

## Morphological Characteristics and Somatic Incompatibility of *Ganoderma* from Infected Oil Palm from Three Inland Estates

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### ABSTRACT

The morphological characteristics of *Ganoderma basidiomata* from infected oil palms from three inland estates showed some variations, but all fall within the description of *G. boninense*, based on Steyaert's classification system (1967, 1975). Pairings of *G. boninense* isolates from the same estate showed that there was somatic incompatibility among the isolates which indicated that the isolates were distinct individuals and not clones of single genotypes.

**Keywords:** *Ganoderma*, oil palm, somatic incompatibility

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### INTRODUCTION

Basal stem rot caused by *Ganoderma* is the most serious disease of oil palm in Malaysia. The disease was first reported by Thompson (1931) and was initially considered a disease of older palms. However, with successive planting of oil palm in the same field and planting in areas that previously supported coconut, the disease began to appear in much younger palms of 10 to 15 years old (Turner, 1981). Now, *Ganoderma* has been found to infect oil palms of 1 to 2 years old, particularly in replanted areas (Singh, 1990).

The highest disease incidence is in coastal areas (Navaratnam, 1964; Turner, 1981; Khairudin, 1990). This is attributed to planting in previous coconut stands (Turner, 1981) and the nature of the soil and its water table (Turner, 1981; Singh, 1990; Khaurudin, 1990). In the past ten years, the disease has also been detected in inland areas (Singh, 1990; Khairudin, 1990; Benjamin and Chee, 1995). The disease usually occurs in the valleys and occasionally on the hill slopes (Singh, 1991). Disease incidence between 2% to 5% had been reported in two inland areas i.e. Gomali Estate, Johor, and Regent Estate, Malacca (Khairudin, 1990). Other disease incidences in inland areas were in Batu Anam, North Johor, where 29% to 50% of 27-year-old oil palms planted in Munchong soil series were affected by the disease and in Gemencheh, Negeri Sembilan, where 11.7% of 27-year-old oil palms planted on lateritic soil were also infected by the disease (Benjamin and Chee, 1995). Pathogenicity test of *G. boninense* on oil palm seedlings grown on inland and coastal soil types showed mortality rate of 60% to 70% which indicated that oil palm seedlings grown on inland soils are as easily infected by the disease as those grown on coastal soils (Ho and Khairudin, 1995).

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A number of studies have been carried out on species identification of *Ganoderma* in the coastal areas but there is very limited information on *Ganoderma* from the inland areas. Thus, the present investigation was conducted to study the morphological characteristics of *Ganoderma* from three inland estates and to determine the somatic incompatibility of the *Ganoderma* isolates from the same estate to establish whether they were different genotypes or clones of single genotypes.

### MATERIALS AND METHODS

#### Morphological Studies

A total of 182 *Ganoderma basidiomata* were collected from living infected oil palms from three inland estates, namely, Bukit Serampang Estate, Tangkak, Johor; Regent Estate, Tampin, Negeri Sembilan and Paya Lang, Segamat, Johor. Most of the infected oil palms showed obvious skirting symptom. Collection of basidiomata was made from the same tree as well as adjacent trees.

The morphological characteristics used to study the *Ganoderma basidiomata* were the same as those described by Steyaert (1967, 1975) and Ho and Nawawi (1986).

#### Isolation of *Ganoderma* Mycelium

Isolation of mycelium was made from the basidiomata. Small pieces of basidiomata (4 mm x 4 mm x 5 mm) were cut and surface sterilised with 5% sodium hypochloride for 3 min, 0.1% HgCl<sub>2</sub> for 1 min, and 2% sodium hypochloride for 2 min, blotted dry and plated on malt agar (MA) with streptomycin sulphate in Petri dishes. Visible mycelia grown from the pieces of basidiomata were subcultured onto fresh MA medium in Petri dishes.

### Somatic Incompatibility Studies

The *Ganoderma* isolates studied are listed in Table 1. The isolates were paired in all combinations i.e. from the same oil palm tree or from different trees within the same estate or different estates. The procedures used were similar to those described by Latiffah *et al* (2002), and the interactions between the isolates were rated as degrees of antagonism according to the ratings by Adaskaveg and Gilbertson (1987) i.e. 0 = compatible, 1 = weak, 2 = moderate and 3 = strong.

**Table 1:** *Ganoderma boninense* isolates used in somatic incompatibility study

Bukit Serampang (BS) Estate	Ganoderma Isolate	
	Regent (R) Estate	Paya Lang (PL) Estate
09BS2	11RG1	13PL1
09BS3	11RG2	13PL2
09BS4	15RG1	13PL4
22BS1	15RG2	23PL1
22BS2	15RG3	23PL2
39BS3	39RG1	32PL2
39BS4	39RG2	32PL3
39BS5	04RG2	38PL2
13BS1	04RG3	38PL4
14BS2	04RG4	14PL4
16BS1	17RG3	07PL2ii
21BS1	18RG1	39PL1
37BS2	19RG2	
40BS2	37RG2	
10BS1D		
14BS3D		

Note: Isolates with the same alphabets and the same numbers in front of the alphabets are from the same oil palm tree. The last number denotes the individual isolates.

## RESULTS

### Morphological Characteristics

The morphological characteristics varied among the basidiomata collected within the same estate as well as between the three estates. The shape of the basidiomata could be sub-ungulate, flabellate, diamidiate or irregular with corrugations and concentric rings. The basidiomata were either sessile or stipitate.

The size of basidiomata from Bukit Serampang Estate ranged from 2.5 to 11.0 cm in diameter. The

majority of the basidiomata were very glossy (index of 4 to 5) and the colour ranged from reddish brown (94.8%) to yellowish brown (5.15%). The context was 0.25 to 2.5 cm thick and brownish in colour. The pore tube layer was slightly lighter than the context layer and the thickness ranged from 0.14 to 2.0 cm. The basidiospores were ellipsoidal, 8.5 x 12.5 to 6.0 x 7.5  $\mu\text{m}$ , yellow to yellowish brown with short, thin echinules. The pores were circular, 100 to 130  $\mu\text{m}$  in diameter and the dissepiments were 25 to 120  $\mu\text{m}$  thick. The pore surface colour ranged from white to orange.

Basidiomata from Regent Estate were 4.3 to 23.5 cm in diameter, very glossy (index of 4 to 5), and the colour ranged from reddish brown (90.9%) to yellowish brown (9.1%). The thickness of the context layer varied from 0.14 to 2.0 cm and the pore tube layer from 0.35 to 1.50 cm. The basidiospores were 9.0 x 12.5 to 5.5 x 11.5  $\mu\text{m}$ , yellowish brown, ellipsoidal with thin short echinules. The pores were circular, 100 – 200  $\mu\text{m}$  in diameter and the dissepiments ranged from 20 to 100  $\mu\text{m}$  thick. The colour of the pore surface ranged from white to orange.

The basidiomata from Paya Lang Estate varied from 4.5 to 19.0 cm in diameter, colour ranged from reddish brown (86.1%) to yellowish brown (13.8%) with an index of glossiness from 3 to 5. The context layer ranged from 0.6 to 1.9 cm and the pore tube layer was 0.5 to 1.5 cm. Like the basidiospores from Bukit Serampang Estate and Regent Estate, the basidiospores were yellow to yellowish brown, ellipsoidal, 8.5 x 12.0 to 4.5 x 6.5  $\mu\text{m}$ . The pores were also circular about 85 to 300  $\mu\text{m}$  in diameter and the dissepiments, 20 to 100  $\mu\text{m}$  thick. The pore surface was white.

Although two major colour-types i.e. reddish brown and yellowish brown were observed in the basidiomata from the three estates, variations existed in other morphological characteristics of the basidiomata within each colour-type, and overlapping of morphological characteristics also occurred between the two colour-types. Based on Steyaert's classification system (1967, 1975), the morphology of the basidiomata from the three estates falls within the description of *G. boninense*

### Somatic Incompatibility

Moderate to strong incompatible (antagonistic) reactions were produced in all pairings of isolates except for self-pairs (Tables 2, 3 and 4). The incompatible reactions between two interacting isolates varied from a sparse zone between the isolates, to a clear line of demarcation produced by a raised line of dense hyphae (Figures 1 and 2). Not only isolates of different palms from the same estates or different estates produced incompatible reactions but also isolates from the same palm tree. Incompatible reactions were visible after 7 days and became more distinct after about 10 to 14 days. Compatible reactions in which the mycelia of the two isolates merged and formed a single colony were only observed in self-pairs.

**Table 2:** Somatic incompatibility of *G. boninense* isolates from Bukit Serampang Estate

	09BS2	09BS3	09BS4	22BS1	22BS2	39BS3	39BS4	39BS5	13BS1	14BS2	16BS1	21BS1	25BS1	37BS2	40BS2	10BS1D	14BS3D
09BS2	0																
09BS3	2	0															
09BS4	2	2	0														
22BS1	2	3	2	0													
22BS2	3	3	3	3	0												
39BS3	2	3	3	3	3	0											
39BS4	3	3	3	2	2	3	0										
39BS5	3	3	3	3	2	2	2	0									
13BS1	3	3	3	3	2	3	3	3	0								
14BS2	2	2	3	2	2	2	3	2	3	0							
16BS1	3	3	3	3	3	2	2	2	3	3	0						
21BS1	3	3	3	3	2	3	2	2	2	3	3	0					
25BS1	3	3	3	3	3	2	2	3	2	3	2	2	0				
37BS2	1	3	1	3	3	3	3	3	3	3	3	2	2	0			
40BS2	2	3	3	2	2	3	2	2	3	3	3	2	2	3	0		
10BS1D	2	2	3	3	3	2	3	3	2	2	3	3	3	2	2	0	
14BS3D	2	3	3	3	3	2	2	3	3	3	2	3	3	3	2	2	0

Note: 1 = weak interaction, 2 = moderate interaction, 3 = strong interaction

**Table 3:** Somatic incompatibility of *G. boninense* isolates from Paya Lang Estates

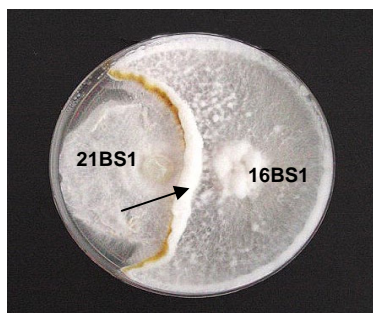
	13PL1	13PL2	13PL4	23PL1	23PL2	32PL2	32PL2ii	38PL2	38PL4	14PL4	07PL2ii	39PL1
13PL1	0											
13PL2	2	0										
13PL4	2	2	0									
23PL1	2	2	2	0								
23PL2	2	2	2	3	0							
32PL2	3	3	3	3	3	0						
32PL3	3	3	3	3	3	2	0					
38PL2	3	2	3	2	2	3	3	0				
38PL4	3	2	3	2	2	3	3	3	0			
14PL4	3	3	3	3	3	2	2	3	3	0		
07PL2ii	2	2	2	2	2	3	3	3	2	3	0	
39PL1	2	3	2	3	2	3	3	2	2	3	2	0

Note: 1 = weak interaction, 2 = moderate interaction, 3 = strong interaction

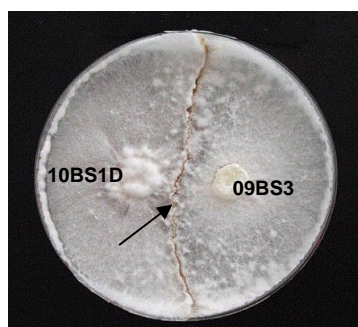
**Table 4:** Somatic incompatibility of *G. boninense* isolates from Regent Estate

	11RG1	11RG2	15RG1	15RG2	15RG3	39RG1	39RG2	04RG2	04RG3	04RG4	17RG3	18RG1	19RG2	37RG1
11RG1	0													
11RG2	2	0												
15RG1	3	2	0											
15RG2	2	2	2	0										
15RG3	2	2	2	2	0									
39RG1	3	3	2	3	3	0								
39RG2	3	3	2	2	3	2	0							
04RG2	2	3	2	2	2	2	2	0						
04RG3	3	3	2	2	2	2	3	2	0					
04RG4	3	3	3	3	3	3	3	3	3	0				
17RG3	2	3	3	2	3	2	2	3	2	3	0			
18RG1	3	2	3	3	3	3	3	3	3	3	3	0		
19RG2	3	3	3	3	3	3	3	3	3	3	3	3	0	
37RG1	3	2	2	2	2	2	2	2	2	3	3	3	3	0

Note: 1 = weak interaction, 2 = moderate interaction, 3 = strong interaction



**Figure 1:** Incompatible reaction between two *G. boninense* isolates, 21BS1 and 16BS1, showing a line of dense and raised hyphae (arrow).



**Figure 2:** Incompatible reaction between two *G. boninense* isolates, 10BS1D and 09BS3, showing a line of demarcation (arrow) between the two isolates

## DISCUSSION

Although variations existed in the morphological characteristics of basidiomata from the three inland estates, the overall morphology of the basidiomata falls within the characteristics of *G. boninense* based on Steyaert's species description (1967, 1975). Variations in basidioma characteristics within a species have been reported for several species of *Ganoderma* and the variations may be caused by different environmental conditions and developmental stages of the basidiomata (Ho and Nawawi, 1985; Adaskaveg and Gilbertson, 1987; Zhao, 1989; Ryverden, 1994).

*Ganoderma boninense* was first reported by Ho and Nawawi (1985) to be the most common species associated with the basal stem rot of oil palm. Later, *G. boninense* was also found to be the most common and virulent species in areas with a high disease incidence, such as in the coastal areas (Idris *et al.*, 2000). The results of the present study showed that *G. boninense* is also the species found on infected oil palm in the inland areas.

Somatic incompatibility reactions between all paired isolates (except self-pairs) observed in this study indicate

that *G. boninense* isolates within each palm and each estate are genotypically distinct individuals and are not clones of genotypic individuals.

Similar results on somatic incompatibility of *Ganoderma* isolates within the same estates have been reported by Arifin *et al* (1996) and Miller *et al* (1999) which indicated that the *Ganoderma* populations are highly heterogenous over localized areas.

The presence of different genotypically distinct individuals suggests that the spread of the basal stem rot disease within an estate may not have occurred by mycelial spread from neighbouring infected palms. The numerous distinct individuals could have arisen via basidiospores. Although the role of basidiospores in disease infection is still unclear, they can colonise dead coconut and oil palm stumps and trunks left on the ground or buried in the soil which then can serve as inoculum for the basal stem rot disease of oil palms (Turner, 1981). The occurrence of genetically distinct individuals within an oil palm estate may also have originated from secondary inoculum sources such as infected debris left from the previous crop. A study by Abdullah (2000) suggested that disease infection and spread was from infected residues left in the soil.

In conclusion, although variations occurred in the morphological characteristics of *Ganoderma* basidiomata from the three inland estates, the *Ganoderma* falls within the description of *G. boninense* based on Steyaert's classification (1967, 1975). Somatic incompatibility shown by the isolates within each estate and within individual palms indicates that they are genotypically distinct individuals.

## ACKNOWLEDGEMENT

We would like to thank IOI Plantation for providing the *Ganoderma* basidiomata from the three inland estates for this study.

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