African Journal of Biotechnology Vol. 10(66), pp. 14718-14723, 26 October, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.1096
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

First identification of *Ganoderma boninense* isolated from Sabah based on PCR and sequence homology

K.P. Chong[1], M.S. Lum[2], C.P. Foong[3], C.M.V.L. Wong[3], M. Atong[2] and S. Rossall[4]

[1]School of Science & Technology, Universiti Malaysia Sabah, 88400, Kota Kinabalu, Sabah, Malaysia.
[2]School of Sustainable Agriculture, Universiti Malaysia Sabah, 88400, Kota Kinabalu, Sabah, Malaysia.
[3]Biotechnology Research Institute, Universiti Malaysia Sabah, 88400, Kota Kinabalu, Sabah, Malaysia.
[4]Division of Plant and Crop Sciences, School of Biosciences, University of Nottingham, Sutton Bonington Campus, Loughborough, Leicestershire, LE12 5RD, United Kingdom.

Accepted 13 July, 2011

Basal stem rot (BSR) of oil palm (*Elaeis guineensis*) is caused by *Ganoderma boninense*, and, commercially, is one of the most devastating diseases in South East Asia. Losses of more than 80% of stands by the time they are halfway through their normal economic life have been reported. High incidence of BSR results in economic losses due to zero yields from dead palms and significantly reduced weight and number of fruit bunches in infected but living palms. Due to the importance of oil palm industry to Malaysia's economy, the transfer of any materials that are related to *Ganoderma* is strictly prohibited from Peninsular Malaysia to Sabah and Sarawak. No basic identification method using molecular techniques for the identification of *G. boninense* exist in this study for *G. boninense* isolates from Sabah. The only report on this pathogens isolate in Sabah was based on their morphology and pathogencity. In conjunction with the morphological similarities among the different isolates, there are numerous opinions on the aggressiveness of the pathogen in Sabah. The isolates of *G. boninense* from Sabah were claimed to be less aggressive compared to those from Peninsular Malaysia. This may be due to lower incidence of BSR in Sabah and Sarawak compared to Peninsular Malaysia. Since the oil palm industry is a fast income-generating tool, data related to it may be highly sensitive. Researchers, plantation managers and entrepreneurs are not forthcoming with the information on oil palm genetic materials or isolates of *G. boninense* with others. The current speculations on the Sabah isolates need further investigation. In this study, we report the identity of isolates *Ganoderma* from Langkon Oil Palm Estate in Sabah, Malaysia. The identity of these isolates was confirmed using DNA sequence analysis after PCR amplification. The latter method shows that the Sabah isolates were very similar to aggressive *G. boninense* strains FA5017 or FA5035 from West Malaysia, with a maximum similarity of 98%.

Key words: Aggressive, FA5017, FA5035, G1_5, isolate.

INTRODUCTION

Basal stem rot (BSR) disease in oil palm is caused by the attack of the pathogen called *Ganoderma boninense*. This fatal disease is considered the most serious disease of oil palm in Malaysia and some estates in South East Asia (Idris et al., 2000 a, b; Susanto, 2009) where losses can reach up to 80% after repeated planting cycles. Almost 90% of the estates in West Malaysia were reported with the presence of *G. boninense* (Khairuddin and Chong, 2008) but only 4% in Sabah (Idris A.S. personal communication). However, this is distant from the real situation in Sabah estates. Many methods have been attempted to control BSR, but to date, no method gives good control of *Ganoderma* infection in established

*Corresponding author. E-mail: chongkp@ums.edu.my.

Abbreviation: BSR, Basal stem rot.

plantations and some have technical limitations in application. In Malaysia, the pathogen attacking oil palm was originally identified as *G. lucidum* (Thompson, 1931), however, after decades of research of the general consensus, it now appears to be that *G. boninense* is the main species pathogenic to the oil palm, especially in South East Asia (Moncalvo, 2000). *In vitro* studies on the morphological characteristics of *G. boninense* by Idris et al. (2000a) found the colonies of *G. boninense* were white in colour on the surface and the reverse was darkened (pigmented). Cultures of *G. boninense* had an undulating surface in the darkened regions. The first indication of basidiomata formation was the appearance of a white mycelium after one to three weeks of incubation on rubber wood blocks, which then developed into a small, white, button-like structure. The apical end began expanding rapidly giving rise to bracket-like structures which were generally white when first formed, but as their length and width increased rapidly, the upper surface developed various yellowish-brown colour with concentric zonations (Idris, 2009). Until today, although, several methods based on biochemistry approach were used to detect *Ganoderma* infection, for example, using *Ganoderma* selective medium (GSM) or molecular DNA based technique such as polymerase chain reaction (PCR) amplification (Idris et al., 2003; Kandan et al., 2009; Latifah et al., 2002; Miller et al., 2000), the arguments on the difference of disease intensity due to aggressiveness of pathogen isolates between Peninsular Malaysia and Sabah remained unclear. To date, there is no report on molecular identification of *G. boninense* isolates from Sabah. The only report on Sabah isolates was merely based on their morphology and pathogenicity (Idris et al., 2001). Due to the importance of oil palm industry to Malaysia's economy, the transfer of any materials that are related to *Ganoderma* is strictly prohibited from Peninsular Malaysia to Sabah and Sarawak. In conjunction with the morphological simi-larities among the different isolates, there are numerous opinions on the aggressiveness of the pathogen in Sabah. The isolates of *G. boninense* from Sabah were claimed to be less aggressive compared to those from Peninsular Malaysia. This study is the first report on the molecular identity of *Ganoderma* isolates from Langkon Oil Palm Estate of Sabah, one of the prominent areas of *G. boninense* attack in Sabah.

MATERIALS AND METHODS

Fungal isolates and growth

Isolate of *G. boninense* was obtained from basidiocarp at the base of diseased oil palms from Langkon Estate, Sabah, Malaysia. Internal tissues of fruiting bodies were excised and cultured on *Ganoderma* selective medium (GSM). The medium was prepared as described by Ariffin and Idris (1992) in two parts. Part A comprised of bacto peptone (Difco) 5.0 g, agar extra pure powder (QRëC) 20.0 g, $MgSO_4.7H_2O$ (Merck) 0.25 g, K_2HPO_4 (QRëC) 0.5 g and distilled water pH 5.5 900 ml. Part B consisted of streptomycin sulphate (Sigma) 300 mg, chloramphenicol (Sigma) 100 mg, pentachloronitrobenzene (PCNB) pure (Aldrich) 285 mg, Ridomil (25% WP) 130 mg, Benlate T20 150 mg, ethanol 95% (Sigma) 20 ml, lactic acid (Sigma) 50% 2 ml, tannic acid (R and M Chemicals) 1.25 g and distilled water pH 5.5 80 ml. Part A was stirred on a hot plate at 100°C until dissolved before being autoclaved for 15 min. Part B was stirred for about 2 h at room temperature. Later, Part B was added to Part A when the autoclaved medium has cooled down to 45 to 50°C. GSM provides a useful tool for isolating *Ganoderma*, free from other contaminants. The content of fungicides and antibiotics is optimal to control growth of bacteria and other contaminating fungi, while allowing *Ganoderma* to thrive. The fungi which successfully grew on the GSM after 5 days were isolated, transferred and maintained in potato dextrose broth (PDB) at 25°C.

DNA extraction

DNA was isolated using the modified mini protocol for purification of total DNA from plant tissues by Qiagen. Approximately, 100 mg of *G. boninense* mycelium, originally grown in PDB, was harvested and homogenized using a bead and vortex at 1000 rpm for 4 to 5 min to disrupt the tissues. Buffer AP 1 (400 µl) and 4 µl of RNase A stock solution (100 mg ml^{-1}) were added to a maximum of 100 mg (wet weight). The disrupted fungal tissues were again vortexed vigorously. The mixture was incubated for 30 min at 65°C and mixed two to three times during the incubation by inverting the tube. This step lyses the cells. Buffer AP2 (130 µL) was added to the lysate, mixed and incubated for 5 min on ice. This step precipitates detergent, proteins and polysaccharides. The lysate was centrifuged for 5 min at 13,000 × *g* and lysate was then pipetted into the QIAshredder Mini spin column (lilac) placed in a 2 ml collection tube and centrifuged at 13,000 × *g*. The flow-through fraction from previous step was transferred into a new tube without disturbing the cell-debris pellet. Volumes (1.5 ml) of buffer AP3/E were added to the cleared lysate and mixed with a Gilson pipette. An aliquot of 650 µl of the mixture from the previous step, including any precipitate formed, was then pipetted into the DNeasy Mini spin column placed in a 2 ml collection tube. The tube was centrifuged for 1 min at 6000 x g and the flow-through was discarded. The collection tube was used for the next step. The previous step was repeated with the remaining sample and flow through and collection tube was discarded. The DNeasy Mini spin column was placed into a new 2 ml collection tube and mixed with 500 µl Buffer AW and centrifuged for 1 min at 6000 × *g*. The flow through was discarded and the collection tube was reused for the next step. Buffer AW (500 µl) was added to the DNeasy Mini spin column and centrifuged for 2 min at 13,000 × *g* to dry the membrane and finally the DNeasy Mini spin column was transferred to a 1.5 ml microcentrifuge tube and 50 µl of Buffer AE was pipetted onto the DNeasy membrane and incubated for 10 min at room temperature (25°C) and then centrifuged for 1 min at 6000 × *g* to elute.

Quantification of DNA concentration

DNA concentration was quantified using a NanoVue™ UV/Visible absorbance spectrophotometer at 260 nm (GE Healthcare). The absorbance recorded was 0.235 with a concentration of DNA 5.8 ng $µl^{-1}$.

Concentration = Abs260 × Factor

NanoVue will default to factors 50 for double stranded DNA, 40 for RNA and 33 for single stranded DNA and oligonucleotides.

Purity of DNA based on ratio A260/A280

Purity of the DNA was calculated based on the 260/280 nm absorbance ratio. This ratio gives an indication of purity; however, it is only an indication and not a definitive assessment. Pure DNA and RNA preparations have expected ratios of 1.7 to 1.9 and ≥ 2.0. The purity ratio obtained was 1.917.

PCR amplification of fungal ITS 1 and ITS 2 regions and the 5.8S gene

PCR amplification of the fungal DNA was done on ITS 1 and ITS 2 regions and the 5.8S gene using primers ITS1 and ITS4. TAE agarose 1.5% (w/v) was used for the agarose gel electrophoresis with 70 V for 40 min with a size of PCR amplicon approximately 650 bp. The concentration of PCR reagents were 1× PCR buffer, 1.5 mM $MgCl_2$, 200 µM dNTPs, 0.4 µM forward and reverse primers, one unit of *Taq* polymerase (GoTaq Flexi DNA polymerase, Promega) and 18 ng of DNA template.

Primer sequences

The primer sequences used were ITS1: 5'- TCC GTA GGT GAA CCT GCG G -3' and ITS4: 5'- TCC GCT TAT TGA TAT GC -3' with a PCR protocol as described by Latifah et al. (2002). The initial denaturation was set at 95°C for 5 min, followed by 35 cycles of denaturation at 95°C for 1 min, annealing at 53°C for 40 s, and extension at 72°C for 50 s. The final extension was set to 72°C for 10 min.

PCR product purification (modification)

Before proceeding to cloning, the PCR product was purified using QIAquick PCR Purification Kit (Qiagen) according to the manual. PCR product (20 µl) was added to 100 µl of Buffer PB and later transferred to QIAquick spin column, centrifuged at 13,000 × *g* for 1 min. The flow through was discarded and added to 750 µl Buffer PE and centrifuged at 13,000 × *g* for 1 min. The flow through was discarded again and the empty column was spun at 13,000 x g for one min. Finally, 15 µl of Buffer EB was added to the empty column and left to stand vertically for 10 min before being centrifuged at 13,000 × *g* for 2 min.

Cloning

The purified PCR product was ligated with pJET1.2/blunt vector as described in manual from CloneJET PCR Cloning Kit (Fermentas).

Blunting reaction

The blunting reaction was started by the addition of 10 µl 2× reaction buffers with 5 µl of nuclease-free water and 2 µl of purified PCR product together with 1 µl of DNA blunting enzyme to make up a final volume of 18 µl. The combination of them was incubated at 70°C for five min and chilled briefly on ice.

Ligation

For ligation, 1 µl of pJEmT1.2/blunt cloning vector was added together with 1 µl of T4 DNA ligase to make up a final volume of 20 µl and further incubated at 22°C for 30 min.

Competent cell preparation

E. coli TOP10 strain (Invitrogen) was picked from a single colony on the agar plate and cultured in LB broth at 37°C with continuous rotation at 200 rpm overnight. Overnight bacterial culture (1 ml) was transferred to 3 ml of LB broth and shaken at 200 rpm for 1 h. After that 1.5 ml of culture was centrifuged at 4°C at 6,000 × *g* for 1 min and the supernatant was discarded. The pellet was washed with ice-cold 50 mM calcium chloride, the washings were discarded. The pellet was then suspended in 1 ml of ice-cold 50 mM calcium chloride and spun at 4°C at 6,000 × *g* for 1 min. Finally, the supernatant was discarded and the cells were suspended in 100 µl of ice-cold 50 mM calcium chloride and stood for 20 min on ice.

Transformation and plating

Ligation mixture (5 ml) was added to 50 µl of chemically competent *E. coli* with gentle mixing and incubated on ice for 5 min. The cells were subjected to heat-shock at 42°C for 40 s without shaking and the tube was immediately transferred to ice for 5 min. After that 50 µl of transformants were plated on Luria-Bertani agar containing 100 µg ml^{-1} of ampicillin. The plates were incubated at 37°C overnight.

Picking colonies and subculture

After culturing overnight, colonies were picked randomly using a sterile toothpick, streaked onto a fresh agar medium LBA plate containing 100 µg ml^{-1} of ampicillin and incubated at 37°C overnight.

Plasmid isolation (Miniprep)

Plasmid isolation was done using GeneJET Plasmid Extraction Kit (Fermentas).

DNA sequencing

The DNA sequencing was done by First BASE Laboratories Sdn Bhd at Selangor, Malaysia.

Sequence analysis

The sequence obtained from First BASE Laboratories Sdn Bhd was trimmed with BioEdit software and later BLAST searched for closest matches in NCBI GenBank database. Furthermore, a phylogenetic tree was constructed to show the relationships among the homologous microorganisms. The evolutionary history was inferred using the Neighbor-Joining method (Saitou and Nei, 1987). The optimal tree with the sum of branch length was equal to 0.18107685. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) was shown next to the branches (Felsenstein, 1985). The tree was drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura et al., 2004)

Table 1. The most homologous nine microorganisms from NCBI gene bank in comparison to the Langkon estate isolate. Note the *G. boninense* strain FA5017 and 5035 with maximum identification of 98% to this isolate.

Accession	Description	Maximum score	Total score	Query coverage (%)	E Value	Maximum indent (%)
EU841913.1	Ganoderma boninense strain FA5017 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, complete sequence; and 5.8S ribosomal RNA gene, partial sequence	506	505	95	6e-140	98
EU701010.1	Ganoderma boninense strain FA5035 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, complete sequence; and 5.8S ribosomal RNA gene, partial sequence	505	505	95	6e-140	98
AJ627585.1	Ganoderma mastoporum 18S rRNA gene (partial), 5.8S rRNA gene, 26S rRNA gene (partial), ITS1 and ITS2, isolate FRIM 98	429	429	100	4e-117	93
AY220544.1	Ganoderma sp. SB-1 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence	422	422	89	6e-115	95
AY569450.1	Ganoderma cupreum isolate SUT H1 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 25S ribosomal RNA gene, partial sequence	422	422	100	6e-115	92
AY593865.1	Ganoderma japonicum AS5.69 type 2 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	420	420	100	2e-114	92
AY593864.1	Ganoderma japonicum AS5.69 type 1 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	414	414	100	1e-112	92
DQ421109.1	Uncultured soil fungus clone 133-14 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	411	411	100	1e-111	92
AF255093.1	Ganoderma sp. CBS187.31 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence	411	411	100	1e-111	92

and are in the units of the number of base substitutions per site. All positions containing gaps and missing data were eliminated from the dataset (Complete deletion option). There were a total of 253 positions in the final dataset. Phylogenetic analyses were conducted in MEGA4 (Tamura et al., 2007).

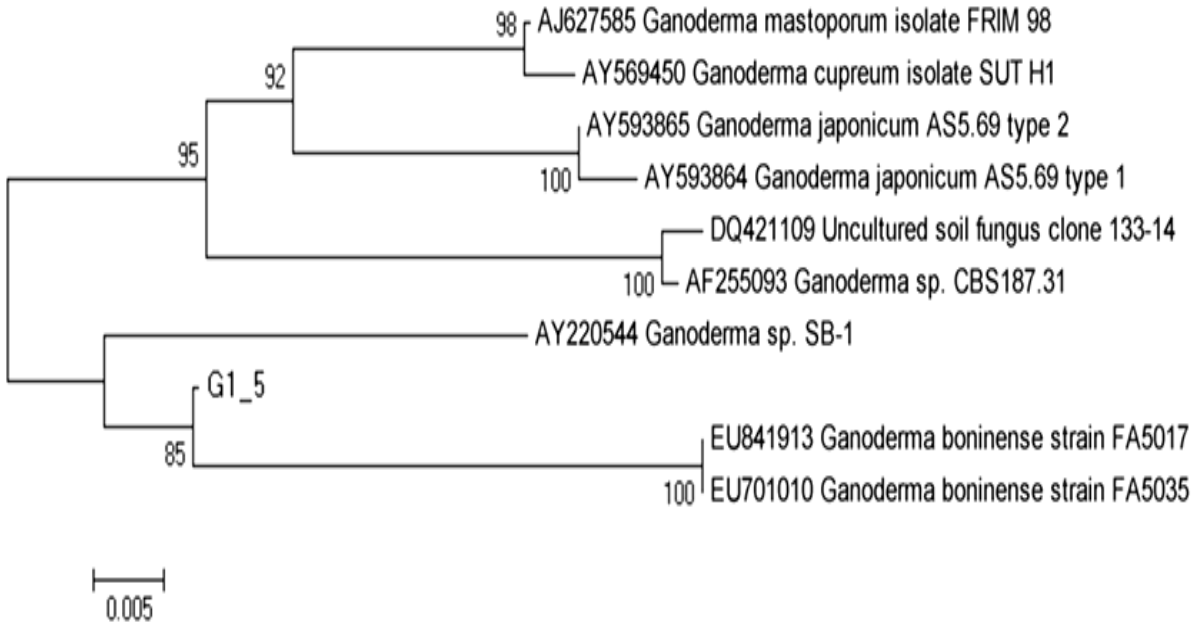

Figure 1. Evolutionary relationships of 10 taxa among isolate of *G. boninense* from Langon estate, Sabah, Malaysia with other nine homologous microorganisms. The evolutionary history was inferred using the Neighbor-Joining method. The optimal tree with the sum of branch length = 0.18107685 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site. All positions containing gaps and missing data were eliminated from the dataset (Complete deletion option). There were a total of 253 positions in the final dataset. Phylogenetic analyses were conducted in MEGA4. Note: G1_5 is the isolate from Langkon, Sabah.

RESULTS

The nine possible microorganisms from the NCBI GenBank database are shown in Table 1. Out of these nine possible microorganisms, *G. boninense* strain FA5017 and FA5035 gave a maximum identification homology of 98% with isolate from Langkon. Figure 1 shows a phylogenetic tree constructed to show the relationships among the homologous microorganisms.

There was strong evidence that the G1_5 isolate from the Langkon estate is very similar to *G. boninense* strains FA5017 and FA5035 (Figure 1). The small differences detected at the 3' end of the sequence may reflect anomalies in the gene bank *G. boninense* sequences (alignments not presented). Bases 427 to 438 showed variation between the FA5017 and FA5035 isolates, compared to other *Ganoderma* sequences at these positions. This may reflect sequencing error or primer incorporation for these *G. boninense* sequences. The sequences of Langkon isolate were divergent with other *Ganoderma* spp. Therefore, it was concluded that the isolate collected from Langkon estate was *G. boninense*, it has very high homology to known isolates from Peninsular Malaysia and most probably has similar aggressiveness as them.

DISCUSSION

In recent years, progress has been made in the early detection and identification of this pathogen through more reliable methods such as enzyme-linked immunosorbent assays (ELISA) (Idris and Rafidah, 2008; Kandan et al., 2009; Utomo and Niepold, 2000) as well as polymerase chain reaction (PCR) based techniques involving certain non-specific *Ganoderma* primers (Idris et al., 2003; Kandan et al., 2009; Latifah et al., 2002; Miller et al., 2000). Molecular techniques exploiting variations in the ribosomal DNA (rDNA) have been used extensively for systematic and phylogenetic studies of fungal pathogens. Different regions of the rDNA diverged at different rates allowing the regions to be exploited at different taxonomic levels (Bruns et al., 1991; Latifah et al., 2002). Polymerase chain reaction using the ITS1 and ITS4 primers showed that isolates from Langkon Estate produced a PCR fragment of approximately 650 bp. The same fragment size does not necessarily indicate sequence similarity. The sequence was trimmed for a

better BLAST search from the NCBI gene bank.

Due to the importance of the oil palm industry to Malaysia's economy, the transfer of any materials that are related to *Ganoderma* is strictly prohibited from Peninsular Malaysia to Sabah and Sarawak. This basic identification which needed to be carried out for this project to confirm the identification of *G. boninense*, using PCR techniques, has never been done before in Sabah. The only report on *G. boninense* isolates in Sabah was merely based on their morphology and pathogenicity (Idris et al., 2001). In conjunction with the morphological similarities among the different isolates, there are numerous opinions on the aggressiveness of *G. boninense* in Sabah. The Head of Research for Borneo Samudera oil palm plantation (the largest plantation group in Sabah) stated that isolates of *G. boninense* from Sabah are less aggressive compared to those from Peninsular Malaysia (Hoong, H.W. personal communication). This may be due to lower incidence of BSR in Sabah and Sarawak compared to Peninsular Malaysia (Ariffin and Idris, 2002).

Besides speculation on the different aggressiveness of the isolates, the difference in the severity of outbreaks may due to the different re-planting factors. In Peninsular Malaysia, most of the oil palm estates are undergoing the second or the third re-planting. The increase in inoculum that remains as debris in the soil after the first and second re-planting may provide a good source for further infection of the newly planted palms through root contact (Chong, 2010). To date, it was reported that almost 90% of the estates in West Malaysia showed the presence of *G. boninense* (Khairuddin and Chong, 2008). In Sabah and Sarawak, most of the estates are in their first planting, providing alternative hypothesis for the lower incidence of pathogen attack in these areas. The disease incidences were only reported to be 4% in Sabah, but this is distant from the real situation in estates who stated it is considerably higher (Idris, A.S. personal communication). Big multi-billion ringgit companies that are involved in the oil palm industries are suspected of not reporting the real statistic of the incidence in Sabah, which currently has the largest plantation areas in Malaysia, in order to avoid losses in their market share (Hoong, H.W. personal communication). Since the oil palm industry is a fast income-generating tool, data related to it may be very secretive. Researchers, plantation managers and entrepreneurs do not share information, oil palm genetic materials or isolates of *G. boninense* with others. This report should reduce the speculation of the different disease intensity in Sabah compared to Peninsular Malaysia due to different aggressiveness of *G. boninense*.

REFERENCES

Ariffin D, Idris AS (2002). Progress and research on *Ganoderma* basal stem rot of oil palm. Proc. Seminar on Recent Progress in the Manage. Peat and *Ganoderma* May 2002. Bangi, Malaysia. 50.

Ariffin D, Idris AS (1992). The *Ganoderma* selective medium (GSM). PORIM Information Series. ISSN 0128-5726.

Bruns TD, White TJ, Taylor JW (1991). Fungal molecular systematics. Ann. Review of Ecol. Syst. 22: 525-564.

Chong KP (2010). The role of phenolics in the interaction between oil palm and *Ganoderma boninense* the causal agent of Basal Stem Rot. Univ. Nottingham, UK. PhD thesis (submitted).

Felsenstein J (1985). Confidence limits on phylogenies: An approach using the bootstrap. Evolution. 39: 783-791.

Idris AS (2009). Basal Stem Rot in Malaysia-Biology, economic importance, epidemiology, detection and control. Proc. of the Inter. Workshop on Awareness, Detection and Control of Oil Palm Devastating Diseases November 2009. Kuala Lumpur Convention Centre, Malaysia.

Idris AS, Ariffin D, Swinburne TR, Watt TA (2000a). The identity of *Ganoderma* species responsible for BSR disease of oil palm in Malaysia-Morphol. characteristics. MPOB Infor. Series MPOB TT. 77a.

Idris AS, Ariffin D, Swinburne TR, Watt TA (2000b). The identity of *Ganoderma* species responsible for BSR disease of oil palm in Malaysia-Pathogenicity test. MPOB Infor. Series MPOB TT. 77b.

Idris AS, Mohd Tayeb D, Ariffin D, Zulkhifli D, Zulkhifli M, Mohd Rafil Y, Affandi M (2001). Morphology and pathogenicity of *Ganoderma* basal stem rot of oil palm obtained from Sabah and Sarawak. Proc. Malaysian Sci. Technol. Congress 2001 September 2001. Kota Kinabalu, Sabah.

Idris AS, Rafidah AR (2008). Polyclonal antibody for detection of *Ganoderma*. MPOB Information Series MPOB TT. 405 (4).

Idris AS, Yamaoka M, Hayakawa S, Basri MW, Noorhasimah I, Ariffin D (2003). PCR technique for detection of *Ganoderma*. MPOB Inf. Series MPOB TT. 188.

Kandan A, Radjacommare R, Ramanathan A, Raguchander T, Balasubramanian P, Samiyappan R (2009). Molecular biology of *Ganoderma* pathogenicity and diagnosis in coconut seedlings. Folia Microbiol. 54: 147-152.

Khairuddin H, Chong TC (2008). An overview of the current status of *Ganoderma* basal stem rot and its management in a large plantation group in Malaysia. The Planter. 84: 469-482.

Latifah Z, Harikrishna K, Tan SG, Abdullah F, Ho YW (2002). Restriction analysis and sequencing of the ITS regions and 5.8S gene of rDNA of *Ganoderma* isolates from infected oil palm and coconut stumps in Malaysia. Annals of Appl. Biol. 141: 133-142.

Miller RNG, Holderness M, Bridge PD (2000). Molecular and morphological characterization of *Ganoderma* in oil palm plantings. In: Ganoderma diseases of perennial crops, J. Flood, Bridge PD, Holderness M (eds). pp. 159-182. CABI Pub., Wallingford.

Moncalvo JM (2000) Systematics of *Ganoderma*. In: Ganoderma diseases of perennial crops, J. Flood, Bridge PD, Holderness M (eds). pp. 23-45. CABI Pub., Wallingford.

Saitou N, Nei M (1987). The neighbor-joining method: A new method for reconstructing phylogenetic trees. Mol. Biol. Evolution. 4: 406-425.

Susanto A (2009). Basal stem rot in Indonesia. Biology, economic importance, epidemiology, detection and control. Proceedings of International Workshop on Awareness, Detection and Control of Oil Palm Devastating Diseases November 2009. Kuala Lumpur Convention Centre, Malaysia. Univ. Putra Malaysia Press.

Tamura K, Dudley, Nei M, Kumar S (2007). MEGA4: Molecular evolutionary genetics analysis (MEGA) software version 4.0. Mol. Biol. and Evolution.

Tamura K, Nei M, Kumar S (2004). Prospects for inferring very large phylogenies by using the neighbor-joining method. Proceedings of the Natl. Academy of Sci. 101: 11030-11035.

Thompson A (1931). Stem-rot of the oil palm in Malaya. Bull. Department Agric., Straits Settlements and FMS. Sci. Series 6.

Utomo C, Niepold F (2000). The development of diagnostic tools for *Ganoderma* in oil palm. In: Ganoderma diseases of perennial crops, J. Flood, Bridge PD, Holderness M (ed). pp. 235-248. CABI Pub., Wallingford.