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Investigation on *Ganoderma* Infection in Oil Palm Based on the Cultural Characteristics and Somatic Compatibility: A Case Study in Sandakan, Sabah

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Sustainability of the oil palm in Malaysia is threatened by *Ganoderma* species causing stem rot. There were many studies conducted to understand the etiology and epidemiology of the disease in West Malaysia, however none of them reported the situation in Sabah, one of the leading producers. Moreover, the mode of *Ganoderma* spread in oil palm is least understood. Thus, the aims for this study were to investigate the infection mode of *Ganoderma* species in oil palm based on the *in-vitro* cultural characteristics and somatic compatibility. A total of 21 *Ganoderma* basidiocarps were isolated from stem rot infected palms in an estate in Sandakan, Sabah. These samples were obtained (i) within infected palms; (ii) among infected neighbouring palms; and (iii) five clusters of infected palms. *In-vitro* morphology of the *Ganoderma* isolates was characterized based on 21 characteristics via a dendrogram. Somatic compatibility was accessed to investigate the genetic heterogenicity. There was a narrow variability (93 to 100%) in terms of the cultural characteristics, and the variations exhibited among the isolates regardless of their origin. The isolates may exhibit similar phenotype, but not necessarily have similar genotype, and vice versa. Based on the somatic compatibility test, it was found that all pairings showed incompatible reactions except in self-pairing and between isolates C5P3-1 and C5P3-2 which were isolated from the same infected palm (genetically identical). These findings indicated that infection of *Ganoderma* in a single palm and neighbouring palms generally were caused by multiple unidentical strains. This further concludes that spread of *Ganoderma* species in oil palm via root-to-root contact is uncommon. Thus, the basidiospores may play an important role in the disease epidemiology, and further research and management strategies of the disease should focus on this.

Keywords: *Ganoderma* species; stem rot disease; oil palm; somatic incompatibility; cultural characteristics

I. INTRODUCTION

The African oil palm (*Elaeis guineensis* Jacq.) is one of the most important oil producing crop which contributed 36% of the total global vegetable oil supplies, and Malaysia is producing 28% of the total global palm oil in 2018 (USDA, 2019). Sabah is one of the major states in Malaysia planted with oil palm. However, the sustainability of oil palm cultivation in Malaysia as well as other major planting countries such as Indonesia and Thailand are seriously threatened by *Ganoderma* species, a basidiomycete fungus that causes stem rot in oil palm (Rees *et al.*, 2012; Rakib *et al.*, 2014). Stem rot disease reduces the yield and shortens the economic life of a palm.

There are many studies carried out based on the morphological characteristics and somatic compatibility of *Ganoderma* in oil palm in West Malaysia (Miller *et al.*, 1999; Latiffah & Ho, 2005; Nusaibah *et al.*, 2010) and in

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Papua New Guinea (Pilotti *et al.*, 2004), and one study in Sawarak (East Malaysia) by Rakib *et al.* (2014). However, there is no information found based on a study in Sabah. Moreover, the mode of *Ganoderma* spread in oil palm is least understood. It is known that infection of *Ganoderma* initiate at the root of an oil palm, and the infection could spread to other healthy neighbouring palms through root-to-root contact (Flood *et al.*, 2005; Khairudin and Chong, 2008). However, the root-to-root infection mode is considered uncommon since *Ganoderma* species in oil palm plantations are genetically heterogeneous and basidiospores may have played an important role to establish infection.

Effective disease management is an important aspect to sustain the oil palm industry. Lack of knowledge of the pathogen may lead to inaccurate disease control strategies. Thus, the aims for this study were to investigate the infection mode of *Ganoderma* species in oil palm based on their *in-vitro* cultural characteristics and somatic compatibility.

II. MATERIALS AND METHOD

A. Collection and Isolation of Ganoderma Species

A total of 21 *Ganoderma* basidiocarps were collected from an oil palm estate located in Sandakan, Sabah. The oil palms were planted in an equilateral triangular system, with a distance between palms of 8.97 m and planting density of 143 palms per hectare. The samples were collected from (i) within an infected palm; (ii) among infected neighbouring palms; and (iii) five clusters of infected palms. An ID was given to each sample where the letter C indicating cluster, P indicating palm, number and followed by a dash (-) indicating different samples within an infected palm cluster, as illustrated in Figure 1. All samples were kept in a paper bag separately, and then brought to a microbiology laboratory in the Faculty of Sustainable Agriculture, Universiti Malaysia Sabah for isolation. The samples were isolated using *Ganoderma* selective medium (GSM) and pure cultures were maintained on potato dextrose agar (PDA) as described by Rees *et al.* (2007) for further analysis.

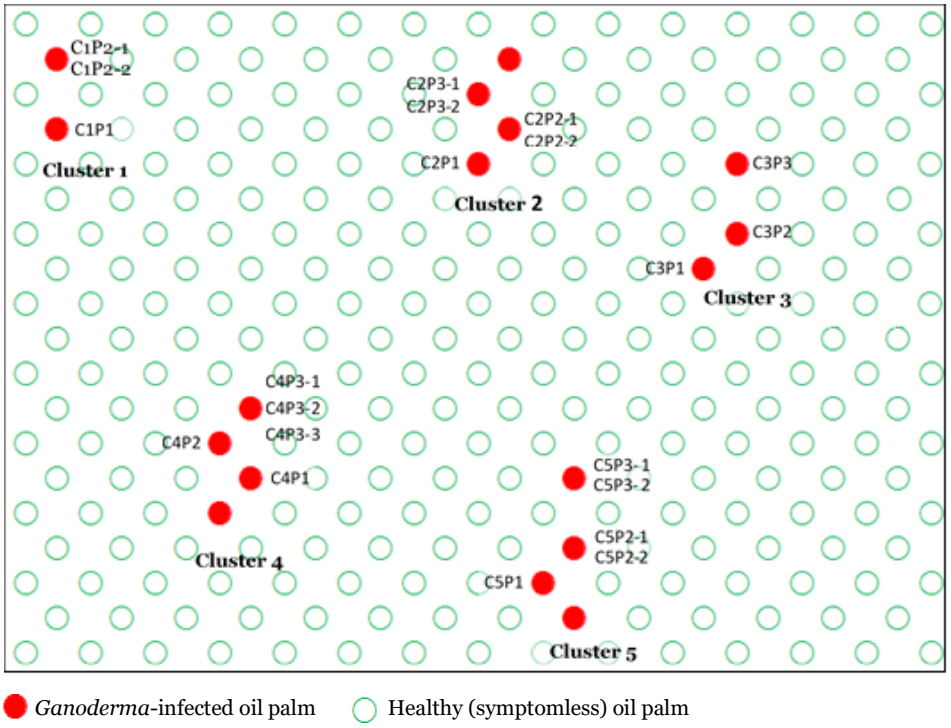

Figure 1. An illustration showing sample of *Ganoderma* basidiocarps collected within an infected palm, among neighboring infected palms, at five different clusters

B. In-vitro Cultural Characterization of Ganoderma Species

The *in-vitro* cultural morphology of *Ganoderma* isolates were characterized according to Rakib *et al.* (2014). Dikaryotic mycelia plug (8 mm) from a 7-days-old active *Ganoderma* pure culture was transferred onto the centre of a standard (90 mm) PDA plate (pH adjusted to 5.5 with HCl), and then incubated in the dark at room temperature (25-30 ºC). The experiment was conducted simultaneously for four replicates to avoid biasness due to external factors. The colony texture, appearance of zone, surface and reverse colour using Munsell soil colour chart were recorded after 7 days of incubation.

Qualitative data were transformed into 21 codes (Table 1) adapted from Rakib *et al.* (2014), and then a binary matrix was generated. The binary data was subjected to cluster analysis using multivariate statistical package (MVSP version 3.22). Similarity matrices were calculated using the simple matching coefficient and a dendrogram was generated using the unweighted pair group method of arithmetic averages (UPGMA) (Pilotti *et al.*, 2004).

Table 1. Cultural characters and their corresponding codes used to describe *Ganoderma* isolates for assessment of *in-vitro* cultural characteristics

Character	Description	Code
Mycelia density	Thin	1
	Dense	2
	Dense at centre only	3
Mycelia texture	Smooth	4
	Rough	5
	Fluffy	6
Surface texture	Adpressed	7
	Moderately wavy	8
	Strongly wavy	9
Colony concentric ring	Absence	10
	Presence	11
Surface pigmentation	No pigmentation (white)	12
	Pale yellow	13
	Yellow	14
	Yellowish brown	15
	Dark yellowish brown	16
Reverse pigmentation	No pigmentation	17
	Pale yellow	18
	Yellow	19
	Brownish yellow	20
	Yellowish brown	21

Source: Rakib *et al.* (2014)

C. Somatic Compatibility of Ganoderma Species

Somatic compatibility test of the *Ganoderma* isolates was tested using a modified method by Miller *et al.* (1999), using PDA instead of 1% malt extract agar (MEA). Dikaryotic mycelia plugs (8 mm) were placed 2 cm apart on PDA plate in all possible combination for the isolates within each cluster, and self-pairing served as the control. All PDA plates were incubated in darkness at room temperature (25-30 ºC). Observation was made after 14 days of incubation and rated either compatible or incompatible. Compatible isolates merged into single colony. Whereas incompatible isolates formed inhibition zone or barrage.

III. RESULTS AND DISCUSSION

The cultural characteristics in terms of mycelia density, mycelia texture, surface texture, colony concentric and colour of the *Ganoderma* isolates were varied. The variations exhibited among the isolates regardless whether they were isolated within the same infected palm, between the palms within a cluster, or across the clusters. Among the isolates, only isolate C5P2-2 showed distinct characteristics, where the mycelia and surface texture were rough and strongly wavy, respectively (Table 2). However, the dendrogram generated based on the cultural characteristics showed a minimum variation among the isolates, where the similarity ranged from 93 to 100% (Figure 2). 100% similarity was found between isolates C2P2-2 and C2P3-2, where these isolates originated from neighbouring infected palms within same cluster.

A similar study by Rakib *et al.* (2014) using a total of 46 *Ganoderma* isolates reported that culture characteristics exhibited wider similarity ranged from 60 to 100%, which could be related to a larger number of samples and samples collected from distinct diseases and locations. The cultural variability is also common in several other phytopathogens, such as in *Pyrenophora tritici-repentis* (Benslimane *et al.*, 2017), *Fusarium oxysporum*, and *Sphaeropsis sapinea* (Hausner *et al.*, 1999).

Table 2. *In-vitro* cultural morphology characteristics of *Ganoderma* on potato dextrose agar (PDA) assessed on 14 days after incubation

Isolate	Mycelia density	Mycelia texture	Surface texture	Colony concentric ring	Surface pigmentation (colour and code)	Reverse pigmentation (colour and code)
C1P1	Dense	Smooth	Adpressed	Absence	Dark yellowish brown (3/6)	Pale yellow (8/3)
C1P2-1	Dense	Fluffy	Moderately wavy	Presence	White (8/1)	White (8/1)
C1P2-2	Dense at centre	Smooth	Adpressed	Presence	Yellow (8/6)	Pale yellow (8/3)
C2P1	Dense	Fluffy	Moderately wavy	Absence	Pale yellow (8/3)	Pale yellow (8/3)
C2P2-1	Dense	Fluffy	Adpressed	Absence	Pale yellow (8/3)	Yellowish brown (5/8)
C2P2-2	Thin	Smooth	Adpressed	Presence	White (8/1)	White (8/1)
C2P3-1	Thin	Smooth	Moderately wavy	Absence	Yellowish brown (5/8)	Brownish yellow (6/6)
C2P3-2	Thin	Smooth	Adpressed	Presence	White (8/1)	White (8/1)
C3P1	Dense	Fluffy	Adpressed	Absence	White (8/1)	Yellow (8/6)
C3P2	Thin	Fluffy	Adpressed	Absence	White (8/1)	Brownish yellow (6/6)
C3P3	Thin	Smooth	Adpressed	Absence	Dark yellowish brown (3/6)	Yellow (8/6)
C4P1	Dense at centre	Fluffy	Moderately wavy	Absence	Yellowish brown (5/8)	Brownish yellow (6/6)
C4P2	Dense	Smooth	Adpressed	Absence	Dark yellowish brown (3/6)	White (8/1)
C4P3-1	Dense at centre	Smooth	Moderately wavy	Absence	Yellowish brown (5/8)	Yellowish brown (5/8)
C4P3-2	Dense at centre	Smooth	Adpressed	Absence	White (8/1)	Brownish yellow (6/6)
C4P3-3	Thin	Smooth	Adpressed	Presence	Dark yellowish brown (3/6)	Yellow (8/6)
C5P1	Dense at centre	Fluffy	Moderately wavy	Absence	Yellow (8/6)	Brownish yellow (6/6)
C5P2-1	Dense at centre	Smooth	Moderately wavy	Absence	Yellow (8/6)	Brownish yellow (6/6)
C5P2-2	Dense at centre	Rough	Strongly wavy	Absence	Dark yellowish brown (3/6)	Brownish yellow (6/6)
C5P3-1	Thin	Fluffy	Adpressed	Presence	White (8/1)	Brownish yellow (6/6)
C5P3-1	Dense at centre	Fluffy	Adpressed	Presence	White (8/1)	Brownish yellow (6/6)

The cultural variability was mostly related to isolates collected from different geographical origin and different samples of infected plants. According to Jain & Fries (2009), cultural variability also related to phenotypic changes which are common in fungi to allow adaptation to a constantly changing environment. Phenotypic changes could be differentiated into three types, which are (i) morphological transition that induced by environmental changes; (ii) phenotypic switching that randomly occurred, reversible and represents an epigenetic state that is not necessarily induced by external signals; and (iii) antigenic variation involves alternating the expression of surface proteins which happens on cellular level (Jain *et al.*, 2008; Hewitt *et al.*, 2016).

Based on the assessment of somatic compatibility, all possible pairing of samples showed incompatibility as they formed either inhibition zone or barrage line, except in self-paired (control), and reaction between isolates C5P3-1 and C5P3-2 where the colonies merged into single colony (Figure 3). Somatic compatibility between different isolates are rarely found in *Ganoderma*. However, the somatic compatibility between isolates C5P3-1 and C5P3-2 in this study exhibited evidence that the formation of multiple *Ganoderma* basidiocarps or spread of the fungi within an infected palm due to asexual reproduction that generates genetically identical individual are possible. Incompatible somatic reactions between the isolates that were isolated within an infected palm, and between neighbouring infected palms within a cluster indicated that they were genetically distinct or heterogeneous (genotypic variation) and originated from different inoculum. This further indicated that infection by multiple strains of *Ganoderma* was possible within a palm and spread of *Ganoderma* to the neighbouring palm was due to basidiospores that generate genetically distinct isolates, and not due to root-to-root contact.

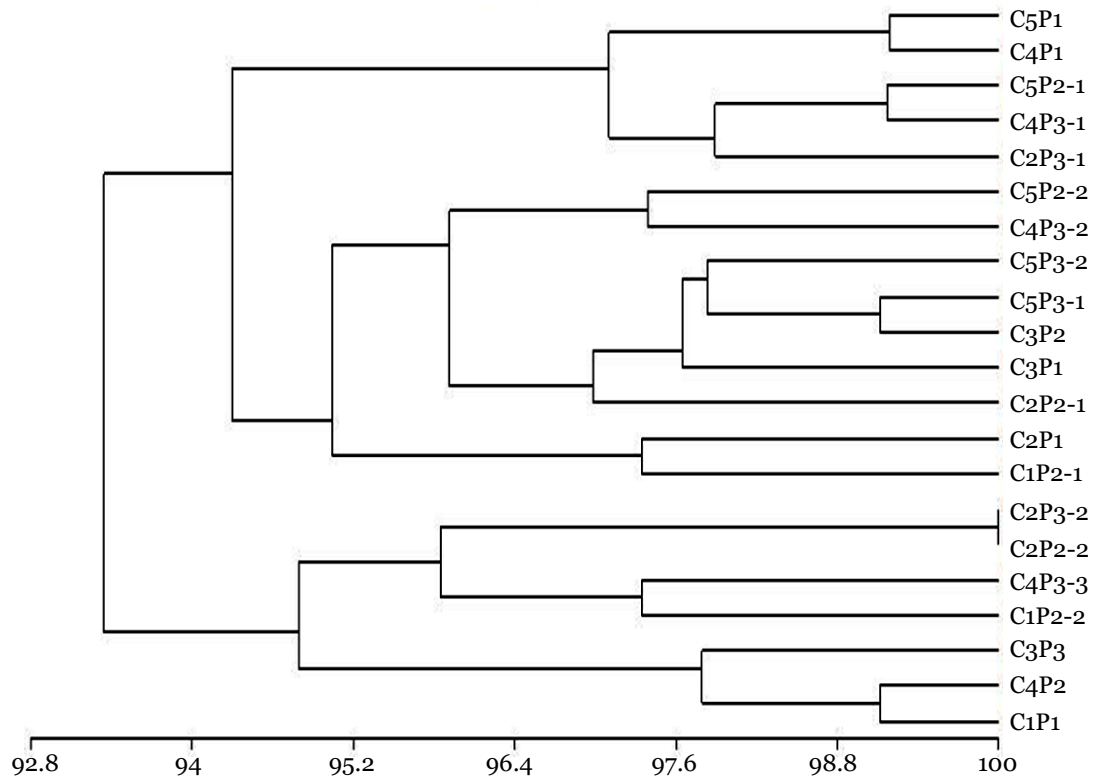

Figure 2. Dendrogram (UPGMA) generated using 21 cultural morphology characteristics of *Ganoderma* isolates

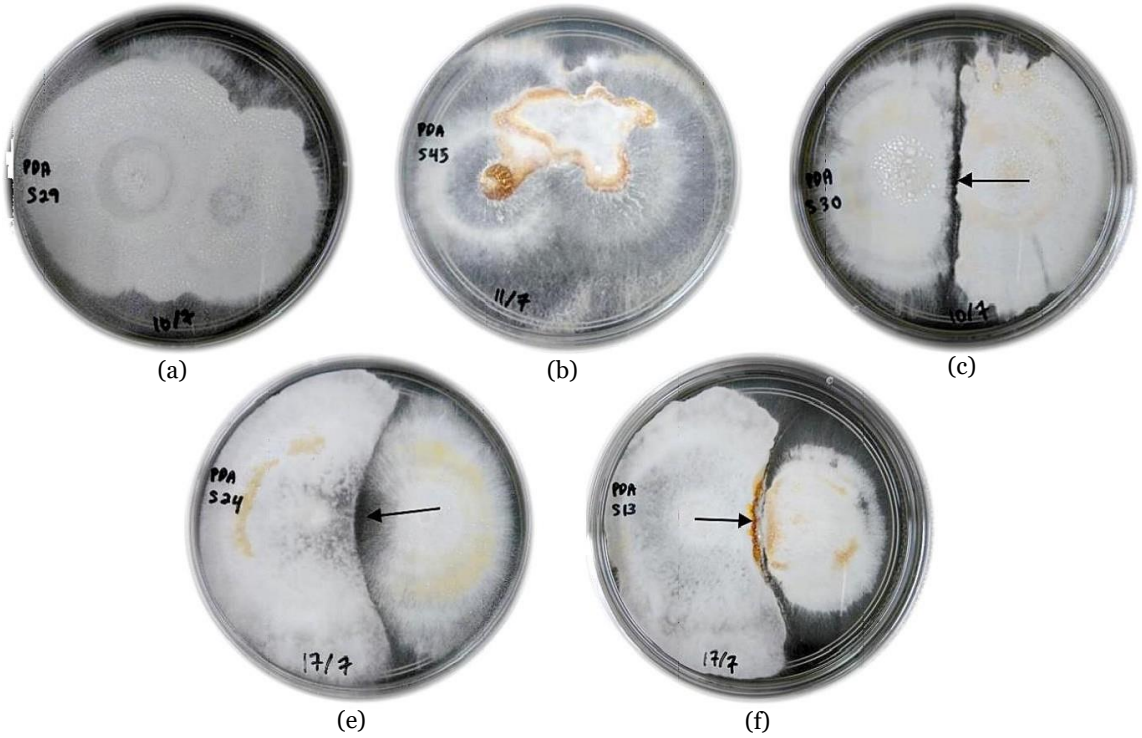

Figure 3. Somatic compatibility of *Ganoderma* on 9 cm (diameter) PDA plate, where arrows showing formation of inhibition zone or barrage. (a) Compatible reaction of self-pairing (control) between C4P3-2; (b) Compatible reaction between isolates (C5P3-1) and C5P3-2) within an infected palm; (c) incompatible reaction between isolates (C4P3-2 and C4P3-3) within an infected palm; and (d and e) incompatible reactions between isolates (C4P2 C4P3- 2, and C3P1 C3P3) of neighbouring palm or within a cluster

Latiffah *et al.* (2005) reported that genetic variation occurred in pathogens originating from the same species or closely related species. Compatibility between different samples of *Ganoderma* species in oil palm plantation were rare because this occurred only in very few samples and most of the time none of them were compatible (Miller *et al.*, 1999; Pilotti, 2005; Latiffah & Ho, 2005; Nusaibah *et al.*, 2010). *Ganoderma* species in the oil palm plantations were genetically heterogeneous due to sexual reproduction that involved dikaryotization of basidiospores and produced distinct individuals (Chan *et al.*, 2011). The findings based on somatic incompatibility also have been re-confirmed with Amplified fragment length polymorphism (AFLP) analysis by Nusaibah *et al.* (2010). This suggests that dispersal of basidiospores plays an important role in the epidemiology of disease as the basidiospores become infective through the build-up of inoculum source (Abdullah, 2000; Hasan *et al.*, 2005). Hence, preventive measures to minimize basidiospores spread should be taken for better disease management, such as removing as much inoculum sources (infected plant tissues) as possible in and around the planting areas and removing existing basidiocarps.

Moreover, the phenotypic similarity between isolates C2P3-2 and C2P2-2 does not corresponded to the genotypic similarity. On the other hand, genotypic similarity between isolates C5P3-1 and C5P3-2 does not corresponded to the phenotypic expression.

IV. CONCLUSION

There was narrow a cultural morphological variability (93 to 100% similarity) among the *Ganoderma* isolates, and complete similarity between C2P2-2 and C2P3-2 does not associated to the somatic compatibility. The isolates may exhibit similar phenotype, but not necessarily have similar genotype, and vice versa. Among all the isolates, only C5P3-1 and C5P3-2 isolated from the same infected palm demonstrated somatic compatibility (genetically identical). Generally, an infection of *Ganoderma* in a single palm and neighbouring palms were caused by multiple unidentical strains. This further conclude that spread of *Ganoderma* species in oil palm due to root-to-root contact is not common, and the basidiospores dissemination may have play an important role in the disease epidemiology, and further research and management strategies of the disease should focus on this.

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