

# ***Ganoderma* diversity from smallholder oil palm plantations in peatlands of Kampar District, Indonesia based on mycelia morphology and somatic incompatibility**

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**Abstract.** Hamzah A, Saputra R, Puspita F, Nasrul B, Irfandri, Depari NS. 2021. *Ganoderma* diversity from smallholder oil palm plantations in peatlands of Kampar District, Indonesia, based on mycelia morphology and somatic incompatibility. *Biodiversitas* 22: 16-22. Basal Stem Rot disease is caused by the pathogenic fungus *Ganoderma boninense* which has caused major economic losses in the palm oil industry. *Ganoderma boninense* has been reported not only infecting crops in the field, but also attacking at the immature phase of the plant even in the nursery. Studies related to *Ganoderma* diversity in oil palm plantations in Riau, Indonesia have not been widely reported. *Ganoderma* genetic diversity is important because it provides information regarding the mechanism of infection and the spatial distribution. The *Ganoderma* spp. isolates were collected from three blocks of smallholder oil palm plantation in Deli Makmur Village, Kampar District, Riau Province, Indonesia and brought to Plant Disease Laboratory, Faculty of Agriculture, Riau University, Pekanbaru, Indonesia. This experimental research was conducted using six *Ganoderma* isolates (Gan1, Gan2, Gan3, Gan4, Gan5, and Gan6) on the parameters of colony diameter and growth speed, while for the parameters of the morphological diversity of *Ganoderma* mycelium and the diversity based on somatic incompatibility assay was carried out descriptively. The results of this study show that oil palm plants in smallholder plantations in Kampar District are infected by two groups of *Ganoderma* based on morphology characteristics using UPGMA dendrogram, but different among isolates based on genetically using somatic incompatibility assay. For the next confirmation, it is necessary to further identify whether the six isolates are different species using molecular identification.

**Keywords:** Basidioma, diversity, *Ganoderma*, somatic incompatibility

## **INTRODUCTION**

Riau Province has the largest oil palm plantation area in Indonesia. Based on data from the Central Bureau of Statistics (2019), the area of oil palm plantations in Riau has reached 2,850,003 ha of the total land area, namely 14,996,010 ha or 19% of the total national area, where 39% of the total area of oil palm plantations is found on peatland. However, in its development, oil palm plantations are still faced with one major obstacle, namely the incidence of stem rot disease which causes losses due to plant mortality. Basal Stem Rot (BSR) disease is a major disease that is still difficult to control in oil palm plantations, especially in Indonesia. This disease is caused by the pathogenic fungus *Ganoderma* spp. which has caused major economic losses in the palm oil industry, because it can cause death by up to 50% resulting in a decrease in oil palm production (Darmono 1998).

*Ganoderma* spp. has been reported not only infecting crops in the field, but also attacking at the immature phase of the plant even in the nursery. In Indonesia, several oil palm plantations reported plant mortality which reached more than 35% in Tanjung Selamat (Susanto et al. 2008). In Malaysia, Idris et al. (2003) reported that plant mortality can reach more than 80% of oil palm trees that have half their economic life span. This pathogen is known to not

only cause stem rot, Susanto et al. (2013) have reported that in Labuhan Batu, North Sumatra, *Ganoderma* can also cause Upper Stem Rot (USR) symptoms which are predominantly found on peatlands, and Rakib et al. (2014a) also reported the occurrence of upper stem rot disease in Sarawak, Malaysia.

To date, there are 15 species of *Ganoderma* spp. causes of Basal Stem Rot disease have been reported, including *G. boninense* (Cooper et al. 2011), *G. chalconum*, *G. miniactocinctum*, *G. tornatum*, and *G. zonatum* (Elliott and Broschat 2001). *G. boninense* is more virulent than other species as a cause of BPB in some cases. In South Africa, root rot is caused by *G. lucidum* Karst. while in Nigeria it was caused by *G. zonatum*, *G. encidum*, *G. colossus*, and *G. applanatum*. In Malaysia, the species identified as causing stem rot is *G. boninense* Pat., while *G. tornatum* is only found growing in the highlands with high rainfall. In Indonesia, the *G. boninense* Pat. identified as the most common fungal species attacking oil palm (Jing 2007).

*Ganoderma* spp. is a soil-borne pathogen, with three ways of spreading, namely contact of plant roots with *Ganoderma* spp. inoculum sources, through the air with basidiospore, and through secondary inoculum in the form of oil palm stumps as alternative hosts (Susanto et al. 2013). Pinaria and Assa (2017), explain that the pathogens can be spread horizontally in the soil up to about 2 meters

from the infected source to the healthy plants root. To date, more than 250 species from the genus *Ganoderma* spp. around the world. This fungus has a porous basidioma at the bottom. The basidioma varies widely in size, shape, and color. The size of the fruit body can reach a diameter of 15 cm and a thickness of 5 cm, the color of the fruit body ranges from light brown to dark brown and some even orange. The upper part of the basidioma is slightly shiny with a white underside (Henessy and Daly 2007).

*Ganoderma* spp. can be identified by observing the character macroscopic of basidioma and the colony on medium, and also microscopic by the mycelia. The fruiting body of *Ganoderma* spp. macroscopically appears as a small white bulb at first and develops into a fan-like, thick and hard shape. The color of the upper surface of the basidioma varies from light brown to dark brown, usually looks shiny, especially when it is young. The outer surface is white, the lower surface is dull white, when touched it will immediately turn bluish-gray and the lower layer of the fruiting body consists of a layer of pores, where basidium and basidiospores are formed (Peterson 2006). Studies related to *Ganoderma* diversity in oil palm plantations in Riau have not been widely reported. Purnamasari et al. (2012) have carried out *Ganoderma* isolation and molecular identification from infected palm oil in Padang and Pontianak. *Ganoderma* genetic diversity is important to know because each has specifications in causing disease in plants. Lack of information on the diversity of *Ganoderma* species causes inaccurate in determining control measures. This genetic diversity will also provide information regarding the mechanism of infection and spread in the land, so this study is necessary.

## MATERIALS AND METHODS

### Study area

This research was conducted from August to November 2020. The *Ganoderma* basidioma was collected from the smallholder oil palm plantations in Deli Makmur Village, Kampar Timur Subdistrict, Kampar District, Riau Province, Indonesia. The sample was taken from infected oil palm tree on the three blocks of oil palm plantations (Figure 1), The first block is located on the site 0°30'18"N - 101°11'09"E, the second block is located on the site 0°30'16"N - 101°11'05"E and the third block is located on the site 0°30'23"N - 101°11'04"E. The basidioma samples were brought to Plant Disease Laboratory, Faculty of Agriculture, Riau University, Pekanbaru, Indonesia for further assessment.

### Procedures

#### Sample collection and isolation

*Ganoderma* spp. collected from infected plants of smallholder oil palm plantations in the peatlands of the Kampar District. The field samples were isolated from the basidioma using Potato Dextrose Agar (PDA). The basidioma was cut to a size of about 1 cm × 1 cm, then the fruit body is soaked in 70% alcohol for 1 minute. The pieces of the fruit body are transferred to 0.5% NaOCl solution for 1 minute then rinsed using sterile water 3 times and dry. The basidioma was placed in PDA medium and then incubated at 28°C. A total of six isolates of *Ganoderma* sp. namely: Gan1, Gan2, Gan3, Gan4, Gan5, and Gan6 were purified.



**Figure 1.** The diversity of *Ganoderma* spp. basidioma from infected oil palm tree in Deli Makmur Village, Kampar District, Riau, Indonesia

**Table 1.** Morphological characterization to describe the similarity coefficient of *Ganoderma* isolates

| Character            | Description             | Character              | Description             |
|----------------------|-------------------------|------------------------|-------------------------|
| Mycelia density      | Thin                    | Mycelia texture        | Smooth                  |
|                      | Dense                   |                        | Rough                   |
|                      | Dense at center only    |                        | Fluffy                  |
| Surface texture      | Adpressed               | Colony concentric ring | Absence                 |
|                      | Moderately wavy         |                        | Presence                |
|                      | Strongly wavy           |                        |                         |
| Surface pigmentation | No pigmentation (White) | Reverse pigmentation   | No pigmentation (white) |
|                      | Pale yellow             |                        | Pale yellow             |
|                      | Yellow                  |                        | Yellow                  |
|                      | Yellowish-brown         |                        | Brownish-yellow         |
|                      | Dark yellowish brown    |                        | Yellowish-brown         |

#### *Morphological diversity of Ganoderma spp. mycelium*

*Ganoderma* morphological diversity was carried out by observing the mycelium growing on PDA media. The observations carried out included: days for full plate, mycelia density, mycelia texture, surface texture, colony concentric ring, surface and reverse pigmentation color. That characteristic then used as quantitative data adopted from Rakib et al. (2014b) and describe in Table 1. The similarity coefficient was shown on dendrogram using unweighted pair group method of arithmetic averages (UPGMA) (Pilotti et al. 2004).

#### *Diameter and growth speed of Ganoderma sp. isolates*

Observation of *Ganoderma* spp. growth was done by measuring the colony diameter and growth rate of fungal isolates grown on PDA medium. The colony diameter of *Ganoderma* spp. measured daily until the mycelial growth of one of any isolate filled the petri dish. The average growth speed was calculated. Growth speed (V) calculation of *Ganoderma* spp. carried out based on the formula  $V = \frac{D(n+1) - D_n}{n}$ , based on the diameter (D) of the colony form 1 day up to 7 days after inoculation (n).

#### *Ganoderma spp. diversity based on somatic incompatibility assay*

The somatic incompatibility test was carried out using the method of Latifah and Ho (2005) by pairing two different *Ganoderma* fungi isolates on PDA media in a petri dish (9 cm in diameter). The inoculum block was taken from the initial plate with a size of  $\pm 3 \text{ mm}^2$ , placed with a distance of  $\pm 2 \text{ mm}$  between the two isolates. The tissue cultures were incubated at 25°C in the incubation room. The results of the somatic incompatibility test were evaluated after 5 days of planting by assessing the incompatibility reactions between isolates. Compatible isolates merged into single colony (self-pairing), whereas incompatible isolates formed inhibition zone or barrage.

#### **Data analysis**

The diameter and growth speed of *Ganoderma* sp. isolates data obtained were analyzed statistically by ANOVA using SPSS then followed by Duncan New Multiple Range Test (DNMRT) at the 5% level by using SAS 9.4. The morphological diversity data of *Ganoderma* spp. mycelium and somatic incompatibility assay were analyzed descriptively and the data presented on tables and figures. The similarity coefficient dendrogram then

analyzed using UPGMA with Numerical Taxonomy System (NTSYS-pc) program.

## **RESULTS AND DISCUSSION**

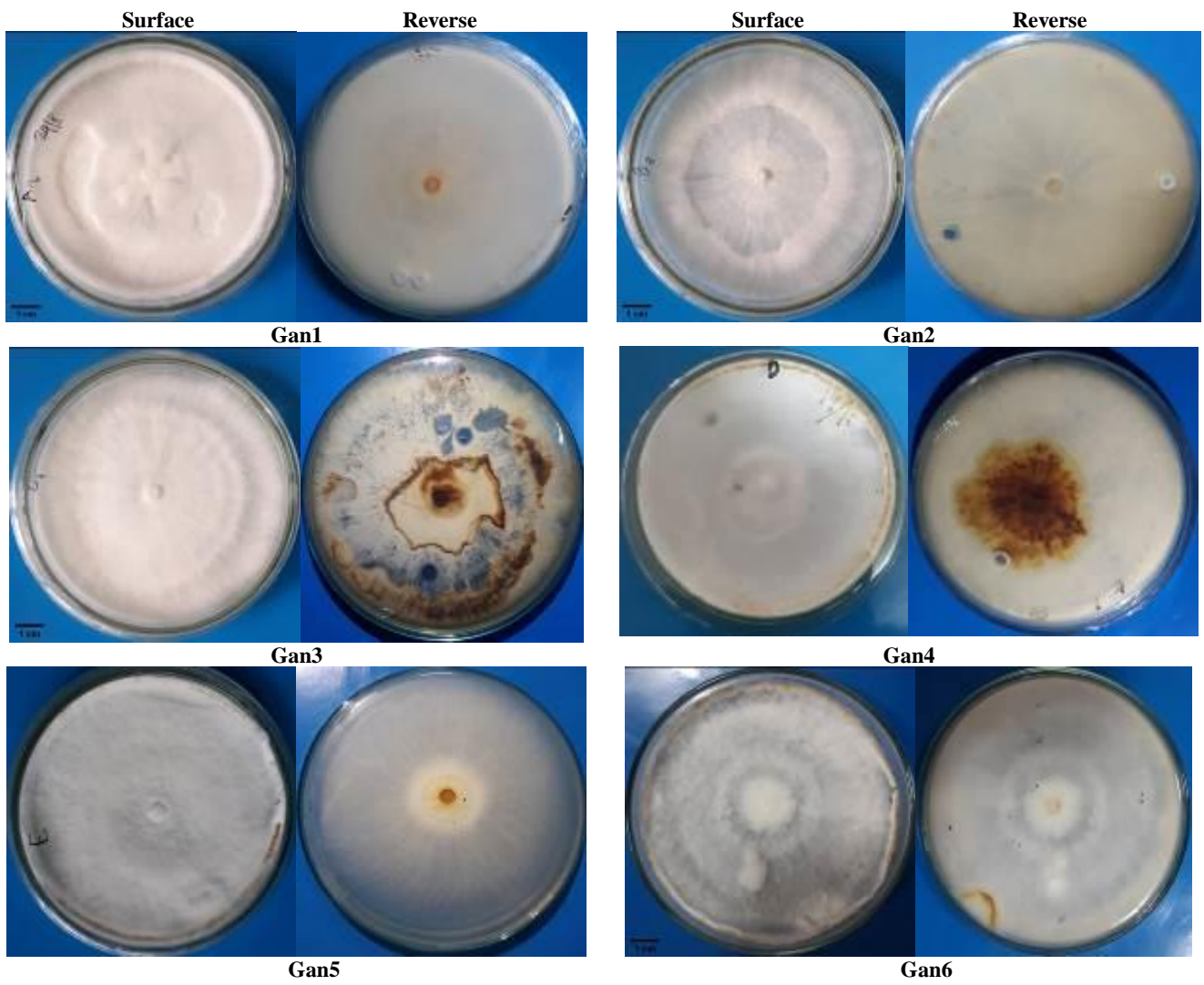
#### **Morphological diversity of *Ganoderma* spp mycelium**

The data in Table 2 shows the diversity of *Ganoderma* spp. based on the morphology of mycelium that grows on PDA medium. The Gan6 isolate was the one that filled the petri dish the fastest with 6 days after inoculation. Meanwhile, Gan2 and Gan3 isolates took care of the Petri dishes for 7 days after inoculation, while other isolates took more than 7 days after inoculation. Mycelium morphology, of the 6 isolates that were observed showed a variety of mycelia density and mycelia structure (Figure 2). However, the surface texture was only Gan3 which showed strongly wavy, and Gan5 which did not show any concentric colony rings. Two *Ganoderma* isolates showed yellowish-brown to brownish-yellow and others have shown no surface pigmentation (white) color, and only Gan3 and Gan4 showed a brownish yellow color on the reverse pigmentation color.

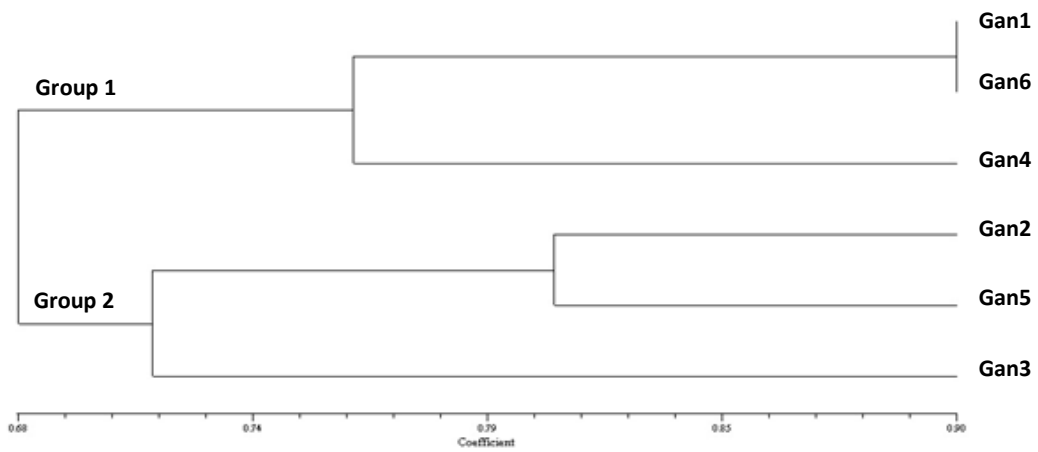
Figure 3 shows the dendrogram of the similarity in morphological characteristics of 6 *Ganoderma* isolates obtained and there are 2 major groups where a cutoff value of 0.70. In Group 1 there were 2 isolates that had a similar morphological similarity with a 90% similarity coefficient, namely the Gan1 and Gan2 isolates, while the Gan4 isolates were separated with a similarity coefficient of only about 76%. Group 2 are the Gan2 and Gan5 isolates which have a similarity coefficient of about 80%, and the Gan3 isolates are separated from the two isolates with a similarity coefficient of only 72%.

#### **Colony diameter and growth speed of *Ganoderma* sp. isolates**

Table 3 showed that the *Ganoderma* sp. isolate Gan6 showed the highest growth rate compared to other isolates, so that this isolate was able to produce a colony diameter of 9 cm at 6 days after inoculation. Figure 4 shows a graph of the development and growth of *Ganoderma* sp. isolates of Gan6 were increasing, while isolates of Gan2 and Gan3 showed moderate growth rates, and isolates of Gan1, Gan4, Gan5 showed slower growth rate which meant slower colony development than other isolates.



**Figure 2.** Morphological characteristic of six *Ganoderma* spp. isolates on PDA medium



**Figure 3.** Dendrogram (UPGMA) based on morphological characteristics generated using 6 *Ganoderma* spp. isolate mycelia

**Table 2.** Morphological diversity of *Ganoderma* spp. mycelia on PDA medium

| <i>Ganoderma</i> sp. isolates | Days for full plate | Mycelia density      | Mycelia texture | Surface texture | Colony concentric ring | Surface pigmentation color | Reverse pigmentation color |
|-------------------------------|---------------------|----------------------|-----------------|-----------------|------------------------|----------------------------|----------------------------|
| Gan1                          | >7                  | Dense                | Smooth          | Moderately wavy | Presence               | White                      | White                      |
| Gan2                          | 7                   | Dense                | Fluffy          | Moderately wavy | Presence               | Yellowish-brown            | White                      |
| Gan3                          | 7                   | Dense                | Fluffy          | Strongly wavy   | Presence               | White                      | Brownish-yellow            |
| Gan4                          | >7                  | Dense at center only | Smooth          | Moderately wavy | Presence               | Dark yellowish brown       | Brownish-yellow            |
| Gan5                          | >7                  | Dense                | Fluffy          | Moderately wavy | Absence                | White                      | White                      |
| Gan6                          | 6                   | Dense at center only | Smooth          | Moderately wavy | Presence               | White                      | White                      |

### *Ganoderma* spp diversity based on somatic incompatibility assay

Based on somatic incompatibility assay, all six isolates except self-pairing isolates showed incompatibility reaction. The pairing reaction of two different *Ganoderma* sp. isolates showed in Table 4. This data shows that the six isolates are genetically different from each other although they are morphologically similar based on colony diameter and growth speed on PDA medium. Data in Table 4 showed there are two forms of somatic incompatibility result in several *Ganoderma* sp. isolates, namely barrage and inhibition zones. Some pairing result shows the pigmentation on the barrage (Table 5 and Figure 5). This somatic incompatibility assay result showed that the smallholder oil palm plantations in Deli Makmur Village, Kampar District were infected by several different *Ganoderma* species.

### Discussion

The use of basidioma morphological information in determining *Ganoderma* diversity is considered insufficient. Seo and Kirk (2000) stated that the identification process is not sufficient based solely on differences in the shape of the basidioma, because some basidioma has a similar shape, therefore it is necessary to pay attention to other criteria, namely spore, and mycelia morphology characteristics. The observations results of the morphological characteristics on the mycelia showed similar characteristics to the study conducted by Rakib et al. (2014b) who showed the species *G. boninense* and *G. zonatum* that confirm by molecular identification.

*Ganoderma* sp. mycelial growth rate of Gan6 isolate on PDA medium provides information regarding the ability of *Ganoderma* to develop. This rapid growth of fungal mycelia causes disease covering plant parts quickly. The presence of fungal mycelium in the plant transport tissue causes the inhibition of water transportation, and nutrients needed by plants, as a result, the initial symptoms appear in the form of chlorosis on the leaves followed by necrosis (Riantin 2009). The growth rate will be in line with the colony's wide diameter which will affect the ability of microbes to fill space and absorb nutrients (Saputra et al. 2019; Elfina et al. 2016).

**Table 3.** Growth speed and colony diameter of *Ganoderma* sp. isolates at several days after inoculation on PDA medium

| <i>Ganoderma</i> sp. isolates | Growth speed of <i>Ganoderma</i> spp. (cm.day <sup>-1</sup> ) | Colony diameter on 6 days (cm) |
|-------------------------------|---|--------------------------------|
| Gan1                          | 1.07 ± 0.15 b   | 6.43 ± 0.93 b                  |
| Gan2                          | 1.43 ± 0.08 a   | 8.55 ± 0.45 a                  |
| Gan3                          | 1.38 ± 0.03 a   | 8.30 ± 0.20 a                  |
| Gan4                          | 0.96 ± 0.09 b   | 5.75 ± 0.55 b                  |
| Gan5                          | 0.85 ± 0.01 b   | 5.08 ± 0.03 b                  |
| Gan6                          | 1.50 ± 0.00 a   | 9.00 ± 0.00 a                  |

Note: In a column, the numbers followed by unequal lowercase letters are significantly different according to the DNMRT test at the 5% level of probability

**Table 4.** Somatic incompatibility result of *Ganoderma* spp. on PDA medium

| <i>Ganoderma</i> sp. isolate | Gan1 | Gan2 | Gan3 | Gan4 | Gan5 | Gan6 |
|------------------------------|------|------|------|------|------|------|
| Gan1                         | √    | x    | x    | x    | x    | x    |
| Gan2                         | x    | √    | x    | x    | x    | x    |
| Gan3                         | x    | x    | √    | x    | x    | x    |
| Gan4                         | x    | x    | x    | √    | x    | x    |
| Gan5                         | x    | x    | x    | x    | √    | x    |
| Gan6                         | x    | x    | x    | x    | x    | √    |

Note: √ = self-pairing, x = incompatible

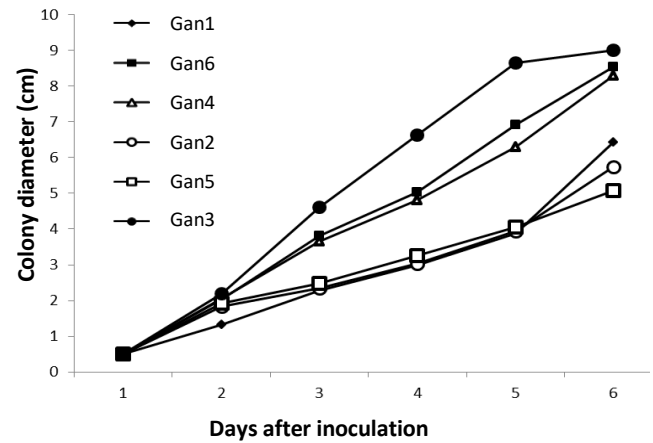
**Table 5.** Somatic incompatibility form of *Ganoderma* spp. isolates on PDA medium

| <i>Ganoderma</i> spp. isolates | Somatic incompatibility form | Pigmentation form |
|--------------------------------|------------------------------|-------------------|
| Gan1 x Gan2                    | Barrage                      | Absence           |
| Gan1 x Gan3                    | Barrage                      | Presence          |
| Gan1 x Gan4                    | Barrage                      | Absence           |
| Gan1 x Gan5                    | Barrage                      | Presence          |
| Gan1 x Gan6                    | Barrage                      | Absence           |
| Gan2 x Gan3                    | Barrage                      | Absence           |
| Gan2 x Gan6                    | Barrage                      | Absence           |
| Gan3 x Gan6                    | Barrage                      | Absence           |
| Gan4 x Gan5                    | Barrage                      | Absence           |
| Gan4 x Gan6                    | Barrage                      | Absence           |
| Gan5 x Gan6                    | Barrage                      | Absence           |
| Gan2 vs Gan4                   | Inhibition zone              | Absence           |
| Gan2 vs Gan5                   | Inhibition zone              | Presence          |
| Gan3 vs Gan4                   | Inhibition zone              | Presence          |
| Gan3 vs Gan5                   | Inhibition zone              | Presence          |

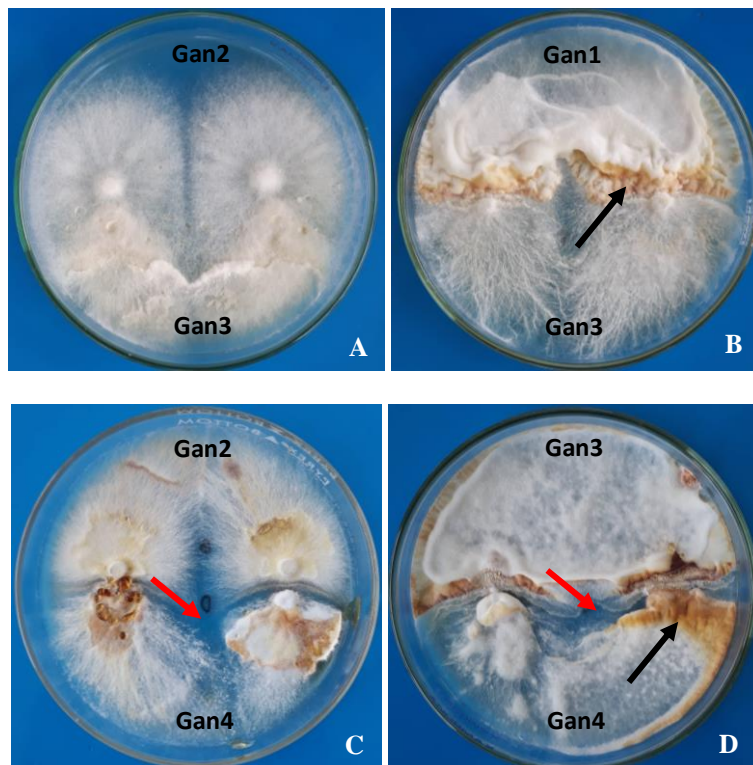
Another method in determining the genetic diversity of *Ganoderma* that can be used is somatic incompatibility. Hidayati and Nurrohmah (2015) stated that somatic incompatibility in basidiomycetes is the rejection of genetically different mycelia which functions to keep genetic changes in an individual. Somatic incompatibility regulates the rejection and recognition of alleles or corresponding and incompatible genes that follow the formation of cells body in a group of organisms. The study of somatic incompatibility can be used to determine the distribution of genotypes in a population, besides that, it can also be used to determine whether each tree affected by the fungus comes from the same or different fungal clones which can later be used to analyze the population and distribution of fungi in the field (Puspitasari and Rimbawanto 2010; Fries 1987). Rakib et al. (2014b) tested 46 *Ganoderma* samples, where somatic incompatibility formed from all tested isolates that showed a genetic difference from one another even in isolates from the same species.

Latiffah and Ho (2005) also stated that these genetic variations occur in pathogens originating from the same or close to species. Incompatibility reactions can be shown in various forms of demarcation, namely the phenomenon of inhibition zone formation between paired mycelium, the formation of dam zones and formation of demarcation lines accompanied by pigmentation formation. The shape of the demarcation line is formed in almost all pairs of isolates

that have different types of mycelia or are not genetically identical to either monosporous or secondary mycelia (Worall 1997). In some conditions, the demarcation and barrage lines are accompanied by pigmentation. Guler (2008) explains that the presence of this pigmentation after the pairing process of two isolates is important because this pigmentation indicates an incompatibility reaction at the meeting of two different mycelia.



**Figure 4.** Growth curve trend of *Ganoderma* colony on PDA medium



**Figure 5.** Somatic incompatibility form of *Ganoderma* isolates. A. Barrage, B. Barrage with pigmentation (black arrow), C. Inhibition zone (red arrow), D. Inhibition zone with pigmentation (black arrow)

Based on Figure 3 and Table 5, Gan1 and Gan6 isolates showed similar on UPGMA but also have barrage formation on somatic incompatibility. Rakib et al. (2019) also found the same case and stated that the isolates may exhibit similar morphology but genotypically different. The results of this study show that oil palm plants in smallholder plantations in Deli Makmur Village, Kampar District, Indonesia are infected by different types of *Ganoderma*. A dendrogram from UPGMA analysis showed there are two groups of *Ganoderma* and the somatic incompatibility assay showed there are six different types with barrage and inhibition variation form. Molecular identification was therefore suggested to identify different species of *Ganoderma* among isolates tested.

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