INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
13–689/2014/16–4–691–699
http://www.fspublishers.org

Full Length Article

Genetic and Morphological Diversity of *Ganoderma* Species Isolated from Infected Oil Palms (*Elaeis guineensis*)

Mohd Rashid Mohd Rakib[1], Choon-Fah Joseph Bong[1*], Ahmad Khairulmazmi[1] and Abu Seman Idris[2]
[1]*Department of Crop Science, Faculty of Agriculture and Food Sciences, Universiti Putra Malaysia Bintulu Sarawak Campus, Nyabau Road, 97000 Bintulu, Sarawak, Malaysia*
[2]*Ganoderma and Diseases Research for Oil Palm (GANODROP) Unit, Biological Research Division, Malaysia Palm Oil Board (MPOB), No. 6, Persiaran Institusi, Bandar Baru Bangi, 43000 Kajang, Selangor, Malaysia*
*For correspondence: josephbcf@upm.edu.my

Abstract

The objectives of this study were to investigate the diversity of *Ganoderma* species isolated from upper stem rot (USR) and basal stem rot (BSR) infected palms in term of their genetic and morphological characteristics. A total of 46 samples of *Ganoderma* species were collected randomly from two oil palm plantations, namely Betong and Miri in Sarawak, Malaysia. The samples were identified using multiplex polymerase chain reaction (multiplex PCR) to reveal three pathogenic *Ganoderma* species (*G. zonatum*, *G. boninense* and *G. miniatocinctum*). *Ganoderma zonatum* was the dominant species (71.7%), followed by *G. boninense* (26.1%) and *G. miniatocinctum* (2.2%). This suggests that *G. zonatum* may have played a more vital role in the epidemiology of the disease than previously believed. The multiplex PCR was a precise identification technique compared to morphological based identification. *Ganoderma* species in the oil palm plantation was genetically heterogeneous based on somatic compatibility test which is suggestive of disease spread via spore dispersal that generates new genetically distinct individuals. There were also significant variations within and between *Ganoderma* species in terms of their cultural morphology and basidiospore characteristics. Cluster analysis of the cultural morphology and scattered plot of basidiospore features also indicated that there was no distinct relationship within and between species, disease types or geographical origins of *Ganoderma* species. © 2014 Friends Science Publishers

Keywords: Basal stem rot; *Ganoderma boninense*; *Ganoderma miniatocinctum*; *Ganoderma zonatum*; Multiplex PCR; Oil palm disease; Somatic compatibility; Upper stem rot

Introduction

Oil palm (*Elaeis guineensis*) is the most important agriculture crop in Malaysia being one of the world's largest producers of palm oil with 5 million hectares of plantation nationwide which contributes to 31.4% of the world's palm oil supplies (USDAFAS, 2012). The state of Sabah and Sarawak in Malaysia are the largest producers with trend showing huge expansion of plantation in the state of Sarawak (MPOB, 2012). It is thus important to sustain the oil palm plantation industry. The major threats to the sustainability of the oil palm plantation are pests and diseases. Among the disease pathogens, *Ganoderma* species which cause basal stem rot (BSR) and upper stem rot (USR) are the most devastating to the oil palm (Hasan *et al.*, 2005; Pilotti, 2005; Rees *et al.*, 2012).

Until recently little was known about USR compared to BSR disease due to lack of study on the disease. The USR disease was considered a relatively minor disease at earlier time and was reported to be caused by *Phellinus noxius*, with *Ganoderma* species as a secondary pathogen (Paul, 1980). Later report by Turner (1981) noted the possibility of *Ganoderma* and *Thielaviopsis* species to cause the disease. USR gained more attention when a few cases of the disease were observed in oil palm plantations with presence of *Ganoderma* species (Abdullah *et al.*, 1999; Pilotti, 2005; Utomo *et al.*, 2005).

Turner (1981) listed 15 species of *Ganoderma* from different parts of the world associated with stem rot disease of oil palm. Among them, seven species were reported in Malaysia namely *G. boninense*, *G. zonatum*, *G. miniatocinctum* and *G. tornatum*, *G. applanatum*, *G. chalceum*, *G. lucidum* and *G. pseudoferreum*. Earlier study in Malaysia by Ariffin *et al.* (1989) assumed that all *Ganoderma* isolates from diseased oil palm were *G. boninense*. Later, Idris *et al.* (2000a) identified four species of *Ganoderma* associated with oil palm in Malaysia, namely *G. boninense*, *G. zonatum*, *G. miniatocinctum* and *G. tornatum*. The first three species were proven to be pathogenic to oil palm, while *G. tornatum* was non-pathogenic. Since both USR and BSR disease were related

To cite this paper: Rakib, M.R.M., C.F.J. Bong, A. Khairulmazmi and A.S. Idris, 2014. Genetic and morphological diversity of *Ganoderma* species isolated from infected oil palms (*Elaeis guineensis*). *Int. J. Agric. Biol.,* 16: 691–699

to the same pathogens, there should be possible features that could be used to distinguish them.

Traditionally, *Ganoderma* species has been identified based on their morphological characteristics (Adaskaveg and Gilbertson, 1989; Pilotti *et al.*, 2004; Latiffah and Ho, 2005). However, studies had shown that *Ganoderma* species were genetically heterogeneous since wide range of genetic variation were reported and caused by outcrossing over generations and different geographical origins (Miller *et al.*, 1999; Pilotti *et al.*, 2003). This leads to variation in their morphological characteristics even within same species (Hong *et al.*, 2001). Recently, molecular approach has been adapted to identify *Ganoderma* species such as through multiplex polymerase chain reaction (PCR) which is a more rapid and precise approach (Idris *et al.*, 2010; Wong *et al.*, 2012).

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. Hence, the aims for this study were to investigate the diversity of *Ganoderma* species isolated from USR and BSR infected oil palms in terms of their genetic and morphological characteristics.

Materials and Methods

Sample Collection and *Ganoderma* Isolation

Sample collection: A total of 46 samples of *Ganoderma* basidiomata were collected randomly from upper stem rot (USR) and basal stem rot (BSR) infected palms of oil palm plantations in Betong (1°54'34.26"N, 111°12'5.52"E) and in Miri (3°55'59.83"N, 113°43'47.79"E) in Sarawak, Malaysia. Fourteen (G1 to G14) and seven (G15 to G21) samples were collected from USR and BSR infected palms in Betong, respectively. Sixteen (G22 to G37) and nine (G38 to G46) samples were collected from USR and BSR infected palms in Miri, respectively. Field samples were brought to laboratory for pure culture isolation.

***Ganoderma* isolation:** The *Ganoderma* was isolated from the basidiomata using *Ganoderma* selective medium (GSM) as described by Ariffin and Idris (1992). Pure cultures of *Ganoderma* obtained were maintained on potato dextrose agar (PDA) for further analysis.

Genetic Diversity of *Ganoderma* Species in Oil Palm Plantations

For species identification of *Ganoderma* using multiplex PCR, the fungi were incubated in potato dextrose broth for 14 days and their mycelia were harvested, lyophilized and ground using liquid nitrogen before used for DNA extraction (Karakousis *et al.*, 2006; Wong *et al.*, 2012). The DNA was extracted using DNeasy extraction kit (Qiagen) and the DNA extract obtained was kept at −20°C until further use. Multiplex polymerase chain reaction (multiplex PCR) was conducted using Ganoderma4 Genotyping kit (Seeplex) supplied by the Malaysian Oil Palm Board (MPOB), which was able to identify four species of *Ganoderma*, namely *G. boninense*, *G. zonatum*, *G. tornatum* and *G. miniatocinctum* (Idris *et al.*, 2010; Wong *et al.*, 2012). The PCR product amplified using dual priming oligonucleotide (DPO) primers which involved 10 primers or five pairs of primer with forward and reverse combination linked with a polydeoxyinosine linker. The DPO technique increases sensitivity and specificity of the reaction by blocking non-specific priming (Chun *et al.*, 2007). Furthermore, 8-methoxypsoralen (8-MOP) provided in the kit function to eliminate template activity of contaminated DNAs, and internal control was also introduced during amplification to identify samples that may contain substances that can interfere with the reaction. The PCR reaction was programed with one cycle of initial denaturation at 94°C for 15 min and 40 cycles of denaturation at 94°C for 30 sec, annealing at 63°C for 90 sec and elongation at 72°C for 90 sec, followed by one cycle of further elongation at 72°C for 10 min (Idris *et al.*, 2010; Wong *et al.*, 2012). The amplified PCR product was evaluated by 1% agarose gel pre-added with ethidium bromide staining. DNA fragments on agarose gel were visualized under ultraviolet light. The five pairs of primer able to yield five bands, and *Ganoderma* species was identified based on the band sizes of 656, 415, 331 and 242 base pairs (bp) for *G. boninense*, *G. zonatum*, *G. tornatum* and *G. miniatocinctum*, respectively. Internal control was amplified at 900 bp.

Assessment of Genetic Similarity using Somatic Compatibility Test

Compatibility of all 46 *Ganoderma* isolates was tested using the modified method by Miller *et al.* (1999) on PDA instead of using 1% malt extract agar. Somatic compatibility was tested by pairing the isolates in all combinations and self-pairing as control. Mycelia plugs (8 mm) were transferred onto standard 9 cm PDA plate (pH adjusted to 5.5 with HCl) and placed 2 cm apart. The plates were incubated for 14 days in dark at ambient room temperature, and assessed and rated as either compatible or incompatible. Compatible isolates merged into single colony, whereas incompatible isolates formed inhibition zone or barrage.

Morphological Diversity of *Ganoderma* Species in Oil Palm Plantations

***In-vitro* cultural characteristics:** Mycelia plug (8 mm) from seven days old active culture was transferred onto the centre of a standard 9 cm PDA plate (pH adjusted to 5.5 with HCl) and incubated for 14 days in the dark at ambient temperature (Idris *et al.*, 2000b). The test for all isolates was run simultaneously to avoid bias due to external factors. The experiment was conducted in four replications.

The diameter was measured daily and the number of days required to fully cover the plate was also recorded. The colony texture, appearance of zone, surface and reverse colour using Munsell soil colour charts were recorded on the seventh day after inoculation. Qualitative data were transformed into code and a binary matrix was generated (Table 1). The binary data was subjected to cluster analysis using multivariate statistical package (MVSP version 3.13). 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). Analysis of variance (ANOVA) and the Duncan's New Multiple Range Test (DNMRT) for means comparison were performed using Statistical Analysis System (SAS version 9.2).

Basidiospore Characteristics

Basidiospores of *Ganoderma* were obtained by culturing them on rubber wood block (RWB) as adapted and modified from Breton *et al.* (2006) and Idris *et al.* (2006). RWB of size 6 x 6 x12 cm were soaked overnight in water, cleaned, and boiled in 0.2% malt extract and autoclaved for 6 h at 121ºC. The wood blocks were then covered with malt extract agar and four mycelia plugs (15 mm) were placed on different side of the block after the agar solidify. The blocks then incubated for 9 weeks in the dark and at ambient temperature until fully colonized with mycelia.

Fully colonize RWB was transferred to polypropylene bags (size 20 x 20 cm) containing unsterilized soil and sand (2:1) mixture medium. Half of the wood block was buried in the soil while other half was exposed to light. Three replications were done for each isolate and the experimental units were placed in an enclosed sheltered house with temperature range of 25-35ºC and relative humidity of 60-80%. Moisture was maintained with regular watering. Observations were made weekly on the development of basidiomata until basidiospores were produced.

Basidiospores were collected on glass slides placed beneath the basidiomata. The basidiospores were then transferred using inoculation needle onto other glass slide, stained with 0.1% lactophenol cotton blue and observed under light microscope at 1000X magnification with a camera attachment. Basidiospore shape was observed. Length and diameter of thirty basidiospores were measured from each basidiomata. Spore shape index (SSI) was calculated, which was the ratio of the diameter to the length of spore (Diameter x 100/Length) (Steyaert, 1980; Pilotti *et al.*, 2004). The data of SSI on spore length was scatter plotted.

Results

Symptoms of USR and BSR Infected Palms in the Field

Ganoderma basidiomata were collected based on appearance of the basidiomata on the infected palms.

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

Character	Description	Code
Days for full plate	<6 days	1
	6-7 days	2
	8-9 days	3
	>9 days	4
Mycelia density	Thin	5
	Dense	6
	Dense at centre only	7
Mycelia texture	Smooth	8
	Rough	9
	Fluffy	10
Surface texture	Adpressed	11
	Moderately wavy	12
	Strongly wavy	13
Colony concentric ring	Absence	14
	Presence	15
Surface pigmentation colour	No pigmentation (white)	16
	Pale yellow	17
	Yellow	18
	Yellowish brown	19
	Dark yellowish brown	20
Reverse pigmentation colour	No pigmentation	21
	Pale yellow	22
	Yellow	23
	Brownish yellow	24
	Yellowish brown	25

Fig. 1: Field symptoms of basal stem rot (BSR) and upper stem rot (USR) disease. (A) BSR infected palm showing *Ganoderma* basidiomata at base of the palm (arrow), (B) USR infected palm showing fractured stem at upper portion of the palm (arrow), (C) USR infected palm showing *Ganoderma* basidiomata at the upper fractured part of the palm, (C) Close up of *Ganoderma* basidiomata on an infected palm that was collected for isolation.

Table 2: Identity of *Ganoderma* isolates from upper stem rot (USR) and basal stem rot (BSR) infected palms in Betong and Miri

Location	Isolate	Disease	Identity
Betong	G1	USR	*G. boninense*
	G2	USR	*G. boninense*
	G3	USR	*G. zonatum*
	G4	USR	*G. zonatum*
	G5	USR	*G. boninense*
	G6	USR	*G. zonatum*
	G7	USR	*G. boninense*
	G8	USR	*G. boninense*
	G9	USR	*G. boninense*
	G10	USR	*G. boninense*
	G11	USR	*G. zonatum*
	G12	USR	*G. zonatum*
	G13	USR	*G. boninense*
	G14	USR	*G. zonatum*
	G15	BSR	*G. zonatum*
	G16	BSR	*G. zonatum*
	G17	BSR	*G. zonatum*
	G18	BSR	*G. zonatum*
	G19	BSR	*G. zonatum*
	G20	BSR	*G. zonatum*
	G21	BSR	*G. zonatum*
Miri	G22	USR	*G. boninense*
	G23	USR	*G. zonatum*
	G24	USR	*G. miniatocinctum*
	G25	USR	*G. zonatum*
	G26	USR	*G. zonatum*
	G27	USR	*G. zonatum*
	G28	USR	*G. zonatum*
	G29	USR	*G. zonatum*
	G30	USR	*G. zonatum*
	G31	USR	*G. zonatum*
	G32	USR	*G. zonatum*
	G33	USR	*G. zonatum*
	G34	USR	*G. zonatum*
	G35	USR	*G. zonatum*
	G36	USR	*G. zonatum*
	G37	USR	*G. zonatum*
	G38	BSR	*G. boninense*
	G39	BSR	*G. boninense*
	G40	BSR	*G. boninense*
	G41	BSR	*G. zonatum*
	G42	BSR	*G. zonatum*
	G43	BSR	*G. zonatum*
	G44	BSR	*G. zonatum*
	G45	BSR	*G. zonatum*
	G46	BSR	*G. zonatum*

Infection point of an USR infected palm was about one meter upwards from the ground, meanwhile the infection was at the base for a BSR infected palm (Fig. 1). This distinct characteristic has been used to distinguish between USR and BSR infected palm in this study.

Identification of *Ganoderma* Isolates

The multiplex PCR analysis was conducted successfully as there were clear bands on internal control at 900 bp for all the samples tested. Single clear band in each column revealed all 46 isolates tested fell into the identifiable genus and species by the multiplex PCR. *Ganoderma boninense*, *G. zonatum* and *G. miniatocinctum* were amplified at 656, 415 and 242 bp, respectively (Fig. 2). Out of the total 46 isolates collected from both oil palm plantations, 12, 33 and 1 isolates were identified as *G. boninense*, *G. zonatum* and *G. miniatocinctum*, respectively. *Ganoderma tornatum* was not detected in both plantations (Table 2). Based on the random sampling, higher population of *G. zonatum* was recoded in both oil palm plantations, which were 62% and 80% in Betong and Miri, respectively, for an overall average of 71.7%, followed by *G. boninense* which constituted 38% in Betong and 16% in Miri, for an overall average of 26.1%. This suggests that *G. zonatum* was the dominant pathogen of both USR and BSR infected palms. *Ganoderma miniatocinctum* comprised only 2.2% of the isolates and this was found on a USR infected palm in Miri.

Genetic Heterogeneity of *Ganoderma* Species

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), where the colonies merged into single colony (Fig. 3). This indicates *Ganoderma* species in the oil palm plantations were genetically heterogeneous.

Cultural Morphological Characters of *Ganoderma* Sp.

Tables 3 and 4 show that there were cultural morphological variations within and between species of *Ganoderma* in Betong and Miri. The colony diameter on fifth day after inoculation was significantly varied, where *G. boninense* ranged from 44.00 mm to 77.21 mm and 53.19 mm to 66.77 mm in Betong and Miri, respectively. *Ganoderma zonatum* ranged from 41.24 mm to 84.44 mm and 42.80 to 84.77mm in Betong and Miri, respectively. Single sample of *G. miniatocinctum* from Miri had produced colony diameter of 65.79 mm. Strongly wavy surface texture characteristic was observed in several isolates of *G. zonatum* as noted in G3, G4, G6, G12, G18, G43 and G45. However, this characteristic was not observed in any of the *G. boninense* and *G. miniatocinctum* isolates.

The dendrogram generated from the cultural morphological characteristics showed clearly the variations among *Ganoderma* species, which ranged from 60 to 100% (Fig. 4). The complete similarity (100%) was found in several samples of *G. zonatum* regardless their disease types and geographical origin, which were between G3 and G4 (isolates from USR infected palms in Betong); G15 and G33 (isolate from BSR and USR infected palms in Betong and Miri, respectively); G19 and G27 (isolate from BSR and USR infected palms in Betong and Miri, respectively); and G30 and G31 (isolates from USR infected palms in Miri). However, there was no distinct relationship between species, disease types or geographical origin of *Ganoderma* in the oil palm plantation in term or their cultural morphological characteristic.

Table 3: *In-vitro* cultural characteristics of *Ganoderma* isolates from upper stem rot (USR) and basal stem rot (BSR) infected palms in Betong assessed on potato dextrose agar (PDA)

Isolate	Disease	Species	*Colony diameter (mm)	Number of days for full plate	**Mycelia density	**Mycelia texture	**Surface texture	**Colony concentric ring	**Surface pigmentation colour	**Reverse pigmentation colour
G1	USR	*G. boninense*	77.21^{b}	6	Dense	Rough	Adpressed	Absence	White	Yellow
G2	USR	*G. boninense*	57.56^{e}	7	Dense	Fluffy	Adpressed	Presence	White	White
G5	USR	*G. boninense*	45.28^{ijk}	8	Thin	Fluffy	Adpressed	Presence	Yellow	Pale yellow
G7	USR	*G. boninense*	46.56^{hij}	9	Dense at centre	Rough	Moderately wavy	Presence	Yellow	Yellow
G8	USR	*G. boninense*	45.62^{ij}	14	Dense at centre	Smooth	Moderately wavy	Absence	Yellow	Brownish yellow
G9	USR	*G. boninense*	44.00^{ijk}	10	Dense	Smooth	Moderately wavy	Absence	Yellow	Brownish yellow
G10	USR	*G. boninense*	64.48^{d}	8	Dense	Fluffy	Adpressed	Absence	Dark yellowish brown	White
G13	USR	*G. boninense*	72.16^{c}	6	Dense	Rough	Adpressed	Absence	Dark yellowish brown	Yellowish brown
G3	USR	*G. zonatum*	46.11^{ij}	11	Dense	Rough	Strongly wavy	Presence	White	Pale yellow
G4	USR	*G. zonatum*	42.62^{jk}	11	Dense	Rough	Strongly wavy	Presence	White	Pale yellow
G6	USR	*G. zonatum*	41.24^{k}	12	Dense at centre	Smooth	Strongly wavy	Absence	White	Yellow
G11	USR	*G. zonatum*	50.57^{fgh}	8	Dense at centre	Smooth	Moderately wavy	Absence	Yellow	Brownish yellow
G12	USR	*G. zonatum*	47.58^{ghi}	9	Dense	Smooth	Strongly wavy	Presence	White	Pale yellow
G14	USR	*G. zonatum*	63.86^{d}	6	Dense	Smooth	Moderately wavy	Absence	White	Brownish yellow
G15	BSR	*G. zonatum*	65.73^{d}	7	Dense	Fluffy	Moderately wavy	Absence	White	Yellowish brown
G16	BSR	*G. zonatum*	84.44^{a}	5	Thin	Smooth	Adpressed	Absence	Yellow	White
G17	BSR	*G. zonatum*	51.65^{fg}	8	Dense at centre	Smooth	Adpressed	Presence	Yellow	White
G18	BSR	*G. zonatum*	52.96^{f}	8	Dense at centre	Rough	Strongly wavy	Absence	White	Brownish yellow
G19	BSR	*G. zonatum*	66.27^{d}	7	Dense	Smooth	Adpressed	Absence	Yellow	Yellow
G20	BSR	*G. zonatum*	78.23^{b}	6	Dense at centre	Smooth	Adpressed	Absence	Yellow	Pale yellow
G21	BSR	*G. zonatum*	71.75^{c}	7	Dense at centre	Rough	Adpressed	Absence	Yellow	Pale yellow

*Mean of colony diameter on the fifth day after inoculation. Mean within column with same superscripts were not significantly different at $p > 0.05$ by DNMRT

**Assessed on the seventh day after inoculation

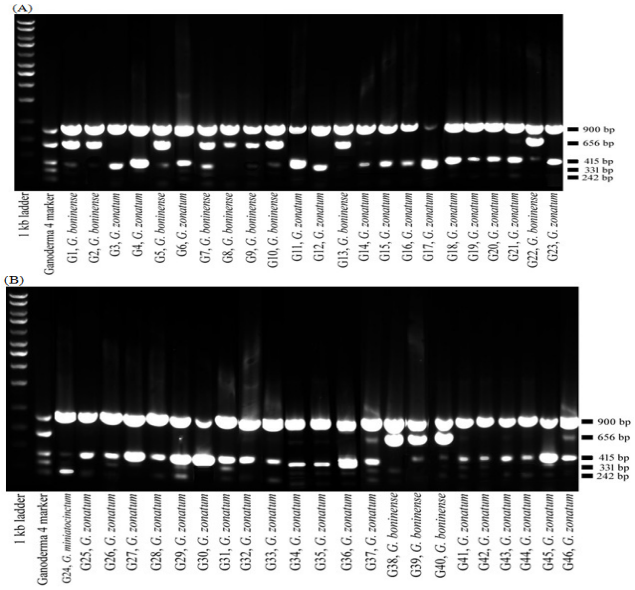

Fig. 2: DNA fragments yielded at 900 bp (internal control), 656 bp (*G. boninense*), 415 bp (*G. zonatum*) and 242 bp (*G. miniatocinctum*) indicating the identity of *Ganoderma* isolates from upper stem rot (USR) and basal stem rot (BSR) infected palms in Betong and Miri using multiplex PCR. (A) Isolate G1 to G23, and (B) G24 to G46

Basidiospore Characteristics of *Ganoderma* Species

Tables 5 and 6 show that there were significant variations of basidiospore length, diameter and spore shape index (SSI) within and between species of *Ganoderma* in Betong and Miri. The spore length, diameter and SSI of *G. boninense* in Betong ranged from 10.51 µm to 11.48 µm, 4.87 µm to 5.57 µm and 45.38 to 48.57 %, respectively, while that of *G. zonatum* ranged from 10.36 µm to 10.89 µm, 4.80 µm to 5.29 µm and 45.89 to 49.49 %, respectively. In Miri, *G. boninense* range from 10.61 µm to 11.23 µm, 4.92 to 5.16 µm and 46.40 to 47.02 % in their spore length, diameter and SSI, respectively. The spore length, diameter and SSI of *Ganoderma zonatum* in Miri range from 10.22 µm to 12.16 µm, 4.66 to 5.32 µm and 43.17 to 49.67 %, respectively. Single sample of *G. miniatocinctum* from Miri have spore length, diameter and SSI of 10.81 µm, 5.09 µm and 47.09%, respectively.

The spore shapes were either ellipsoid or narrowly ellipsoid. The scattered plot of SSI on spore length did not yield any specific pattern and thus it was unable to distinguish the *Ganoderma* species from different geographical origin, disease type or between any of the three species found in this study (Fig. 5).

Discussion

Besides the notorious BSR disease devastating oil palm plantations throughout Southeast Asia, USR disease is also becoming a major threat as USR infected palms were observed in the oil palm plantations in current study. Several studies reported occurrence of USR disease of oil palms such as in Sabah, Malaysia (Abdullah *et al.*, 1999), Papua New Guinea (Pilotti, 2005) and Indonesia (Rees *et al.*, 2012). These reports were not much compared to BSR

Table 4: *In-vitro* cultural characteristics of *Ganoderma* isolates from upper stem rot (USR) and basal stem rot (BSR) infected palms in Miri assessed on potato dextrose agar (PDA)

Isolate	Disease	Species	*Colony diameter (mm)	Number of days for full plate	**Mycelia density	**Mycelia texture	**Surface texture	**Colony concentric ring	**Surface pigmentation colour	**Reverse pigmentation colour
G22	USR	*G. boninense*	66.77^{d}	7	Dense	Rough	Adpressed	Absence	White	White
G38	BSR	*G. boninense*	54.24^{ghij}	8	Thin	Smooth	Adpressed	Presence	Yellow	Yellow
G39	BSR	*G. boninense*	53.19^{hijk}	8	Dense at centre	Rough	Moderately wavy	Absence	Yellowish brown	Yellow
G40	BSR	*G. boninense*	63.23^{de}	7	Dense at centre	Rough	Moderately wavy	Presence	White	Pale yellow
G23	USR	*G. zonatum*	84.77^{a}	5	Dense	Rough	Moderately wavy	Absence	Yellow	Yellow
G25	USR	*G. zonatum*	50.80^{ijk}	8	Dense	Rough	Moderately wavy	Absence	White	Pale yellow
G26	USR	*G. zonatum*	49.04^{jkl}	10	Dense	Smooth	Moderately wavy	Absence	Pale yellow	Yellow
G27	USR	*G. zonatum*	62.11^{def}	7	Dense	Smooth	Adpressed	Absence	Yellow	Yellow
G28	USR	*G. zonatum*	77.96^{bc}	6	Dense	Smooth	Adpressed	Absence	Yellow	White
G29	USR	*G. zonatum*	84.90^{a}	5	Thin	Fluffy	Adpressed	Presence	White	White
G30	USR	*G. zonatum*	53.56^{ghijk}	7	Dense at centre	Smooth	Moderately wavy	Presence	White	Yellow
G31	USR	*G. zonatum*	80.71^{ab}	6	Dense at centre	Smooth	Moderately wavy	Presence	White	Yellow
G32	USR	*G. zonatum*	73.78^{c}	6	Thin	Rough	Adpressed	Presence	Yellow	Pale yellow
G33	USR	*G. zonatum*	77.72^{bc}	6	Dense	Fluffy	Moderately wavy	Absence	White	Yellowish brown
G34	USR	*G. zonatum*	49.05^{jkl}	7	Dense at centre	Smooth	Moderately wavy	Absence	White	Yellow
G35	USR	*G. zonatum*	65.19^{de}	6	Dense at centre	Rough	Moderately wavy	Presence	White	Brownish yellow
G36	USR	*G. zonatum*	66.89^{d}	6	Thin	Smooth	Adpressed	Absence	White	White
G37	USR	*G. zonatum*	59.31^{efgh}	8	Thin	Fluffy	Adpressed	Absence	Yellow	Brownish yellow
G41	BSR	*G. zonatum*	47.30^{kl}	9	Dense at centre	Smooth	Moderately wavy	Presence	Pale yellow	Brownish yellow
G42	BSR	*G. zonatum*	60.00^{efg}	7	Dense	Smooth	Moderately wavy	Absence	Yellow	Brownish yellow
G43	BSR	*G. zonatum*	56.29^{fghi}	8	Dense	Rough	Strongly wavy	Absence	White	Yellow
G44	BSR	*G. zonatum*	74.29^{bc}	6	Dense	Rough	Moderately wavy	Absence	Yellow	Yellow
G45	BSR	*G. zonatum*	42.80^{l}	14	Dense	Rough	Strongly wavy	Absence	Yellow	Brownish yellow
G46	BSR	*G. zonatum*	42.90^{l}	8	Dense at centre	Rough	Moderately wavy	Absence	White	Yellow
G24	USR	*G. miniatocinctum*	65.79^{de}	7	Thin	Rough	Moderately wavy	Absence	White	Yellow

*Mean of colony diameter on the fifth day after inoculation. Mean within column with same superscripts were not significantly different at $p > 0.05$ by DNMRT

**Assessed on the seventh day after inoculation

occurrence reports because USR disease was often misinterpreted as BSR disease since *Ganoderma* species appeared in both diseases. USR infected palm was clearly observable from the single point of stem infection at one meter upwards from the ground with symptomless base as described by Hasan *et al.* (2005).

The multiplex PCR used for identification of *Ganoderma* species from USR and BSR infected palms in this study is a powerful tool. Although single clear bands were observed on the gel electrophoresis, some isolates showed multiple faint bands in a single column (Fig. 2). Wong *et al.* (2012) reported these as a common occurrence in multiplex PCR as several primers were involved in a single reaction which resulted in uneven and unspecific binding because different primer has its different temperature for denaturation, annealing and elongation. Faint band also can be related to some factors such as concentration of primer, template DNA, Mg^{2+} and number of cycle during PCR (Henegariu *et al.*, 1997; Elfath *et al.*, 2000). This suggests that presence of clearest visible band in a single column is sufficient to identify the *Ganoderma* isolates.

Among the three pathogenic species of *Ganoderma* found, *G. zonatum* was found to be the dominant species in both oil palm plantations. This indicate that *G. zonatum* was the major threat to oil palm in Sarawak, contrary to previous reports that suggested *G. boninense* as the most important pathogen (Ariffin *et al.*, 2000; Idris *et al.*, 2004; Sapak *et al.*, 2008; Rees *et al.*, 2009; Bivi *et al.*, 2010). The present finding suggests that *G. zonatum* plays a major role in the stem rot of oil palm and hence should be accorded due recognition and emphasis in research into better management of the oil palm.

Somatic incompatibility of all the isolates tested indicates they were genetically different from one another even in isolates from the same species. Latiffah *et al.* (2005) reported genetic variation occurred in pathogen that originated from the same species or closely related species. Compatibility between different samples of *Ganoderma* species in an oil palm plantations were rare because this occurred only in very few samples and sometimes none of them were compatible (Miller *et al.*, 1999; Pilotti *et al.*, 2003; Pilotti, 2005; Latiffah and Ho, 2005; Nusaibah *et al.*, 2010). This suggested that spread of USR and BSR disease in an oil palm plantation primarily was due to basidiospore spread, where large inoculum source accumulated in dead plant material and generated new individuals that were genetically distinct form their parent (Abdullah, 2000; Hasan *et al.*, 2005).

The wide range of variation in morphological characteristic can be related to the heterogeneity of *Ganoderma* species. In this study, the only cultural characteristic that appeared to distinguish *G. zonatum* from *G. boninense* and *G. miniatocinctum* was the strongly wavy characteristic of the colony in *G. zonatum*. However, this characteristic also varied and was not present in all of the *G. zonatum* isolates. Furthermore, the cultural appearances of fungi are also highly dependent on several factors such as type of media, pH and temperature (Adaskaveg and Gilbertson, 1989; Hoe *et al.*, 2009). Although similar (100%

Table 5: Basidiospore characteristics of *Ganoderma* isolates from upper stem rot (USR) and basal stem rot (BSR) infected palms in Betong

Isolate	Disease	Species	Basidiospore size (µm)		Spore shape index (%)
			Length	Diameter	
G1	USR	*G. boninense*	11.35^{ab}	5.14^{bcdefg}	45.38^{fg}
G2	USR	*G. boninense*	10.59^{defg}	4.94^{fghi}	46.75^{bcde}
G5	USR	*G. boninense*	10.58^{defg}	4.96^{efghi}	46.98^{bcde}
G7	USR	*G. boninense*	10.58^{defg}	4.87^{hi}	46.11^{defg}
G8	USR	*G. boninense*	10.55^{defg}	5.00^{efghi}	47.50^{abcde}
G9	USR	*G. boninense*	10.51^{defg}	5.05^{cdefgh}	48.12^{abcd}
G10	USR	*G. boninense*	11.48^{a}	5.57^{a}	48.57^{abc}
G13	USR	*G. boninense*	11.10^{bc}	5.38^{ab}	48.52^{abc}
G3	USR	*G. zonatum*	10.43^{fg}	5.15^{bcdefg}	49.49^{a}
G4	USR	*G. zonatum*	10.84^{cde}	5.28^{bc}	48.80^{ab}
G6	USR	*G. zonatum*	10.55^{defg}	5.02^{defghi}	47.65^{abcde}
G11	USR	*G. zonatum*	10.76^{cdef}	5.20^{bcde}	48.51^{abc}
G12	USR	*G. zonatum*	10.65^{defg}	5.17^{bcdef}	48.66^{ab}
G14	USR	*G. zonatum*	10.89^{cd}	5.29^{b}	48.74^{ab}
G15	BSR	*G. zonatum*	10.83^{cde}	5.03^{defghi}	46.45^{cdef}
G16	BSR	*G. zonatum*	10.88^{cd}	4.81^{hi}	44.30^{g}
G17	BSR	*G. zonatum*	10.36^{g}	4.91^{ghi}	47.47^{abcde}
G18	BSR	*G. zonatum*	10.78^{cdef}	5.16^{bcdef}	47.94^{abcde}
G19	BSR	*G. zonatum*	10.83^{cde}	5.25^{bcd}	48.61^{ab}
G20	BSR	*G. zonatum*	10.48^{efg}	4.80^{i}	45.89^{efg}
G21	BSR	*G. zonatum*	10.44^{fg}	4.95^{fghi}	47.53^{abcde}

Mean within column with same superscripts were not significantly different at $p > 0.05$ by DNMRT

Table 6: Basidiospore characteristics of *Ganoderma* isolates from upper stem rot (USR) and basal stem rot (BSR) infected palms in Miri

Isolate	Disease	Species	Basidiospore size (µm)		Spore shape index (%)
			Length	Diameter	
G22	USR	*G. boninense*	11.05^{bcde}	5.16^{abc}	46.77^{bcde}
G38	BSR	*G. boninense*	10.94^{bcde}	5.14^{abcde}	47.02^{abcde}
G39	BSR	*G. boninense*	10.61^{efg}	4.92^{def}	46.40^{bcdef}
G40	BSR	*G. boninense*	11.23^{bcd}	5.15^{abcd}	45.97^{bcdefg}
G23	USR	*G. zonatum*	11.01^{bcde}	5.19^{abc}	47.14^{abcde}
G25	USR	*G. zonatum*	12.16^{a}	5.32^{a}	43.91^{fgh}
G26	USR	*G. zonatum*	11.02^{bcde}	5.13^{abcde}	46.56^{bcde}
G27	USR	*G. zonatum*	10.90^{bcde}	4.70^{g}	43.17^{h}
G28	USR	*G. zonatum*	11.03^{bcde}	5.28^{ab}	47.99^{abcd}
G29	USR	*G. zonatum*	11.23^{bc}	5.13^{abcde}	45.76^{cdefg}
G30	USR	*G. zonatum*	10.58^{efg}	5.07^{bcde}	48.24^{abcd}
G31	USR	*G. zonatum*	10.89^{bcde}	5.09^{bcde}	46.81^{bcde}
G32	USR	*G. zonatum*	10.86^{bcdef}	5.00^{cde}	46.09^{bcdefg}
G33	USR	*G. zonatum*	10.52^{efg}	5.01^{cde}	47.72^{abcd}
G34	USR	*G. zonatum*	10.32^{fg}	5.11^{abcde}	49.67^{a}
G35	USR	*G. zonatum*	10.81^{bcdef}	4.72^{gf}	43.73^{gh}
G36	USR	*G. zonatum*	10.23^{g}	4.66^{g}	45.64^{defgh}
G37	USR	*G. zonatum*	10.78^{cdef}	5.09^{abcde}	47.32^{abcde}
G41	BSR	*G. zonatum*	10.22^{g}	4.96^{cde}	48.67^{ab}
G42	BSR	*G. zonatum*	10.50^{efg}	4.91^{ef}	46.88^{bcde}
G43	BSR	*G. zonatum*	10.68^{defg}	5.16^{abc}	48.40^{abc}
G44	BSR	*G. zonatum*	11.29^{bc}	4.92^{def}	43.64^{gh}
G45	BSR	*G. zonatum*	11.34^{b}	5.06^{bcde}	44.72^{efgh}
G46	BSR	*G. zonatum*	10.78^{cdef}	5.16^{abc}	47.94^{abcd}
G24	USR	*G. miniatocinctum*	10.81^{bcdef}	5.09^{bcde}	47.09^{abcde}

Mean within column with same superscripts were not significantly different at $p > 0.05$ by DNMRT

similarity) cultural morphological features were observed between G3 and G4, G15 and G33, G19 and G27, and G30 and G31 based on the dendrogram generated (Fig. 4), they

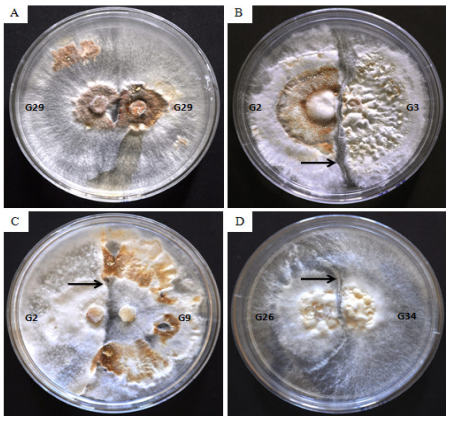

Fig. 3: Compatibility test. (A) Compatible reaction between same isolate (control) of G29 (*G. zonatum*) merged into single colony, (B) Incompatible reaction between G2 (*G. boninense*) and G3 (*G. zonatum*) showing formation of inhibition zone (arrow), (C) Incompatible reaction between G2 (*G. boninense*) and G9 (*G. boninense*) showing formation of barrage (arrow), (D) Incompatible reaction between G26 (*G. zonatum*) and G34 (*G. zonatum*) showing formation of barrage (arrow)

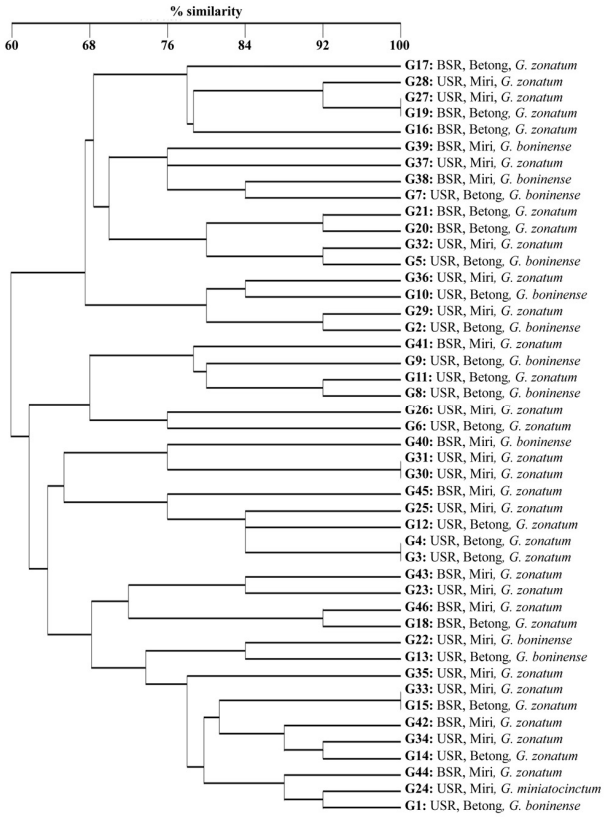

Fig. 4: Dendrogram (UPGMA) generated from 25 cultural morphological characters of *Ganoderma* species isolated from upper stem rot (USR) and basal stem rot (BSR) infected oil palms in the plantation block in Betong and Miri using simple matching coefficient

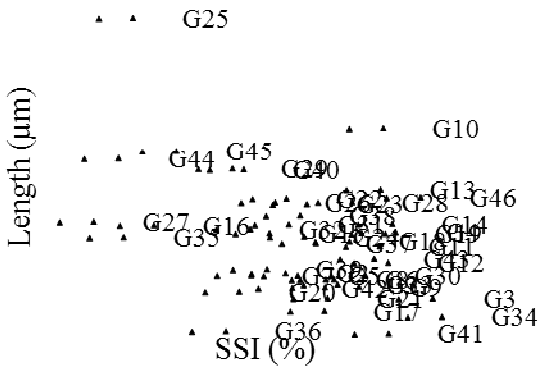

Fig. 5: Distribution of spore shape index (SSI) on spore length for 46 isolates of *Ganoderma* species isolated from upper stem rot (USR) and basal stem rot (BSR) infected palms in Betong and Miri

were still genetically different based on the somatic incompatibility between the isolates. This showed that different genotype in *Ganoderma* species may express similar morphological features (phenotype). The dendrogram also showed same species of *Ganoderma* may be separated by up to 40% dissimilarity, while different species of *Ganoderma* may have up to 92% similarity. This indicates that *Ganoderma* species in an oil palm plantation could not be separated according to their species, disease type or geographical origins based on their cultural morphological features. Hence, cultural characteristics observed in this study may not be useful as an identification tool. More precise tool such as the multiplex PCR should be used to identify the *Ganoderma* species accurately.

The basidiospore shapes of *Ganoderma* species in this study were similar as those reported by Pilotti *et al.* (2004) and the sizes falls within the description of *G. boninense* (Ho and Nawawi, 1985; Pilotti *et al.*, 2004; Latiffah and Ho, 2005). Latiffah and Ho (2005) also reported variation in basidiospore size in *Ganoderma* species from different geographical origins. Hence, the species of *Ganoderma* in an oil palm plantation cannot be differentiated solely based on the basidiospore morphology as wide range of variation occurred.

In conclusion, three pathogenic *Ganoderma* species, namely *G. zonatum*, *G. boninense* and *G. miniatocinctum* were found to be associated with USR and BSR infected palms in Sarawak, Malaysia. Biological studies of the *Ganoderma* isolate showed somatic incompatibility which indicates that the *Ganoderma* species in the oil palm plantation were genetically heterogeneous. The genetic heterogeneity further suggests spore dispersal as the mode of disease spread when genetically distinct individuals were generated through the build-up of large inoculum source for infection. Hence, preventive measures to minimize spore spread should be taken for better disease management. Isolates from same species also varied in term of their cultural and basidiospore morphological characteristics which suggest that morphological features is a less precise method for *Ganoderma* species identification compared to the multiplex PCR approach. Although similar pathogens were associated with URS and BSR, no clear connection was found between isolates isolated from USR and BSR infected palm in terms of their genetic and morphological characteristics that can be used to distinguish them. Among the three species, *G. zonatum* was the most dominant species followed by *G. boninense* and *G. miniatocinctum*. This indicates that *G. zonatum* may have played a more vital role in the epidemiology of the disease than previously believed. The current belief that *G. boninense* is the major causative pathogen of the disease may need to be reviewed to give more emphasis to studies on *G. zonatum* for more effective management of the disease.

References

Abdullah, F., S.B. Liew and N. Malik, 1999. Upper stem rot of oil palms (*E. guineensis*) in Langkon, Sabah. *In: Sustainable Crop Protection Practices in the Next Millennium*. Pp: 101–103. Sidek, Z., S.L. Bong, S.K. Vijaya, C.A. Ong and A.K. Husan (eds.). Malaysian Plant Protection Society, Malaysia

Abdullah, F., 2000. Spatial and sequential mapping of the incidence of basal stem rot of oil palm *(Elaeis guineensiss*) on a former coconut (*Cocos nucifera*) plantation. *In: Ganoderma Diseases of Perennial Crops*. pp. 183–194. Flood, J., P.D. Bridge and M. Holderness (eds.). CABI Publishing, Wallingford, United Kingdom

Adaskaveg, J.E. and R.L. Gilbertson, 1989. Cultural studies of four North American species in the *Ganoderma lucidum* complex with comparisons to *G. lucidum* and *G. tsugae*. *Mycol. Res.*, 92: 182–191

Ariffin, D., A.S. Idris, and H. Abdul Halim, 1989. Significance of the black line within oil palm tissue decay by *Ganoderma boninense*. *Elaeis*, 1: 11–16

Ariffin, D. and A.S. Idris, 1992. The *Ganoderma* selective medium (GSM). *Palm Oil Research Institute of Malaysia Inform. Series 8*. Kuala Lumpur, Malaysia

Ariffin, D., A.S. Idris and G. Singh, 2000. Status of *Ganoderma* in oil palm. *In: Ganoderma Diseases of Perennial Crops*. Pp: 49–68. Flood, J., P.D. Bridge and M. Holderness (eds.). CABI Publishing, Wallingford, United Kingdom

Bivi, M.R., M.S.N. Farhana, A. Khairulmazmi and A.S. Idris, 2010. Control of *Ganoderma boninense*: a casual agent of basal stem rot disease in oil palm with endophyte bacteria *in Vitro*. *Int. J. Agric. Biol.*, 12: 833–839

Breton, F., Y. Hasan, Hariadi, Z. Lubis and H. de Franqueville, 2006. Characterization of parameters for the development of an early screening test for basal stem rot tolerance in oil palm progenies. *J. Oil Palm Res.*, Special Issue: 24–36

Chun, J.Y., K.J Kim, I.T. Hwang, Y.J. Kim, D.H. Lee, I.K. Lee and J.K. Kim, 2007. Dual priming oligonucleotide system for the multiplex detection of respiratory viruses and SNP genotyping of CYP2C19 gene. *Nucleic Acids Res.*, 35: e40

Elfath, M.E., M.A. Ahmed, J.C. Robert and E.K. Paul, 2000. Multiplex PCR: Optimization and application in diagnostic virology. *Clin. Microbiol. Rev.*, 13: 559–570

Hasan, Y., H.L. Foster and J. Flood, 2005. Investigations on the causes of upper stem rot (USR) on standing mature oil palms. *Mycopathologia*, 159: 109–112

Henegariu, O., N.A. Heerema, S.R. Dlouhy, G.H. Vance and P.H. Vogt, 1997. Multiplex PCR: critical parameters and step–by–step protocol. *Bio–Tech.*, 23: 504–511

Ho, Y.W. and A. Nawawi, 1985. *Ganoderma boninense* Pat. from basal stem rot of oil palm (*Elaeis guineensis*) in Peninsular Malaysia. *Pertanika*, 8: 425–428

Hoe, P.K., C.F.J. Bong, K. Jugah and A. Rajan, 2009. Evaluation of *Metarhizium anisopliae* var. anisopliae (Deuteromycotina: Hyphomycete) Isolates and their effects on subterranean termite *Coptotermes curvignathus* (Isoptera: Rhinotermitidae). *Amer. J. Agri. Biol. Sci.*, 4: 289–297

Hong, K.K, S.S. Geon and G.K. Hong, 2001. Comparison of characteristics of *Ganoderma lucidum* according to geographical origins: Consideration of morphological characteristics. *Micobiology*, 29: 80–84

Idris, A.S., D. Ariffin, T.R. Swinburne and T.A. Watt, 2000a. The identity of *Ganoderma* species responsible for basal stem rot (BSR) disease of oil palm in Malaysia – Pathogenicity test. *Malaysian Palm Oil Board Inform. Series 103*, MPOB TT No. 77b

Idris, A.S., D. Ariffin, T.R. Swinburne and T.A. Watt, 2000b. The identity of *Ganoderma* species responsible for basal stem rot (BSR) disease of oil palm in Malaysia – Morphological characteristics. *Malaysian Palm Oil Board Inform. Series 102,* MPOB TT No. 77a

Idris, A.S., A. Kushairi, S. Ismail and D. Ariffin, 2004. Selection for partial resistance in oil palm progenies to *Ganoderma* basal stem rot. *J. Oil Palm Res.*, 16: 12–18

Idris, A.S., S. Kushairi, D. Ariffin and M.W. Basri, 2006. Technique for inoculation of oil palm germinated seeds with *Ganoderma*. *Malaysian Palm Oil Board Inform. Series 321,* MPOB TT No. 314

Idris, A.S., S. Rajinder, A.Z. Madihah and M.B. Wahid, 2010. Multiplex PCR–DNA kit for early detection and identification of *Ganoderma* species in oil palm. *Malaysian Palm Oil Board Inform. Series 531*, MPOB TS No.73

Karakousis, A., L. Tan, D. Ellis, H. Alexiou and P.J. Wormald, 2006. An assessment of the efficiency of fungal DNA extraction methods for maximizing the detection of medically important fungi using PCR. *J. Microbiol. Methods*, 65: 38–48

Latiffah, Z. and Y.W. Ho, 2005. Morphological characteristics and somatic incompatibility of *Ganoderma* from infected oil palm from three inland estates. *Malays. J. Microbiol.*, 1: 46–52

Latiffah, Z., K. Harikrishna, S.G. Tan, A. Faridah and Y.W. Ho, 2005. Random amplified polymorphic DNA (RAPD) and random amplified microsatellite (RAMS) of *Ganoderma* from infeted oil palm and coconut stumps in Malaysia. *Asia Pacific J. Mol. Biol. Biotechnol.*, 13: 23–34

Malaysian Palm Oil Board (MPOB), 2012. *Oil Palm Planted Area, Dec 2012*. MPOB, Bangi, Selangor, Malaysia

Miller, R.N.G., M. Holderness, P.D. Bridge, G.F. Chung and M.H. Zakaria, 1999. Genetic diversity of *Ganoderma* in oil palm plantings. *Plant Pathol.*, 48: 595–603

Nusaibah, S.A., S. Rajinder and A.S. Idris, 2010. Somatic incompatibility and AFLP analysis of four species of *Ganoderma* isolated from oil palm. *J. Oil Palm Res.*, 22: 814–821

Paul, H., 1980. *Fungus Diseases of Tropical Crops*. Cambridge University Press: 330–331

Pilotti, C.A., F.R. Sanderson and E.A.B. Aitken, 2003. Genetic structure of a population of *Ganoderma boninense* on oil palm. *Plant Pathol.*, 52: 455–463

Pilotti, C.A., F.R. Sanderson, E.A.B. Aitken and W. Armstrong, 2004. Morphological variation and host range of two *Ganoderma* species from Papua New Guinea. *Mycopathologia*, 158: 251–265

Pilotti, C.A, 2005. Stem rots of oil palm caused by *Ganoderma boninense*: Pathogen biology and epidemiology. *Mycopathologia*, 159: 129–137

Rees, R.W., J. Flood, Y. Hasan, U. Potter and R.M. Cooper, 2009. Basal stem rot of oil palm (*Elaeis guineensis*); mode of root infection and lower stem invasion by *Ganoderma boninense*. *Plant Pathol.*, 58: 982–989

Rees, R.W., J. Flood, Y. Hasan, M.A. Wills and R.M. Cooper, 2012. *Ganoderma boninense* basidiospores in oil palm plantations: evaluation of their possible role in stem rots of *Elaeis guineensis*. *Plant Pathol.*, 61: 567–578

Sapak, Z., S. Meon and Z.A.M. Ahmad, 2008. Effect of endophytic bacteria on growth and suppression of *Ganoderma* infection in oil palm. *Int. J. Agric. Biol.*, 10: 127–132

Steyaert, R.L, 1980. Study of some *Ganoderma* species. *Bull. du Jardin Bot. Nat. de Belgique*, 50: 135–186

Turner, P.D., 1981. *Oil Palm Diseases and Disorders*. Oxford University Press: 88–110

United States Department of Agriculture, Foreign Agricultural Service (USDAFAS), 2012. *World Agricultural Production*. USDA, USA

Utomo, C., S. Wernrr, F. Niepold and H.B. Deising, 2005. Identification of *Ganoderma*, the causal agent of basal stem rot disease in oil palm using a molecular method. *Mycopathologia*, 159: 159–170

Wong, L.C., C.F.J. Bong and A.S. Idris, 2012. *Ganoderma* species associated with basal stem rot disease of oil palm. *Amer. J. Appl. Sci.*, 9: 879–885

(Received 23 May 2013; Accepted 25 November 2013)