damage at all developmental stages of the plant from the nursery stage to mature trees in the field and includes the development of chocolate brown, cauliflower-like or whip-like galls on the stem, branch, petiole, shoot and pod. Different seed sources may respond differently to gall rust fungus. Thus, the responses of *F. moluccana* seedlings from 6 selected seed sources to gall rust disease caused by *U. tepperianum*, were evaluated, at the Brumas Estate, Malaysia, in terms of gall rust disease severity, mortality, and disease infection rate of the seedlings. Based on disease severity, infection rate and cumulative mortality due to gall rust disease, the wamena was found to be the best seed source in relation to gall rust disease resistance.

Key words: Falcataria moluccana, seed sources, *Uromycladium tepperianum*, artificial inoculation, resistance.

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Introduction

Falcataria moluccana ((Miq.) Barneby and J.W. Grimes (batai)), of the family Fabaceae (alt. Leguminosae), is a fast growing tree frequently planted in plantations forests in the humid tropics (WAGNER *et al.,* 1999). This species is best known as a pulp crop (NAS, 1979), but is also used for fiber and particleboard, packing cases, boxes, matches, chopsticks, light furniture, lightweight veneer and plywood and composite products (CABI, 2000). Since the wood is fairly easy to cut, *F. moluccana* is also suitable for wooden shoes, musical instruments, toys and novelties and also general turnery (PEH and KHOO, 1984). The species has also been used as shade trees to coffee, tea and cacao plants and cattle because of its favorable thin crown. According to SCHUBERT (1985), it is also used as windbreakers for banana plants in Hawaii. Batai is also grown as an ornamental tree, although it seldom survives for more than 50 years (BUDELMAN, 1989).

F. moluccana occurs naturally in Indonesia (Moluccas and Irian Jaya islands), Papua New Guinea, New Britain and the Solomon Islands, ranging from 10° S to 30° N (WAGNER *et al.,* 1999; RICHTER and DALLWITZ, 2000). It is an exotic tree species planted in Brunei, Cambodia, Cameroon, Cook Islands, Fiji, French Polynesia, Japan, Kiribati, Laos, Malaysia, Marshall Islands, Myanmar, New Caledonia, Norfolk Island, Philippines, Samoa, Thailand, Tonga, United States of America, Vanuatu and Vietnam (WAC, 2005). Gall rust disease caused by *Uromycladium tepperianum* (Sacc.) (RAHAYU *et al.,* 2005) is one of the most destructive diseases in these plantations in some parts of the South East Asian region, such as in the Philippines (Braza, 1997), Sabah in Malaysia (LEE, 2004) and some islands in Indonesia (RAHAYU, 2007). *U. tepperianum* is a microcyclic rust that infects more than a hundred host species, *U. tepperianum* also infects *Acacia* spp.. in Australia and New Zealand (OLD *et al.,* 2000), but it has never been reported to have infected *Acacia* spp. in Malaysia. However, in Indonesia, the fungus had been found to infect old *Acacia decurrens* trees on the Ijen plateau in East of Java, at about 2100 m above sea level (SRI RAHAYU, 2007, unpublished data).

In the entire life cycle of the fungus, *U. tepperianum* (Sacc.) McAlp. it produces only one single spore type, namely teliospore. Under nursery conditions, teliospores of the rust fungus cannot themselves infect the host, they first have to germinate to produce basidiospores (DICK, 1985; RAHAYU, 2007). The *Uromycladium* fungi are autoecious, only requiring one host to complete their life cycle (MORRIS, 1987).

The disease causes severe damage at all developmental stages of the plant from the nursery stage to mature trees in the field and this involves the development of chocolate brown, cauliflower-like or whip-like galls on the stem, branch, petiole, shoot and pod. Affected plant parts and severely infected *F. moluccana* trees die prematurely (CRISTOVAO and OLD, 2003). On the other hand, due to its host specificity, the gall-forming rust fungus *U. tepperianum* from Australia was selected as a suitable biological agent for controlling an invasive tree *Acacia saligna* in the Western Cape Province of Africa and was able to decrease tree density by at least 80% at all sites in 1995 (MORRIS, 1997). Experience with rust diseases of other tree genera elsewhere, shows that there may be major differences in resistance by species, provenance, family and seed source levels. However, the impact of the disease has not previously been quantitatively assessed in different seed sources of *F. moluccana* seedlings. In this paper the responses *F. moluccana* seedlings, from different seed sources, to gall rust disease caused by *U. tepperianum*, encompassing gall rust disease severity, mortality and infection rate of the seedlings were evaluated in Tawau, Sabah, Malaysia.

Materials and Methods

Seedlings material

F. moluccana seedlings originating from six seed sources were evaluated. Detailed information of each

Table 1. – List of *F. moluccana* seed sources used to screen for resistance of gall rust.

Seed source	Origin	Altitude (m a.s.l)	Lattitude	Longitude	Seedlot number
Wamena	Papua	1500-2000	4º01'S	138º31'E	A01 A02 A05 A09 A11 C03
Walang Gintang	East Flores	280	8º39'S	122º18'E	508 A 509 A 502 A 550 480 481 483 484 485 486
Morotai	North Moluccas	50	2º22'S	128º25'E	530 533 539 540 541 546 548 555 556 559
Kediri	Central of Java	200	7º49'S	112º01'E	429 430 432 438 439 440 441 443 445 446
East Timor	Timor	900	8º43'S	125º34'E	510 511 516 517 519 524 525 527 528 529
2S/75	Brumas, Sabah	-	-	-	Imported from Sri Lanka

Note: - = no data

seed source is presented in *Table 1*. The seed lot for each number comes from one tree with no outcrossing between each individual tree. The seeds were initially treated with boiling water at 90°C for five minutes, followed by soaking them in tap water for 12 hours before they were sown. The seeds were sown directly in 8 x 8 x 12 cm polyethylene bags containing sand and topsoil in ratios of 3:7 (v/v). The seedlings were maintained in the nursery for 6 weeks. At 6 weeks seedlings with height ranging from 7 to 11 cm, were moved to the field site.

Fungal material

General rust fungus easily forms pathogenic races. However, there is no information on the pathogenic races of *U. tepperianum* found on *F. moluccana* up to now. Spores for inoculum were obtained from galls of infected *F. moluccana* seedlings originating from a single seed source in the nursery. Accordingly the inoculum is expected to be from one race. Crude scrapings of galls were made to collect teliospores which were subsequently suspended in distilled water containing Tween 20 (0.2 ml/l).

Inoculation

Six week-old healthy seedlings were used as experimental units. Inoculum density of the first, second and third inoculation were 23.4×10^6, 3.7×10^6 and 14×10^6 teliospores/ml, respectively. For 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 on the entire seedling using a spray bottle. Control seedlings were treated using the same procedure using distilled water as inoculum. All seedlings of this study were maintained in the nursery and watered twice a day, up to field capacity.

Experimental Design

A Randomized Complete Block Design (RCBD) with 3 blocks, 7 replications in each block and 4 unit samples in each replication were set up separately for the gall rust inoculation and the control. In order to prevent contamination of control seedlings by teliospores from the inoculated seedlings, the distance between the inoculated and control plots was at least 100 m. The plant part observed was the stem of the seedlings. The inoculated treatment consisted of 3 blocks x 7 replications x 4 unit samples x 6 seed sources = 504 seedlings as unit observations, as well as for the control treatment.

Severity, mortality and infection rate

Symptoms exhibited by gall rust on seedling vary in different plant tissue and could appear on the shoot, leaf stalk and stem. The scores for gall rust disease were based on estimations made on the stem since earlier findings indicated that the stem is the most susceptible to it (RAHAYU *et al.,* 2006). In addition, based on the size of gall rust and seedling malformation due to fungus infection, gall rust indices scores were grouped into four categories as presented in *Table 2*. A preliminary study showed that full symptoms of gall rust developed within 6 to 7 days after inoculation (DAI), therefore the scores for gall rust disease was initially recorded every 10 days for 7, 17, 27 and 37 days.

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

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

where:

DS = Disease Severity

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

z_0, z_1, z_2, z_3 = index score 0, 1, 2, 3.

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

The infection rate of gall rust (r) per unit time (t) was calculated using the following formula as suggested by KURT (2002).

$$r = 1/ (t_2 - t_1) \times [(\ln(x_2/1-x_2)) - (\ln(x_1/1 - x_1))]$$

where:

t_1 and t_2 = the dates on which the disease severity measurements were made

x_1 and x_2 = disease severity (%) recorded on these dates

Based on the assumptions about gall rust disease resistance of *F. moluccana* seedlings of polygenic inheritance, the typical mortality curve can be used to differentiate seed sources with potential resistance. Gall rust symptoms with a score of 3, noted as dead seedlings, was calculated for 7 to 37 DAI. The percentage of dead seedlings at each observation period was considered as the cumulative seedling mortality. Based on gall rust disease severity and mortality, the response of *F. moluccana* seedlings to gall rust disease was grouped into four categories as presented in *Table 3*.

Table 2. – Index score for gall rust symptoms on *F. mollucana* seedlings under shade 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

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

Gall rust disease severity and or mortality (%)	Category
> 0 to ≤ 20	Highly resistant (HR)
> 20 to ≤ 40	Resistant (R)
> 40 to ≤ 60	Moderate (M)
> 60 to ≤ 80	Susceptible (S)
> 80 to ≤ 100	Highly susceptible (HS)

Table 4. – Key-outs for ANOVA on gall rust disease severity and mortality of *F. moluccana* seedlings.

Source of variation	d.f.	mean squares	E(MS)
Block (B)	b - 1	MS_B	$\sigma^2_e + \sigma^2_B + r\sigma^2_{SS} + r\sigma^2_{B \times SS}$
Seed Sources (SS)	ss - 1	MS_{SS}	$\sigma^2_e + r\sigma^2_{SS} + r\sigma^2_{B \times SS}$
B X SS	(b-1)(ss-1)	$MS_{B \times SS}$	$\sigma^2_e + r\sigma^2_{B \times SS}$
Error	b(ss-1)	MS_E	σ^2_e

According to Stubbs *et al.* (1986) and Zadoks and Schein (1979), the logarithmic function $(\ln(x/(1 - x))$, which is termed as the logit of x, the development of gall rust disease symptom on each seed source can be plotted as a straight line as shown in the following :

$$y = a + bx$$

where:
y = logit of the amount of gall rust infection
a = gall rust severity at first observation
b = the slope or the disease rate (r)
x = the number of days after the first observation.

Statistical Analysis

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

$$Y_{ij} = \mu + \tau_i + \beta_j + \tau\beta_{ij} + \varepsilon_{ij}$$

where:
Y_{ij} = Disease severity, cumulative mortality, height, or relative growth rate mean of 4 seedlings of seed sources *i* in block *j*.
μ = The population mean
τ_i = The random variable effect for block *j*, j = 1, 2, 3
β_j = The fixed effect for seed source, *i* = 1 s/d six i.e. Wamena, Walang Gintang, Morotai, Kediri, East Timor and 2S/75 seed sources
$\tau\beta_{ij}$ = The random interaction of seed sources by block
ε_{ij} = The error term ~NID (0, σ^2_e; where i = 1, 2, 3 block; j = 1, 2, 3, ... , six seed sources)
Assumptions: $\tau_i \sim NI (0, \sigma^2_t)$, $\beta_j \sim NI (0, \beta_b)$, $\varepsilon_{ij} \sim NI (0, \sigma^2)$

Data were subjected to two way analyses of variance (ANOVA) which was carried out separately for the inoculated and control seedlings. The ANOVA table key-outs with the expected means squares (EMS) used for each treatment are as given in *Table 4*. The analysis utilized the PROC GLM test to measure the significance of these random effects (SAS Institute, 2004).

Results

Severity, Mortality and Infection rate

Summary of the results of the analysis of variance for disease severity, showed that effect of blocks and also seed sources were not significant. However interaction between blocks and seed sources was significantly from 7 DAI to 37 DAI. This finding indicates that cumulative variation within blocks and seed sources was large and that subsequent block conditions particularly the microclimate surrounding the individual seedlings have a great effect on gall rust disease severity. The mean performances of the *F. moluccana* seedlings from the six seed sources for gall rust disease severity are presented in *Fig. 1*. The wamena seed source was found to be the only seed source which showed resistant (R).
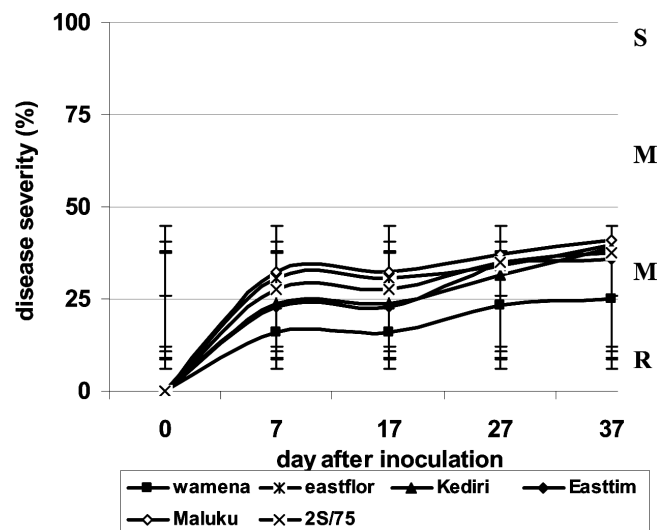

Figure 1. – Mean performance of gall rust disease severity in six different seed sources of *Falcataria moluccana*, inoculated with *U. tepperianum* teliospores, at 7, 17, 27, and 37 DAI, with different categories: S = susceptible (≥75%–100%), M = moderate (≥25%–74%), R = resistant (0%–25%), vertical bars represent standard deviation; bars in the same range are not significantly different at P ≤ 0.05.
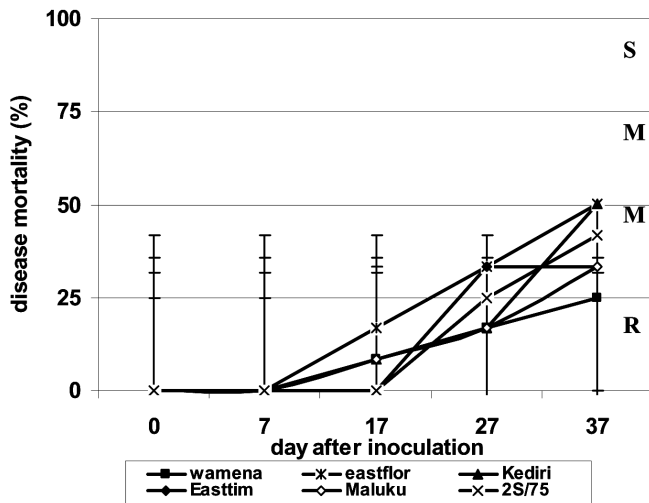
Figure 2. – Mean performance of gall rust disease mortality in six different seed sources of *Falcataria moluccana*, inoculated with *U. tepperianum* teliospores, at 7, 17, 27, and 37 DAI, with different categories: S = susceptible (>75–100%), M = moderate (>25–≤75), R = resistant (0–25%), vertical bars represent standard deviation; bars in the same range are not significantly different at P ≤ 0.05.
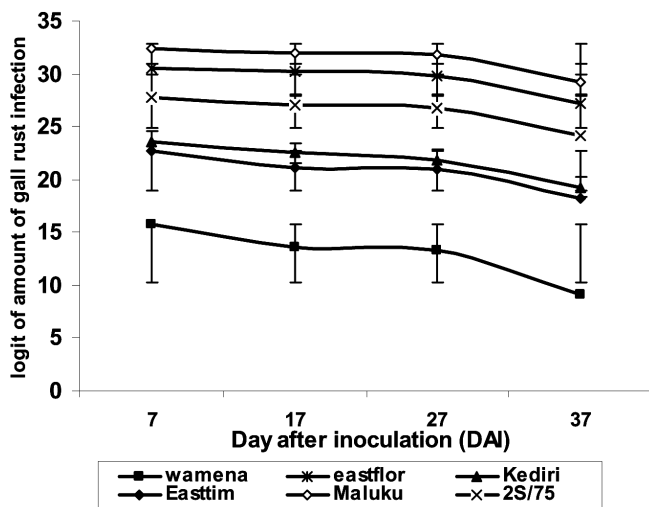

Figure 3. – Logit plot of the gall rust infection rate of *F. moluccana* seedlings from six seed sources at 7 to 37 days after inoculation, vertical bars represent standard deviation; bars in the same range are not significantly different at P ≤ 0.05.

The analysis of variance for cumulative mortality showed that effect of blocks, seed sources and interaction between blocks and seed sources were not significant. However, cumulative mortality of seedlings originating from the wamena seed source showed resistance from 7 to 37 DAI, while the others showed moderate resistance beyond 37 DAI (*Fig. 2*).

Gall rust infection rate (r) from 1 to 37 DAI presented as the logit plot of gall rust infection (*Fig. 3*), shows that seedlings from the wamena seed source had the lowest infection rate, significantly different from the others. Seedlings from the other seed sources showed higher infection rates, with seedlings from the Maluku seed source displaying the highest rate of infection.

Discussion

Gall rust disease caused by *Uromycladium tepperianum* is a major threat to *F. moluccana* plantations in Sabah Malaysia. Affected seedlings usually lose their leaves and become stunted, eventually dying with disease incidence in the nurseries at 90 to 100 percent (DE GUZMAN *et al.,* 1991). Further, 20 days infection of gall rust fungus, separately on the stem, shoot and leaf stalk, on two month old seedlings caused 82%, 70% and 73% mortality, respectively (RAHAYU *et al.,* 2006). This may relate to the abnormal physiological process of the seedlings with galls. Abnormalities are generally caused by either hypertrophy or hyperplasia or both. Hypertrophy is defined as an abnormal over-development due to an increase in cell size whereas hyperplasia is the increase in cell number due to the disease producing agent (TOOTHILL, 1984). In addition, mycelium of the rust fungus is typically perennial in the bark or wood of the host tree. This resident mycelium causes various host malformations, including bending of stem or shoot due to hypertrophy or hyperplasia on the meristematic cells (RAHAYU, 2007). Further gall formation also leads to stoppage of water movement in the xylem (EDMONDS *et al.,* 2000).

Generally, resistance to rust fungus is polygenic, and the characteristics of the mortality can be used to differentiate the potentially useful levels of resistance from susceptible seed sources (STINE, 1999). However, this study found no significant differences in gall rust disease severity and mortality in seedlings from the 6 *F. moluccana* seed sources treated. This evidence may be related to the infection mode of gall rust fungus caused by *U. tepperianum* on *F. moluccana*. According to RAHAYU (2007), basidiospores of *U. tepperianum* are capable of direct penetration through the epidermis by formation of a penetration peg. This is followed by an enzymatic process infection and invasion of the gall rust fungus in the host cells. Thus, all the seedlings from the six seed sources were infected by the rust fungus, although, subsequent fungus development in the cell hosts differs for each seed source. In addition, given that the cell wall is the first line of defense against rust infection, it is not surprising that the plants evolve means to perceive and respond defensively forming galls (MELLERSH and HEATH, 2001). Since the stems of the seedlings of all the seed sources became infected in this experiment, it is apparent that the seedlings in this study lack mechanisms of resistance that eliminate infection. Consequently, it is unlikely that seedlings with a high degree of resistance can be found, meaning that no seedlings will be free from *U. tepperianum* infection.

Based on resistance category, only the wamena seed source showed resistance up to 37 DAI, while the other seed sources displayed moderate resistance. In addition, the gall rust infection rate of the seedlings from the wamena seed source at 1 to 37 DAI was found to be the lowest and significantly different from the other seed sources. In their natural distribution, *F. moluccana* trees in Wamena grow in groups around the Wamena village at about 2000 m above sea level. In that area,

fogs are frequent and when clouds blow through the foliage, nutrients may be 'fog-stripped' leaching them out unless the plants have protective impermeable cuticles. Fog is one of the most significant factors supporting gall rust disease development in *F. moluccana* (RAHAYU, 2007). Due to the seed source from Wamena having adapted to foggy conditions, their anatomical and morphological properties such as impermeable cuticles and intracellular modification are thus more resistant to gall rust fungus infections. In addition, based on histopathological observations, there was also evidence of hypersensitive reaction inside infected stem cells of seedlings from the wamena seed source. Cells, particularly xylem cells with houstorial cells die swiftly as in the seedlings cells from wamena, while in other seed sources, houstorial cells developed rapidly and occupied all the xylem cells. According to GOODMAN and NOVACKY (1994) and LEVINE *et al.* (1994), hypersensitivity is an effective defence mechanism against biotrophs and non-biotrophs alike because of the accompanying upregulation of a multitude of defense genes that produce a highly antimicrobial environment in and around the dead cells. Further intensive research on the hypersensitive reactions of some provenances, progenies or seed sources of *F. moluccana* is needed in order to ascertain the materials resistant to gall rust fungus.

There is a correlation between the result of artificial inoculation and the performance of the same materials in the field (POWERS *et al.,* 1982). Highly susceptible materials in the greenhouse were also highly susceptible in field plantings. The problem usually involves families rated as moderate in resistance. This seems to be particularly true for loblolly pine. It was found that some loblolly pine families which appeared to be relatively susceptible to fusiform rust in inoculation tests were moderately resistant under field conditions. Nevertheless the reverse was not found to be true. Those conditions may be due to the seedlings in the green house being subjected to strong treatments in order to stimulate the disease. On the other hand, under field conditions, varying factors occur which either stimulate or inhibit disease development. Thus, in the case of gall rust disease screening, there is a good chance that the seedlings from seed sources which demonstrated resistance (wamena) in artificial inoculation may also show resistance in the field. Since wamena was found to be the most promising resistant seed source in this test, further tests should be done with regards to its resistance to gall rust disease in the field.

Conclusions

Based on artificial inoculation under field conditions on *F. moluccana* seedlings, wamena was found to be the best seed source in relation to gall rust disease resistance. Further tests should be done with regards to its resistance to gall rust disease in the field.

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Characterization of *Juglans nigra* (L.), *Juglans regia* (L.) and *Juglans* x *intermedia* (Carr.) by SSR markers: a case study in Italy

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Abstract

Juglans nigra and *Juglans regia* are economically important species in Europe, Asia and North America. Natural hybrids between the two species, known as *Juglans* x *intermedia* (Carr), are valued for timber production. We tested ten nuclear microsatellite markers to (1) identify new *J*. x *intermedia* hybrids and characterize their parentage species *J. regia* and *J. nigra* (2) detect *J. nigra* genotypes with a spontaneous crossing ability with *J. regia* in a mixed Italian population. This study was also designed to confirm the transferability of ten black walnut SSR loci to Persian walnut All ten microsatellites amplified in both species, producing fragments of variable size; eight (7.14%) were common, 68 (60.7%) amplified in *J. nigra* and 36 (32.1%) in *J. regia* only (private alleles). Indices of genetic diversity revealed high level of variability. The Principal Coordinate Analysis on the basis of total 112 alleles divided the total sample set into three main groups: *J. nigra*, *J. regia* and *J*. x *intermedia* hybrids. Performing the microsatellite fingerprinting, a triploid hybrid plant with two genome parts of *J. nigra* and one part of *J. regia* was identified. The cytological analysis proved this triploid state showing 48 somatic chromosomes. The mother testing analysis of the 7 diploid hybrids by exclusion method indicated one putative hybridogenic mother plants. The sequence analysis of amplified fragments confirmed the cross-species amplification of SSR. Inter-specific differences between alleles were due not only to simple changes in the number of repeats but also to mutations in the flanking regions.

Key words: Juglans, interspecific hybrids, microsatellites, cross-species amplification, sequencing.

Introduction

Juglans nigra (Eastern black walnut) and *Juglans regia* (common or Persian walnut) are highly economically important species in Europe, Asia and North America. The Persian walnut, an indigenous species in Eurasia from the Balkans to southwest China, is cultivated throughout the temperate regions of the world for its high quality wood and edible nuts. Black walnut, native to the Eastern part of North America, is a fast growing species with a dark-colored wood used in the manufacture of furniture and other wood products. Beginning in the 17th century, *J. nigra* was imported from the Eastern and Central hardwood forests of the United States to the European continent for ornamental purposes, and subsequently for its rapid growth. In Italy the black walnut is usually found in private and public parks of Pianura Padana where is also used for reforestation and recovering degraded areas (FENAROLI, 1975). Both

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