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Mating Compatibility and Restriction Analysis of *Ganoderma* Isolates from Oil Palm and Other Palm Hosts

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Abstrak: Keserasian mengawan dan analisis pembatasan kawasan *Internal Transcribed Spacer* (ITS) telah dijalankan untuk menentukan pertalian *Ganoderma boninense*, spesies yang sering dikaitkan dengan reput pangkal batang kelapa sawit, dengan pencilan *Ganoderma* daripada kelapa sawit yang dijangkiti, dua palma hiasan iaitu palma pinang merah (*Cyrtostachys renda*) and palma MacArthur (*Ptychosperma macarthurii*), pencilan dari tunggul kelapa (*Cocos nucifera*), *Ganoderma miniatocinctum, Ganoderma zonatum* and *Ganoderma tornatum*. Keputusan kajian menunjukkan *G. boninense* serasi dengan pencilan *Ganoderma* dari kelapa sawit, *G. miniatocinctum* and *G. zonatum*, pencilan daripada palma pinang merah, palma MacArthur dan pencilan tunggul kelapa. *G. boninense* tidak serasi dengan *G. tornatum*. Oleh itu, keputusan kajian mencadangkan pencilan *G. boninense*, *G. miniatocinctum, G. zonatum,* pencilan *Ganoderm*a daripada kelapa sawit, palma hiasan dan tunggul kelapa mewakili spesies biologi yang sama. Analisis pembatasan kawasan ITS menunjukan variasi di mana lima haplotip dijana daripada corak jalur pembatasan. Analisis kluster *unweighted pair-group method with arithmetic averages* (UPGMA) menunjukkan kesemua pencilan *Ganoderma* dikelompokkan dalam 5 kumpulan utama dan nilai kesamaan semua pencilan berjulat antara 97% hingga 100%. Dengan itu, analisis pembatasan kawasan ITS menunjukkan *G. boninense* dan pencilan *Ganoderma* daripada perumah palma yang lain mempunyai pertalian yang rapat. Berdasarkan ujian keserasian mengawan dan analisis pembatasan kawasan ITS yang dijalankan dalam kajian ini, pelbagai kumpulan spesies *Ganoderma* daripada kelapa sawit dan perumah palma yang lain, mempunyai pertalian yang rapat kecuali pencilan *G. tornatum* dan pencilan *Ganoderma* daripada teh dan getah.

Kata kunci: *Ganoderma,* Perumah Palma, Keserasian Mengawan, Kawasan ITS

Abstract: Mating compatibility and restriction analyses of Internal Transcribed Spacer (ITS) regions were performed to determine the relations between *Ganoderma boninense*, the most common species associated with basal stem rot in oil palm and *Ganoderma* isolates from infected oil palm, two ornamental palms, sealing wax palm (*Cyrtostachys renda*) and MacArthur palm (*Ptychosperma macarthurii*), an isolate from coconut stump (*Cocos nucifera*), *Ganoderma miniatocinctum, Ganoderma zonatum* and *Ganoderma tornatum*. The results showed that *G. boninense* was compatible with *Ganoderma* isolates from oil palm, *G. miniatocinctum* and *G. zonatum*, *Ganoderma* isolates from sealing wax palm, MacArthur palm and coconut stump. *G. boninense* was not compatible with *G. tornatum*. Therefore, the results suggested that the *G. boninense*, *G. miniatocinctum, G. zonatum,* and *Ganoderma* isolates from oil palm, ornamental palms and coconut stump could represent the same biological species. In performing a restriction analysis of the ITS regions, variations were observed in which five haplotypes were generated from the

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restriction patterns. An unweighted pair-group method with arithmetic averages (UPGMA) cluster analysis showed that all the *Ganoderma* isolates were grouped into five primary groups, and the similarity values of the isolates ranged from 97% to 100%. Thus, a restriction analysis of the ITS regions showed that *G. boninense* and the *Ganoderma* isolates from other palm hosts were closely related. On the basis of the mating compatibility test and the restriction analysis of the ITS regions performed in this study, a diverse group of *Ganoderma* species from oil palm and other palm hosts are closely related, except for *G. tornatum* and *Ganoderma* isolates from tea and rubber.

Keywords: *Ganoderma*, Palm Host, Mating Compatibility, ITS Regions

INTRODUCTION

Basal stem rot of oil palm that is caused by *Ganoderma* species is the most serious disease of oil palms in South-East Asia, causing severe economic losses in Malaysia (Turner 1981; Singh 1991; Ariffin *et al*. 1996) and North Sumatra (Hasan & Turner 1998). With more extensive planting and replanting from old oil palm estates, the infection of young palms has also been noted (Sanderson *et al*. 2000).

Several species of *Ganoderma* have been associated with basal stem rot in South-East Asia. Turner (1981) stated that as many as seven species were involved, namely *G. applanatum*, *G. boninense*, *G. chalceum*, *G. lucidum*, *G. miniatocinctum*, *G. pseudoferreum* and *G. tornatum*. Ho and Nawawi (1985) reported that *G. boninense* was the most common species to infect oil palms in Peninsular Malaysia. Based on morphological studies and pathogenicity tests, Idris *et al*. (2000a, b) reported that three species, *G. boninense, G. miniatocinctum* and *G. zonatum* are pathogenic. However, *G. miniatocinctum* and *G. zonatum* were significantly less aggressive than *G*. *boninense.*

The mating system of *G. boninense* was determined to be heterothallic and tetrapolar with multiple alleles at both mating type loci (Pilotti *et al*. 2002, 2003). By crossing the monokaryotic mycelia of different *Ganoderma* isolates, a mating test could be performed to determine the compatibility of those isolates and will thus assist in defining the *Ganoderma* species that are involved in causing basal stem rot in oil palms. Therefore, the present study was conducted to determine the mating compatibility of *G. boninense* with *Ganoderma* isolates that were isolated from oil palm, ornamental palm, coconut stump, *G. miniatocinctum, G. zonatum* and *G. tornatum* by using mating compatibility tests; the genetic relations among the *Ganoderma* isolates from different palm hosts in northern Peninsular Malaysia were also observed by using a restriction analysis of the internal transcribed spacer (ITS) region and the 5.8S gene of ribosomal DNA.

MATERIALS AND METHODS

Mating Compatibility Test

Ganoderma cultures of *G. boninense* (Per 71), *G. miniatocinctum* (337035), *G. tornatum* (NPG1) and *G. zonatum* (Por 68) were obtained from the Malaysian Palm Oil Board (MPOB) of Bandar Baru Bangi in Selangor, Malaysia. From these cultures, basidiocarps were artificially induced to obtain basidiospores for the generation of monokaryon cultures. The *Ganoderma* basidiocarps were induced by using rubber wood block as a substrate according to the method described by Idris *et al.* (2000b).

The basidiospores used to generate monokaryon cultures were obtained from two basidiocarps of oil palms (*Elaeis guineensis* - LPOP78/5/3, LPOP78/5/5), two ornamental palms, namely sealing wax palm (*Cyrtostachys renda* - SWP) and MacArthur palm (*Ptychosperma macarthurii* - MAP) and coconut stump (*Cocos nucifera* - BPC).

A single spore isolation was performed to obtain monokaryotic mycelia. The basidiocarp was cut vertically to reveal the hymenium layer. The surfaces of the spore-bearing tubes were gently scraped with extremely fine nichrome wires to dislodge the spores, which were then suspended in sterile distilled water. The spore suspension was then streaked onto water agar. After 24–48 h, the spores germinated, producing visible colonies. Each colony was then examined with an inverted microscope to ensure that it originated from a single spore. A single hyphal tip was carefully cut and transferred to potato dextrose agar (PDA). All colonies were subsequently checked under a compound microscope to confirm that the colonies were monokaryotic.

Based on the formation of dikaryons, monokaryons representing each mating type were identified; and finally, tester strains were selected to represent each mating type. A selection of the mating type was performed for all the *Ganoderma* isolates in a similar manner.

Selections for tester strains were performed according to the methods described by Pilotti *et al.* (2002). Tester strains for each isolate were obtained by crossing two identical sets of nine sibling monokaryons from the same basidiocarp. Compatible mating types arising from these random combinations were recorded, and for each isolate, four monokaryons were selected, each of which represented the putative genetic entities of A_1B_1, A_1B_2, A_2B_1 and A_2B_2. After tester strains were selected for all the isolates, mating was performed by crossing the selected tester strains between different isolates (Table 1).

G. boninense was crossed with *Ganoderma* isolates from oil palm, sealing wax palm, McArthur palm, coconut stump, *G. miniatocinctum*, *G. zonatum* and *G. tornatum*. *G. tornatum* was also separately crossed with these sets of isolates.

Table 1: List of monokaryon cultures used in mating compatibility tests.

Isolate	Host	Location	Tester strains
POP78/5/3	Oil palm (*Elaeis guineensis*)	Basidiocarp collected in Pelam Estate, Kedah (same trunk)	15,19,20,22
LPOP78/5/5	Oil palm (*Elaeis guineensis*)		23,24,25,29
SWP	Sealing wax palm (*Cyrtostachys renda*)	Basidiocarp collected at Batu Uban, Pulau Pinang	13,14,18,19
MAP	MacArthur palm (*Ptychosperma macarthurri*)	Basidiocarp collected at Minden, Pulau Pinang	2,4,6,8
G. boninense (Per71)	Oil palm (*Elaeis guineensis*)	Dikaryotic cultures obtained from MPOB (basidiocarps were induced)	2,3,5,6
G. miniatocinctum (337035)	Oil palm (*Elaeis guineensis*)		1,3,6,9
G. tornatum (NPG1)	Oil palm (*Elaeis guineensis*)		4,6,8,9
G. zonatum (Por68)	Oil palm (*Elaeis guineensis*)		1,4,7,9
BPC	Coconut stump *(Cocos nucifera)*	Basidiocarp collected at Balik Pulau, Pulau Pinang	1,2,4,8

Restriction Analysis of ITS Regions

A total of 21 isolates, which include all the *Ganoderma* isolates from oil palm, sealing wax palm, MacArthur palm, coconut stump, rubber and tea, were used in a restriction analysis of the ITS region of rDNA. Isolates from tea (TEA1) and rubber (03RG2) were also included in the analysis. Both isolates were obtained from the culture collection of the Rubber Research Institute of Malaysia (Sungai Buloh, Selangor). Both monokaryotic and dikaryotic isolates were used for isolates from oil palm, sealing wax palm and MacArthur palm. The isolates were cultured on PDA for 7 days, after which the mycelia were harvested and DNA was extracted by using a Dneasy® Mini Plant Kit (Qiagen, Hilden, Germany) DNA extraction kit.

The ITS+5.8S were amplified with ITS1 (5' TCC GTA GGT GAA CCT GCGG 3') and ITS4 (5' TCC GCT TAT TGA TAT GC 3') primer pairs (White *et al.* 1990). After optimisation, the amplification was conducted in a 50 µl reaction mixture with a final concentration of 1X PCR buffer, 0.1 µM of each primer, 4.0 mM $MgCl_2$, 10 mM dNTP mix, 2 units of *Taq* polymerase (Promega, WI, USA) and 4 ng of DNA. A polymerase chain reaction amplification was performed by using a PTC-100® Peltier Thermal Cycler (MJ Research Inc., MA, USA), with an initial denaturation of 2 min at 95°C followed by 35 cycles of 1 min denaturation at 94°C, 1 min annealing at 56°C and 2 min extension at 72°C, and a final extension of 10 min at 72°C.

The PCR products were digested with *Alu* I, *Bsu* 15I, *Eco* RI, *Hind* III, *Hin* fI, *Msp* I and *Taq* I (Fermentas, Vilnius, Lithuania) by using the reaction conditions recommended by the manufacturers. These restriction enzymes were

selected as representative four and six-base cutter enzymes. The restriction fragments were separated by electrophoresis in a 1.5% agarose gel by using Tris Borate EDTA (TBE) as a running buffer. The gels were run at 80 V and 500 mA for 160 min, and a 100 bp marker (Gene Ruler™, Fermentas) was used as a standard marker. After electrophoresis, the gels were stained with ethidium bromide, and the restriction patterns were visualised and photographed with a GeneSnap image acquisition system Syngene, (MD, USA). The restriction analysis was repeated twice to ensure that consistently digested fragments were obtained.

Each isolate was assigned to a composite haplotype as defined by the combination of the restriction patterns generated by the seven restriction enzymes. A binary data matrix based on the presence (1) and absence (0) of restriction fragments were analysed by using the Numerical Taxonomy System of Multivariate Program (NT-SYS) software package version 2.2 (Exeter Software, NY, USA) (Rohlf 2005). A genetic similarity matrix was then constructed by using a simple matching coefficient, and an unweighted pair-group method with arithmetic averages (UPGMA) cluster analysis was generated to infer the relations among the *Ganoderma* isolates from different palm hosts. Bootstrapping was performed to estimate the confidence limit of the dendrogram by using WinBoot (International Rice Research Institute, Manila, Philippines) with 1000 bootstrap replications (Yap & Nelson 1996). To test the goodness of fit of the cluster analysis to the data, a cophenetic value was constructed from the dendrogram. The degree of fit was based on Rohlf (2005).

RESULTS AND DISCUSSION

The presence of a clamp connection after mating between two monokaryons was an indication of compatibility, revealing that the alleles at both the A and B loci were different. The results of crosses between sibling monokaryons from a single basidiocarp showed that fertile dikaryons resulted in only one-quarter of the matings (Table 2), which indicated a bifactorial incompatibility with a tetrapolar mechanism (Pilotti *et al*. 2002).

According to the crosses, *G. boninense* was fully compatible with all *Ganoderma* isolates from oil palm, sealing wax palm, McArthur palm, coconut stump and *G. zonatum* (Table 3). The compatibility between *G. boninense* and *G. zonatum* was also reported by Pilotti *et al.* (2004). The results suggested that the alleles involved in the mating were different, resulting in 100% fertile dikaryon formation. These findings indicated that all these isolates were compatible, which also suggested that *G. boninense*, *G. zonatum* and the other isolates could belong to the same biological species.

Table 2: Crosses between sibling monokaryons from a single basidiocarp, and the alleles are assigned arbitrarily.

LPOP78/5/3		15	16	17	18	19	20	21	22	23
	Alleles	A1B1	A1B2	A2B2	A1B1	A2B2	A2B	A2B2	A1B2	A1B1
15	A1B1	–	–	+	–	+	–	+	–	–
16	A1B2			–	–	–	+	–	–	–
17	A2B2				+	–	–	–	–	+
18	A1B1					+	–	+	–	–
19	A2B2						–	–	–	+
20	A2B							–	+	–
21	A2B2								–	+
22	A1B2									–
23	A1B1									–

Note: + : clamp formation, – : clamp not formed

A compatibility of approximately 75% was observed between *G. boninense* and *G. miniatocinctum,* in which *G. boninense* formed 12 fertile dikaryons out of 16 crosses with *G. miniatocinctum*. The results indicated that both species lacked complete compatibility or partial compatibility. Based on an arbitrary standard that could be applied to equate the compatibility percentage to the nomenclatural rank suggested by Petersen and Hughes (1998), a 75% compatibility could represent different varieties of the same species. Partial compatibility was also used by Boidin (1986) as an index to measure the extent of speciation in which partially compatible individuals were in the process of speciation. However, based on the present study, there was not enough evidence to conclude that *G. boninense* and *G. miniatocinctum* represent the same species or different varieties, but based on morphological features by Steyaert (1972, 1975) and Ho and Nawawi (1985), *G. miniatocinctum* was regarded as synonymous with *G. boninense*.

G. boninense was not compatible with *G. tornatum*. No dikaryon was formed within the 16 crosses between *G. boninense* and *G. tornatum,* which indicated that both species were two distinct species. *G. tornatum* was also not compatible with *G. miniatocinctum*, *G. zonatum*, isolates from the oil palm, sealing wax palm, MacArthur palm and coconut stump. *G. tornatum* was saprophytic and only found on dead palms (Idris *et al.* 2000b). Pilotti (2005) reported that basal stem rot in Papua New Guinea was caused primarily by *G. boninense*, and *G. tornatum* was found to be a minor pathogen. Pilotti (2005) also reported that *G. tornatum* has a broad host range whereas *G. boninense* appears to be restricted to palms. The present mating studies were performed in accordance with studies conducted by Idris *et al.* (2000a, b) and Pilloti (2005), which indicated that *G. tornatum* was genetically different from *G. boninense*.

Table 3: Crosses between *G. boninense* tester strains and *Ganoderma* isolates from oil palm, sealing wax palm, McArthur Palm and coconut stump.

	G. boninense (Per 71)			
	2	3	5	6
G. miniatocinctum (337035)				
1	+	+	+	+
3	+	+	+	–
6	+	+	–	–
9	+	+	–	+
G. tornatum (NPG1)				
4	–	–	–	–
6	–	–	–	–
8	–	–	–	–
9	–	–	–	–
G. zonatum (Por68)				
1	+	+	+	+
4	+	+	+	+
7	+	+	+	+
9	+	+	+	+
Oil palm (LPOP78/5/3)				
15	+	+	+	+
19	+	+	+	+
20	+	+	+	+
22	+	+	+	+
Oil palm (LPOP78/5/5)				
23	+	+	+	+
24	+	+	+	+
25	+	+	+	+
29	+	+	+	+
Sealing wax palm (SWP)				
13	+	+	+	+
14	+	+	+	+
18	+	+	+	+
19	+	+	+	+
McArthur palm (MAP)				
2	+	+	+	+
4	+	+	+	+
6	+	+	+	+
8	+	+	+	+
Coconut (BPC)				
1	+	+	+	+
2	+	+	+	+
4	+	+	+	+
8	+	+	+	+

Note: + : clamp formation, – : clamp not formed

A *Ganoderma* isolate from coconut stump was compatible with *G. boninense,* which suggested that *Ganoderma* from oil palm and coconut stumps were closely related and could be from the same species. Similar results were also reported by Pilotti *et al*. (2004), in which *G. boninense* isolates from coconut were completely interfertile with *G. boninense* from infected oil palm. The stumps or trunks of coconut that are colonised by *Ganoderma* reportedly act as inoculum sources to newly planted oil palm (Turner 1981). Molecular analyses of random amplified polymorphic DNA (RAPD), random amplified microsatellite (RAMS) and restriction analyses of ITS regions also indicated that *Ganoderma* isolates from infected oil palm and coconut stumps were closely related (Latiffah *et al.* 2002).

Sealing wax palm and MacArthur palm are planted widely in tropical regions, and *Ganoderma*-causing basal stem rot has been recorded on these palms (Elliot & Uchida 2004). The present study indicated that *Ganoderma* isolates from both ornamental palms were compatible with *G. boninense* from infected oil palm, suggesting that cross infection could occur between the ornamental palms and oil palm.

The PCR product that was amplified with ITS1 and ITS4 primers was approximately 650 bp for all *Ganoderma* isolates from various palm hosts. Five restriction enzymes, namely *Taq* I, *Alu* I, *Bsu* 151, *Eco* RI and *Hin* f1, produced digested fragments for all PCR products for the 21 *Ganoderma* isolates used in this study (Table 4). Only the PCR products of the isolates from tea (TEA1) and rubber (03RG2) produced digested fragments when digested with *Hind* III, which showed that the PCR products of both isolates possessed restriction or cutting sites for *Hind* III. The PCR products of other *Ganoderma* isolates were undigested and exhibited ITS+5.8S region sizes of 650 bp. Similar results were shown when digesting with *Msp* I, when only the PCR products of *G. zonatum* and the isolates of MacArthur palm could be digested by *Msp* I. The PCR products of other isolates were undigested, which indicated that the PCR products did not have restriction sites for *Msp* I. Only consistent restriction fragments were used for the cluster analysis.

The restriction patterns of *G. boninense* from oil palm were similar to the restriction patterns of *G. miniatocinctum*, *G. zonatum*, seven oil palm isolates, three isolates from sealing wax palm, four isolates from MacArthur palm and an isolate from coconut stump. Based on the restriction patterns, five haplotypes were assigned among the *Ganoderma* isolates (Table 4).

Table 4: Haplotypes generated from a restriction analysis of *Ganoderma* isolates from oil palm, sealing wax palm, McArthur palm, coconut stump, tea and rubber.

Isolate	Host	Haplotype	Restriction patterns						
			Msp I	*Taq* I	*Alu* I	*Bsu* 15I	*Eco* RI	*Hind* III	*Hin* fI
G. boninense (Per 71)**	Oil palm	1	NR	A	A	A	A	NR	A
LPOP78/5/3/(15)*	Oil palm	1	NR	A	A	A	A	NR	A
LPOP78/5/3/(17)*	Oil palm	1	NR	A	A	A	A	NR	A
LPOP78/5/3/(15X17)**	Oil palm	1	NR	A	A	A	A	NR	A
LPOP78/5/3*	Oil palm	1	NR	A	A	A	A	NR	A
LPOP78/5/5 (21)*	Oil palm	1	NR	A	A	A	A	NR	A
LPOP78/5/5 (28)*	Oil palm	1	NR	A	A	A	A	NR	A
LPOP78/5/3/(21X28)**	Oil palm	1	NR	A	A	A	A	NR	A
SWP(140)*	Sealing wax palm	1	NR	A	A	A	A	NR	A
SWP(19)*	Sealing wax palm	1	NR	A	A	A	A	NR	A
SWP(14X19)**	Sealing wax palm	1	NR	A	A	A	A	NR	A
BPC**	Coconut stump	1	NR	A	A	A	A	NR	A
G. miniatocinctum (337035)**	Oil palm	2	NR	A	A	A	A	NR	C
G. zonatum (Por68)**	Oil palm	3	A	A	A	A	A	NR	A
MAP(2)*	MacArthur palm	3	A	A	A	A	A	NR	A
MAP(4)*	MacArthur palm	3	A	A	A	A	A	NR	A
MAP(2X4)**	MacArthur palm	3	A	A	A	A	A	NR	A
MAP*	MacArthur palm	3	A	A	A	A	A	NR	A
G. tornatum (NPG1)**	Oil palm	4	NR	A	B	A	A	NR	B
TEA1**	Tea	5	NR	A	A	A	A	A	B
03RG2**	Rubber	5	NR	A	A	A	A	A	B

Note: **dikaryotic isolate; *monokaryotic isolate; NR – no restriction sites in the PCR product; A, B, C – restriction patterns

A UPGMA cluster analysis was performed to estimate the variations observed among the isolates and to group the isolates. A good cophenetic correlation was obtained (r = 0.98) that showed a good fit for the cluster analysis and the data. Five clusters were generated from the dendrogram (Fig. 1). *G. boninense* isolates were clustered in Cluster 1 with an isolate from coconut stump (BPC), seven oil palm isolates [LPOP78/5/3, LPOP78/5/3(15), LPOP78/5/3(17), LPOP78/5/3(15x17), LPOP78/5/5(21x28), LPOP78/5/5(21) and LPOP78/5/5(28)] and three sealing wax palm isolates [SWP(14), SWP(19) and SWP(14x19)], with a bootstrap value of 56 and 100% shared similarity. *G. zonatum* was grouped with four isolates from MacArthur palm [MAP, MAP(2), MAP(4) and MAP(2x4)] in Cluster 3 with a bootstrap value of 41 and 100% shared similarity. *G. miniatocinctum* and *G. tornatum* formed Cluster 2 and Cluster 4, respectively. Isolates from tea (TEA1) and rubber (03RG2) were grouped in Cluster 5.

Despite the low bootstrap support of the branch, the clustering of all the *Ganoderma* isolates generally supports the results of the mating studies, and the grouping was performed according to the 5 haplotypes. Low bootstrap values could also indicate a close relation among the isolates in which the similarity among isolates ranged from 97%–100%. Isolates that were fully compatible and showed 100% similarity were grouped together, which also showed that isolates associated with basal stem rot were closely related. Moreover, in a study by Moncalvo (2000) with ITS parsimony data, a collection of *Ganoderma* isolates from palm were grouped together in a well-supported palm clade.

In the mating compatibility tests, *G. boninense* was found to be partially compatible with *G. miniatocintum*. *G. boninense* formed 12 fertile dikaryons out of 16 crosses with *G. miniatocinctum*, indicating that both isolates shared one common allele. Both species produced different haplotypes in which *G. boninense* exhibited haplotype 1 and *G. miniatocinctum* exhibited haplotype 2. From the restriction patterns between *G. boninense* and *G. miniatocinctum*, only the *Hin* f1 restriction pattern was different. The relation could be observed in the dendrogram where *G. boninense* and *G. miniatocinctum* were clustered in a separate cluster but shared greater than 99% similarity. The results of the mating compatibility and restriction analysis of ITS+5.8S supported the studies by Corner (1983) and Steyaert (1975), in which both authors regarded *G. boninense* and *G. miniatocinctum* as synonymous. Both species were also regarded as synonyms of *G. chalceum* (Corner 1983).

On the basis of morphological and pathogenicity studies, Idris *et al.* (2000a, b) reported that *G. boninense, G. miniatocinctum* and *G. zonatum* were associated with basal stem rot. Although the three species were compatible, some variability was observed based on a restriction analysis of the ITS regions. Although the number of tested isolates was small, this variability could indicate an outbreeding population. A similar study was performed by Pilotti *et al.* (2000) on *Ganoderma* spp. from Papua New Guinea by using RAPD analysis. Pilotti *et al*. (2000) reported that variations among closely related isolates of *Ganoderma* as shown by RAPD analysis are indicative of an outbreeding population, although the number of analysed isolates was small. Based on the present study and a number of morphological, somatic incompatibility and molecular studies, it has

been suggested that the pathogen is quite unlikely to represent a single population within the basal stem rot pathosystem of oil palms in South-East Asia (Miller *et al*. 2000).

Isolates of *Ganoderma* from tea (TEA1) and rubber (03RG2) showed 100% similarity, and they formed Cluster 2 with a bootstrap value of 76. Isolates from tea and rubber were most likely to be *G. philippii,* which was reported to cause red root disease on rubber and tea (Steyaert 1972; Khairudin 1991; Lee 2000). The results of the present study support the report of Ho and Nawawi (1985), which stated that the morphological and physiological characteristics of the *Ganoderma* species that caused basal stem rot on oil palm were clearly different from the species causing red root rot in tea and rubber.

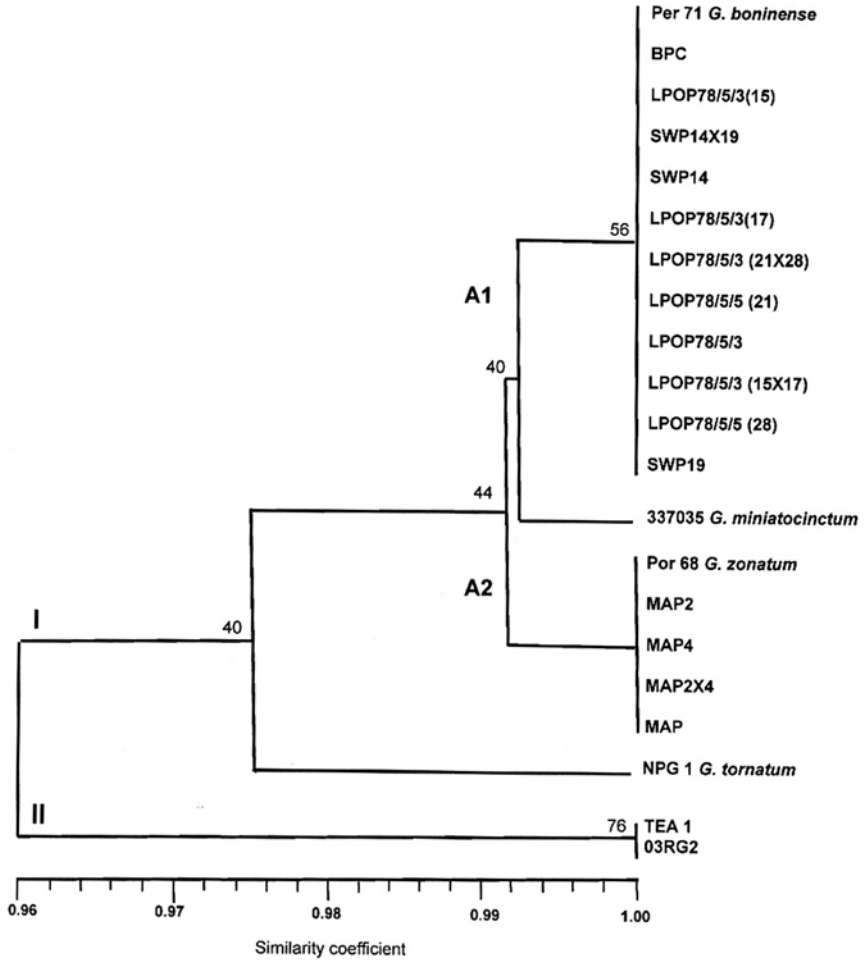

Figure 1: Dendrogram from the UPGMA cluster analysis with a simple matching coefficient based on the restriction fragments of the ITS regions. The percentages of the bootstrap values with 1000 replications are indicated next to the branch.

CONCLUSION

The present study suggested that monokaryotic mycelia in different *Ganoderma* species from palm and non-palm hosts might form dikaryotic mycelia, and under suitable conditions, they could infect susceptible oil palm. Therefore, monokaryotic mycelia might play a role in disease infection and spread. Other palm hosts could also become inoculum sources of basal stem rot infection. However, a mating compatibility test showed that *G. boninense, G. miniatocinctum, G. zonatum* and *Ganoderma* isolates from other palm hosts were compatible, and some variation was observed when using restriction analysis, which suggested an outbreeding population of the pathogens in association with the basal stem rot of oil palm. Therefore, monitoring *Ganoderma* fruiting bodies that were growing on the trees and on any available substrates in the oil palm field may be part of the components needed for the integrated disease management of basal stem rot disease.

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