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Diversity of *Ganoderma* pathogen in Pontianak, West Kalimantan: Characteristics, virulence and ability to infect *Acacia mangium* seedlings

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Abstract. *Suryantini R, Wulandari RS. 2018. Diversity of* Ganoderma *pathogen in Pontianak, West Kalimantan: Characteristics, virulence and ability to infect* Acacia mangium *seedlings. Biodiversitas 19: 465-471.* The study aimed to determine morphological characteristics and virulence of *Ganoderma* isolates. The method that was used: isolation and characterization isolate from *Acacia mangium*, palm oil (*Elaeis guineensis*) and rubber *(Hevea brasiliensis*); inoculation of isolate in *A. mangium*; its influence to seedling dry weight. Results showed that isolated from *A. mangium* is *G. lucidum*, from palm oil is *G. boninense* and isolated from rubber plant is *G. applanatum*. Symptoms were observed within 3 months after inoculation. Symptoms began with chlorosis, necrosis and then seedling death. The *G. lucidum* is of highest virulent (2.08) compare to *G. boninense* (1.42). Whereas the one which isolated from rubber plant is moderately virulent (0.92). *Ganoderma* infection was indicated by decreasing the dry weight of infected seedlings. Difference type of isolates did not significantly effect to the decreasing of seedling dry weight 3.82 g (inoculated by *G. lucidum*), 4.01 g (inoculated by *G. boninense*), 5.02 g (inoculated by *G. applanatum*). These results showed that these isolates (especially *G. lucidum*-like) are species to watch out as for *Ganoderma* root rot pathogen. The presence of perennials such as palm oil and infected rubber, can be a potential source of inoculum for *A. mangium*.

Keywords: *A. mangium, Ganoderma,* infection, root rot, virulence

INTRODUCTION

Pulp production in Indonesia has decreased from 1.65 million m3 (in quarter 1) to 1.05 million m^3 (in post quarter). In the other hand, the raw material for pulp is still dominated by species of acacia (such as *Acacia mangium*). Production of acacia has always fluctuated. In 2015, it reached about 52.22%, while in 2020, the demand for pulp and paper was estimated to be 490 million tons. Based on this, acacia production should be increased. Expansion of acacia plantation is done on marginal land, and aimed to increase the land productivity. But the presence of *Ganoderma* as root rot pathogen has become the obstacle to acacia productivity. In 2003, infection of *G. lucidum* to *A. mangium* was recorded 3-28% in Sumatera and Kalimantan (Irianto et al. 2006). It is possible that the severity level could. In addition to *G. lucidum,* the cause of *A. mangium* death in Indonesia-Malaysia were *G. steyeartanum, G. mastoporum* and *G. philippii* (Glen et al. 2009; Hidayat et al. 2014).

Ganoderma infects acacia on the second rotation with the plant life of 3-5 years. But infection of *Ganoderma* may occur earlier with higher severity. In Sumatera, *G. philipii* attacked *Eucalyptus* extensively (Gafur et al. 2011) and *Paraserianthes falcataria* in Central Java (Herlyana et al. 2012). This shows that *Ganoderma* is pathogen which has wide range of host. This is related to the easy spread of *Ganoderma* by direct contact with the infected root, (except species of *G. zonatum,* this species is the pathogen-host specific) (Pilotti 2005).

Hidayat et al. (2014) estimated that the number of *Ganoderma* species ranges from 250 to > 400 species. According to Hidayat et al. (2014) the high similarity of basidiocarp features may be the cause why *Ganoderma* is the most difficult genus to accurately identify species of all polypores. Therefore, *Ganoderma* pathogen still has high potential to study. The ability of *Ganoderma* that infects various woody plants is due to the ability to produce lignolitic enzyme. Degradation of cell wall enzymatically is the first process of *Ganoderma* infection. This pathogen will colonize all root tissues. Then the root become brownish red as it is covered with mycelium of *Ganoderma.*

The early symptoms are less noticeable until loss of leaf occurs. Symptom developed slowly. Therefore, this attack of *Ganoderma* is latent, but it has the high mortality rate, such as palm oil, rubber plant, and acacia. Three of this plant species have been widely developed as plantation crop in West Kalimantan (Pontianak). Financial loss due to *Ganoderma* infection has not been felt as in Sumatra plantation. This is due to the rotation of *A. mangium,* rubber and palm oil plantation is in the 1st rotation. The development of disease with high severity usually occurs in the 2nd rotation and up. The duration of crop rotation will affect pathogenicity and virulence of pathogen. Virulence of *Ganoderma* is different depending on the species or isolate and host of pathogen. Therefore, this study aimed to

obtain information regarding the diversity and virulence of *Ganoderma* to infect *A. mangium* seedling.

MATERIALS AND METHODS

Procedures

Isolation and identification of Ganoderma

Ganoderma spp. were obtained from infected *A. mangium,* palm oil, and rubber plant in Pontianak, West Kalimantan. Gills of *Ganoderma* were cut (0.5-1 cm). Four up to five of the pieces were cultured in PDA added amoxicillin, incubated in 28ºC, for 30 days. The isolate identification consisted of morphology (color, concentric rings, hypha texture and spore) and the day filled Petri dish. Identification was based on The Fungi ID app. (Arbtalk 2007) and Munsell Color Soil Chart (Munsell 1975)

Incompatibility test

This test used to confirm the relationship of isolates. Two isolates of *Ganoderma* were PDA cultured in pairs. Each isolate was placed 1 cm from the edge of the petri dish (6 cm) (Figure 1). They were incubated 28ºC for 10 days. The different isolates are characterized by the inhibition zone (incompatible isolates), marked with '-'. The same isolates are not characterized by inhibition zone (compatible isolates), marked with "+". The antagonistic relationship between isolates which formed the inhibition zone were categorized as weak, medium and strong (Pilloti et al. 2003).

Observation of Ganoderma *infection in roots in vitro*

Microscopic observation was performed by inoculated *Ganoderma* in the acacia seedling roots, then incubated under aseptic condition. The inoculation site of root was observed under microscope. The observation time was 3 days, 5 days and 7 days after inoculation.

Experimental design

The were four treatments namely: *A. mangium* seedlings were planted without the *Ganoderma* inoculation as control (G0), seedlings were inoculated by *G. boninense* from palm oil (G1), seedlings were inoculated by *G. lucidum* from *A. mangium* (G2), and seedlings were inoculated by *G. applanatum* from rubber plant (G3). Completely randomized design with five replicates was applied to the experiment design. *Ganoderma* inoculation in seedlings was done by attaching one plug (diameter 0.5 cm) of isolates to the surface of seedling roots (3 months old) and closed aseptically. Seedlings that had been inoculated by *Ganoderma,* was planted in sterile medium (soil). The watering and weeding were done every day for three months.

Data analysis

There was two kind of data namely qualitative and quantitative. The qualitative data was the micro and macromorphology of root inoculated *Ganoderma.* The quantitative data was pathogen virulence and dry weight of seedlings. Virulency determination is based on the disease severity index (DSI). The scoring of symptoms was based on Izzati and Abdullah (2008) (Table 1). The quantitative data were analyzed using Analysis of Variance (ANOVA). When the result is significant then Duncan Test was applied.

$$DSI = \sum \frac{score\ x\ the\ number\ of\ plants\ in\ the\ score}{the\ number\ of\ all\ the\ plants\ tested}$$

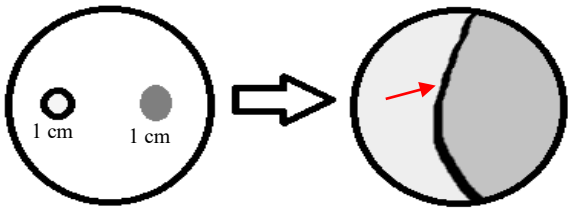


Figure 1. The incompatibility test

Table 1. Scores of the symptoms of acacia seedlings on a disease scale of 0-3 with reference to Izzati and Abdullah (2008).

Diseases class	Symptom of infection
0	Healthy plants with green leaves without appearance of fungal mycelium on any part of plants
1	Appearance of white fungal mass on infected region (stem) without chlorotic leaves
2	Appearance of white fungal mass on infected region (stem) with chlorotic leaves (1-3 yellowing leaves)
3	Appearance of white fungal mass on infected region (stem) with chlorotic leaves (1-3 browning leaves)

RESULTS AND DISCUSSION

Characteristic of morphology for *Ganoderma* isolates

Ganoderma is a pathogen that has a high genetic variance. In this study we obtained three isolates of *Ganoderma*: from palm oil (G1), *A. mangium* (G2) and rubber plant (G3) with different characteristics(Table 2 and 3).

Ganoderma were isolated from *A. mangium,* rubber and palm oil stands. Each species has both micro and macromorphological differences. Generally, *Ganoderma* has a fan-like shape with variations in size and surface colour of the pilleus. G1 isolate has a fan-like shape, wavy in the margin. G2 isolate has a radial furrows shape, and G3 has a wrinkled pilleus surface. The size of basidiocarp G1, G2, and G3 are 7 x 6.5 cm, 5 x 7.8 cm and 3 x 4 cm, respectively. The size of basidiocarp varied. This was due to environmental differences and habitat, so it was not specific character of *Ganoderma* species. Figure 2 showed a morphology difference in the basidiocarp of *Ganoderma*. Based on that character of isolates, the G1 and G2 isolates are a laccate basidiocarp fungi while G3 isolate is a non-laccate basidiocarp fungus.

Table 2. Morphological characteristic of basidiocarp for *Ganoderma* isolates from infected woody plants

Parameters	G1	G2	G3
Host	*A. mangium*	Palm oil	Rubber plant
Colour pilleus	5RP 2/3 purplish brown, yellow-margined	10RP 2/8 purplish red	10RP 5/3 light brown-10RP 3/3 dark brown, alternately
Pileus surface	Smooth, shiny, a fan-like shape	Smooth, shiny, a radial furrows shape	Wrinkled, a fan-like shape, like bracket
Concentric zone in pilleus	Well developed	Well developed, multiple smooth, wavy	Well developed
Margin pilleus	Wavy, color: 10YR 9/4 pale	Smooth	Smooth, color: 5YR 9/1 whitish
Stipe	Short	Short	No

Table 3. Characteristic of *Ganoderma* isolates from infected woody plants on PDA medium

Parameters	G1	G2	G3
The day filled Petri dish (days)	16	22	22
Miselia density	Dense	Dense	Rare
Texture	Rough	Rough	Smooth
Pigmentation	N9 White on the top 10YR 5/4 brown on the bottom	10YR 9/1 Pale on the top 10YR 9/3 Yellow on the bottom	2,5Y 9/1 White on the top 2,5Y 9/3 Pale on the bottom
Spore shape	Ellipsoid	Ellipsoid	Ellipsoid
Spore size (µm)	5,15-5,80 x 6,24-7,25	2,28-3,14 x 3,46-5,00	3,18-4,22 x 3,82-6,08

A **B** **C**

Figure 2. *Ganoderma* spp.: A. *Ganoderma* sp. from infected *A. mangium*; B. *Ganoderma* sp. from infected palm oil; C. *Ganoderma* sp. from infected rubber trees.

Morphological characteristics of G1, G2, and G3 isolates showed a difference in species significantly. The growth of G1 was faster than G2 and G3 isolates. That is based on the day's filled petridish (Table 3). G1 miselium filled petri dish for 16 days, while G2 and G3 need 33 days to fill petridish. Pigmentation isolates were shown by the color on the top/bottom of colony surface, based on Munchen Color (1975). Pigmentation of G1 isolate was N9 (on the top surface) and 10YR 5/4 (on the bottom surface). Pigmentation of G2 and G3 isolates was 10YR 9/1 and 2.5

Y 9/1 (on the top surface) respectively. Generally, characteristics of spore, conidium, and hypha of isolates have intra-species similarity withGenus of *Ganoderma.* Spore of each species relatively had the same shape, namely ellipsoidal (2.28-5.80 x 3.46-7.25 µm in size). G1 and G2 isolates have white spore while G3 has brown spore.

Incompatibility somatic

Based on the incompatibility test, the three isolates had genetic differences. The same pair of isolates would occur microscopically a hyphal fusion. Macroscopically, this was indicated by the absence of borderline at the mycelium meeting between G1 with G1, G2 with G2, G3 with G3 (Table 4).

Table 3. and Figure 3. showed that there was the borderline formation on the mycelia meeting of pair isolates. Pair of G1 vs G2 formed barrage (borderline) unclearly. A barrage of G2 vs G3 looked clearer than a pair of G1 vs G2, while a barrage of G1 vs G3 isolates was more clear and visible than other isolate pairs. This indicated that all isolates of *Ganoderma* (G1,G2, G3) showed the antagonistic relationship (G1 vs G2 is weak, G1 vs G3 is strong, G2 vs G3 is medium). Therefore G1, G2 and G3 isolates are different isolates clone. Observation of microscopic incompatibility test showed that there was a fusion between hypha G1 with G2 followed by cell death. Isolates pairs of G1 vs G3 and G2 vs G3 only occurred in contact without any hypha fusion (unpublished data).

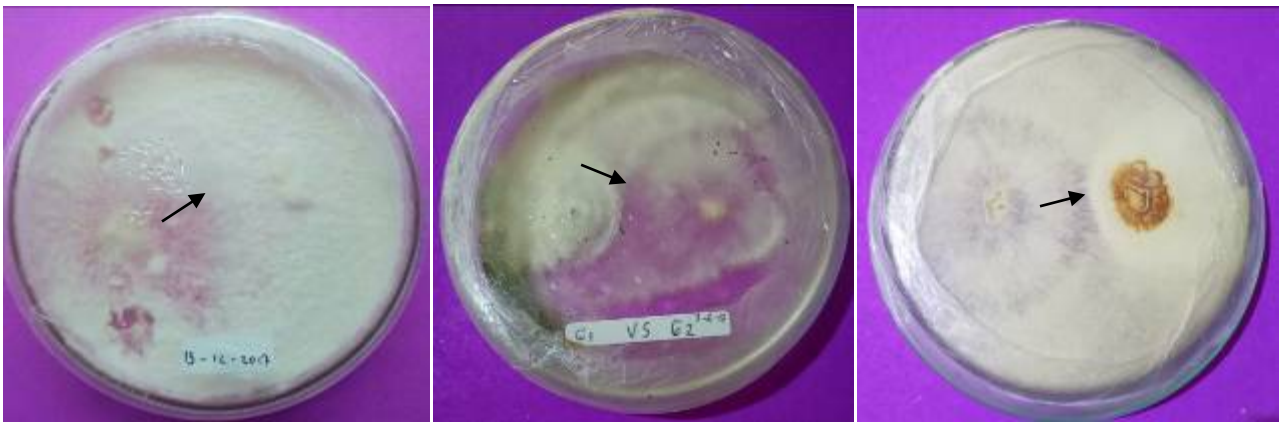
Virulence of *Ganoderma* in *A. mangium* seedlings

Infection of *Ganoderma* in root seedlings occurred on the 7th day after inoculation. On the microscopic observation, this infection was evidenced by the presence of swelling around the infection site (in root epidermis) (Figure 4.A). Then, this infection was continued with the presence of symptom in seedlings. The early symptom was chlorosis (yellowing) in leaves (Figure 4.B), followed by necrosis that started from the tip of the leaf (Figure 4.C). Finally, seedlings dropped the necrotic leaves until seedlings death of (seedlings looked as if they were burned) (Figure 4.D).

Table 4. Incompatibility of pair isolates of *Ganoderma* spp.

***Ganoderma* isolates**	**G1**	**G2**	**G3**
G1	-	+	+
G2		-	+
G3			-

Note: -: nothing barrage (borderline formation), no antagonism, compatible reaction. + : barrage (borderline formation), antagonism, incompatible reaction



A **B** **C**

Figure 3. Incompatibility test of G1, G2, G3 isolates (arrow lie showed border line or barrage). A. G1 x G2, B. G1 x G3, C. G2 x G3

Figure 4. Symptom's development of *Ganoderma* infection in three months old *A. mangium*. A. Swelling at the infection site of root epidermis, B. Chlorosis, C. Browning and necrosis at the tip of the leaf, D. Seedling looks as if they were burned

Results showed that symptoms were seen in seedlings aged three months. Incubation time of each isolate (G1, G2, and G3) was different. Incubation time of G1, G2 and G3 were two, six and seven weeks, respectively. This symptom development until the death seedling was relatively slow (± 3-7 weeks). But seedling that was infected by G1 isolate, had the fastest symptom development (three weeks) than others. G2 isolate had high virulence with 2.08 of disease severity index (DSI). G1 and G3 isolates were virulence and moderate virulence (Table 4).

Effect of *Ganoderma* infection on seedling dry weight

Results showed that the isolates infection effected by the decreasing of *A. mangium* dry weight significantly ($\rho < 0.001$). The dry weight decreased by 49.9%-61.88% of the dry weight of control treatment (10.02 g) (Figure 5).

Figure 5 showed that the smallest seedling dry weight was seedlings that were infected by G1 (3.82 g). Infection G3 in seedlings caused their dry weight to be lower than G2infected seedlings. However, the difference of *Ganoderma* isolates did not significantly affect ($\rho < 0.001$) to the seedling dry weight. This is perhaps correlated with disease severity in seedlings that was caused by G1, G2, and G3 infection. Table 4 showed that the disease severity index (DSI) by G1 did not differ significantly with DSI by G2 and G3.

Table 4. Virulence of G1, G2, and G3 isolates in *A. mangium* aged three months.

***Ganoderma* isolates**	**DSI**	**Virulence**
G0 (no *Ganoderma* isolate)	0.00 ± 0.00^{a}	Avirulence
G1 (isolate from *A. mangium*)	2.08 ± 0.38^{c}	High virulence
G2 (isolate from palm oil)	1.42 ± 0.52^{bc}	Virulence
G3 (isolate from rubber plant)	0.92 ± 0.52^{b}	Moderate virulence

Note: The values of DSI represented mean ± standard error for 3 replicates. DSI with different superscript alphabetic letters was significantly different at < 0.05 by Duncan test

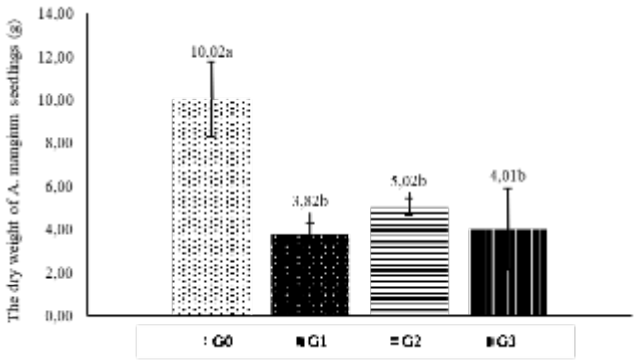


Figure 5. Effect of the *Ganoderma* infection on dry weight of *A. mangium.* Note: G1 isolated from *A. mangium,* G2 isolated from palm oil, and G3 isolated from rubber plants

Discussion

Identification of *Ganoderma* species cannot be based on morphological characteristics alone. Morphological similarity does not indicate genetic similarity. Differences in morphology of *Ganoderma* show that this fungus has high heterogeneity (Suryanto et al. 2005). The high genetic variety in *Ganoderma* is perhaps caused by out crossing over generations and differences of geographical origin (Pilotti et al. 2003; Keypour et al. 2014), such as observed in variation of *G. lucidum* (Wang et al. 2012). Commonly, variation can occur on stipe and pilleus morphology.

Sun et al. (2006) explained that generally, identification of *Ganoderma* is more based on host-specificity, geographical distribution, and basidiocarp macro-morphology. Identification of *Ganoderma* could also be based on spore characteristic as primer taxonomy characteristic. In this study, identification of *Ganoderma* was based more on spore, basidiocarp characteristics and growth of isolates in PDA. Characteristics of *Ganoderma* basidiocarp is sufficient to identified isolates (Wong et al. 2012). Suryanto et al. (2005) explained that *Ganoderma* which has pilleus like as fan-like or kidney-like, brownish red and blackish at the margin, stipes like hood and reddish brown spores, was identified as *G. lucidum.* This characteristic was similar to G1 isolate (the identification based on tree fungiid). *Ganoderma* pilleus is brown and yellow at the margin, has the concentric zone, and brownish white spores, so it was identified as *G. boninense.* This was similar to G2 isolate. *G. applanatum* based on Suryanto et al. (2005), has brown pilleus, stiff, no stipe, similar to G3 isolate.

Identification of *Ganoderma* based on morphological characteristics resulting in incorrect identification such as *G. lucidum* and *G. orbiform* (Glen et al. 2009). *G. lucidum* (isolate from *A. mangium*) (Iriyanto et al. 2006) turned out to be *G. steyeartanum.* Rename occurred after identification was based on morphology of basidiocarp /sporocarp and the sequence rDNA ITS. *G.* orbiform (Fr.) Ryvarden changed to *G. boninense* because of the genetic similarity of both. Prediction of genetic difference could be based on somatic incompatibility reaction such as *Acromyrmex echinatior* (Kooij et al. 2015). Morphologically, the third *Ganoderma* isolates that were identified as *G. lucidum* (G1), *G. boninense* (G2) and *G. applanatum* (G3), showed borderline formation. Pairing of G1 vs G1 (self-pairing)displayed less prevalent borderline which indicated both isolates had genetic similarity. Nusaibah et al. (2010) provided isolates pairing that formed poor borderline (inter species of *G. zonatum, G. miniatocinctum*, and *G. tornatum*), had 100% genetic similarity based on AFLP (Lim and Fong, 2005). Morphology of G1, G2 and G3 basidiocarp appeared to differ from each other, and had a morphological similarity between G1 with *G. lucidum,* G2 with *G. boninense* and G3 with *G. applanatum*.

Penetration of *Ganoderma* in host begins by degrading cell wall of root or basal stem enzymatically and physically. Then, it is continued with tissue colonization. Microscopically, the success of penetration was evidenced by its swelling in epidermis (Figure 4a). That swelling is mycelium sheath. Gill et al. (2016) explained that the mycelial sheath comprised of two different types of tissues. They are an outer melanized layer (<40 µm) and an inner amorphous layer (>100 µm). Deeper observation (Gill et al. 2016) showed that *G. philipii* infection in *A. mangium* young roots could induce the production of wound periderm with multiple layers of new parenchyma cells. Another form of plant defense responses is callose synthesis. Callose synthesis responsible for stress-induced callose deposition in the plant, and it is influenced by the timing of callose deposition (Ellinger and Voigt 2014). Root rot pathogen, such as *Rhizoctonia solani,* induced callose in *Pinus merkusii* (Suryantini 2014). *P. merkusii* that were resistance, had more callose than susceptible plant, and vice versa. This caused callose induction, disrupting the translocation of xylem tissue. In this study, *Ganoderma* isolates were thought to induce callose in the infected seedling. So it was caused by disruption water and nutrition translocation, characterized by chlorosis (Figure 4b). Then the infected seedling will lose turgor. Eventually, cell death occurred. This event was characterized by necrosis/browning (Figure 4c). The infection ended with plant death (Figure 4d).

In the concept of disease triangle explain that disease occurrence is caused by the interaction of three components (virulent pathogen, susceptible plant and favorable environment). The three components also effect the incubation period and disease symptom development. Thus, the acacia seedlings infected by different isolates, showed different incubation periods and symptoms. *G. lucidum* had the fastest incubation time than the others. It affected virulence of isolates. Table 4. showed that G1 was the most virulent (with 2.08 on diseases severity index) than the G2 and G3 isolates. Diseases severity will further cause death of seedlings (looks like burning).

The previous research provided that *Ganoderma* infection did not influence the palm oil growth (height and diameter) (Goh et al. 2016). This might be due to the swelling effect in the injured roo t or basal stem tissue (Nagy et al. 2000). This swelling was the induced systemic resistance (ISR) in host (pine) to a pathogen (*Ganoderma*) infection. ISR included massive accumulation of resin at the site of damage, accumulation, and deposition of polyphenolics in the young root tissue surrounding the traumatic ducts and presumably with accompanying enhanced anti-fungal activity, production of a physical barrier (lignification). The infected seedlings had decreased dry weight compared to seedlings in control (no infection) (Figure 5). The decreased seedlings dry weight was caused by increased respiratory rate. Miller and Scott (1962) explained that the presence of the pathogen resulted in an increased respiratory rate in susceptible and resistant varieties. In a highly resistant variety, there was a particularly rapid rise in respiration, followed by the early collapse of some mesophyll cells and a return of the respiratory rate to a normal level. So this does not inhibit a plant growth permanently. In a susceptible strain, the respiratory response to the presence of the pathogen was slower, and the increased respiration continued parallel with fungal growth. The fungal growth will block translocation in xylem, beside callose as a plant response. Thus, *A. mangium* is a susceptible plant species to the infection of *G. lucidum* G1 and *G. applanatum* G3, but it is a resistant plant to the infection of *G. boninense* G2

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