THE IDENTITY OF *Ganoderma* SPECIES RESPONSIBLE FOR BSR DISEASE OF OIL PALM IN MALAYSIA - MORPHOLOGICAL CHARACTERISTICS

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Basal stem rot (BSR) caused by species of Ganoderma is a disease of economic importance of oil palm in Malaysia and Indonesia. The genus Ganoderma Karst. is categorised as a higher fungus, a polyporoid genus, within the family: Ganodermataceae, Order:Aphyllophorales, Subclass: Hymenomycetes and Class: Basidiomycetes. The early literature on BSR disease of oil palm reported conflicting opinions on the identity of species of Ganoderma responsible for this disease. The identity of the Ganoderma responsible for BSR disease was investigated using 267 isolates obtained from an extensive survey of 37 oil palm fields located throughout Malaysia. The objective was to determine the differences between types observed *in vivo* could be reproduced *in vitro*, under standardised conditions. To achieve this, techniques were adapted to enable basidiomata to develop in axenic culture for morphological comparisons. Additionally, dimensions of the resulting basidiospores were recorded and growth diameter of mycelial cultures on media measured.

MORPHOLOGICAL CHARACTERISTICS

During collection of isolates of Ganoderma, it was noted that colour of the upper surface of basidiomata recorded from living, or dead oil palms in the field were variable in appearance, but could be assigned to one of three main groups, obviously referred to as types A, B and C (*Figure 1*). The isolates of Ganoderma were grown on Potato Dextrose Agar (PDA, Oxoid) in standard 9cm sterile Petri dishes and incubated at 28°C. The radius of each colony was measured at 5, 10 and 14 days after incubation. The colony characters were also recorded, with descriptions of colony colour on the surface and beneath the mycelia.

A rubber woodblock (RWB, size 3x3x6cm) was used as a substrate to artificially produce basidiomata of Ganoderma. The RWB was first soaked in 2% Malt Extract (ME) for 12 hours, placed in a plastic bag and autoclaved

Figure 1. Basidiomata of Ganoderma collected from oil palm, illustrating the appearance of those classified as Type A - dark shiny red to dusky red (Fig. 1A); Type B - reddish yellow to yellowish red (Fig. 1B); and Type C – dark brown to dull, only found on dead palms and forest trees (Fig. 1C).

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at 121°C for 45 minutes. After sterilisation and cooling, plugs of mycelium were cut from the edge of actively growing mycelium (5-10 days old of cultures grown on PDA), and transferred onto the RWB in plastic bag and then incubated at 28°C for 2.5 months during which time the RWB was completely colonised with Ganoderma inoculum (*Figure* 2). The colonised RWB was then transferred to polypropylene bags (size 15x23cm) containing a mixture of unsterilised soil and sand (2:1) and allowed to sit on glasshouse benches. The RWB inoculum was maintained with regular watering. The temperature and humidity of the glasshouse during the period of study is 30-35°C and 60-70%, respectively. Observation were made weekly on the development and growth of basidiomata formed. After 8-10 weeks, basidiomata formed and their length, width and thickness were measured. The colour of the upper surface of basidiomata was recorded according to the Munsell Soil Colour Chart (1990). Basidiospores were collected on glass slides placed beneath the basidiomata and were viewed under light microscope after the application of a drop of 0.1% lactophenol cotton blue stain and a cover slip applied. The length and width of basidiospores collected from basidiomata for each isolate was measured using an eyepiece micrometer.

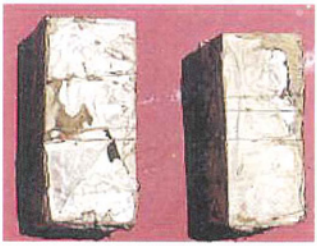

Figure 2. Colonisation of RWB (size 3x3x6cm) with Ganoderma, 2.5 months after incubation at 28°C.

GROWTH CHARACTERISTICS *IN VITRO*

The typical characteristics of the mycelial mats and the reverse colour of isolates of Ganoderma obtained from oil palm are shown in *Figure* 3. Colonies of Ganoderma were white in colour on the surface and the reverse were darkened (pigmented). The cultures of these isolates had an undulating surface in the darkened regions that buckled the agar. There were wide variations between isolates, colonies diameter ranged from 17 to 51mm after 5 days, and 34 to 84mm after 10 days. Most isolates grew to completely cover the Petri dish after 14 days.

BASIDIOMATA CHARACTERISTICS *IN VITRO*

The first indication of basidiomata formation was the appearance of a white mycelium on the top of RWB, after one to three weeks of incubation, which then developed

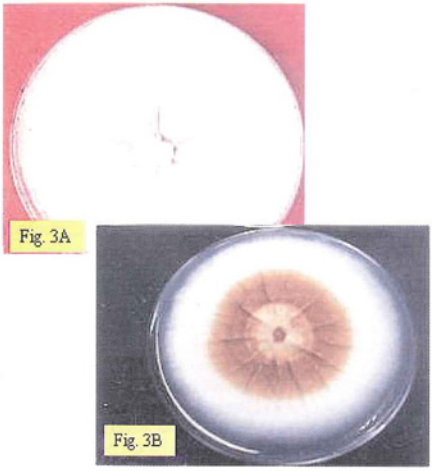

Figure 3. Colonies of Ganoderma grown on PDA, 14 days after incubation at 28°C. White mycelium with slightly sculptured surface (Fig. 3A), and reverse colour (darkened) (Fig. 3B).

Figure 4. Artificially induced basidiomata of Ganoderma on a substrate of RWB, two weeks after transferred into glasshouse. Mycelium white crust (Fig. 4A), and Small white button (Fig. 4B).

into a small white button-like structure (*Figure 4*). The apical end began expanding rapidly giving rise to bracket-like structures which were generally white when first formed but as their length and widths increased rapidly, the upper surface developed various colour with concentric zonations. The basidiomata lengths ranged from 1.36 to 3.64cm, the widths from 1.20 to 3.61cm and the thickness from 0.3 to 1.1cm. Actively growing margins and the undersurface of the basidiomata were white. The observed 'life-span' of most of the basidioma was 3 to 4 months. In comparison, the 'life-span' of Ganoderma basidiomata observed on infected oil palms in the field was 6 to 7 months. The colour and dimensions of the basidiomata that formed was recorded 8-10 weeks after incubation, and from these observations it was possible to distinguish three main types as shown in *Figure 5*.

Figure 5. Artificially induced basidiomata of Ganoderma on a substrate of RWB, 8-10 weeks after incubation in the glasshouse. Type A - dark red to dusky red (Fig. 5A); Type B – reddish yellow to yellowish red, the presence of yellow colours was typical for type B isolates (Fig. 5B); and Type C - dark brown to dull (Fig. 5C).

BASIDIOSPORE CHARACTERISTICS *IN VITRO*

Basidiospores were obtained from all isolates that produced basidiomata in culture. The colour of the spore mass seen on the collection slides was yellowish brown for all isolates. Most isolates produced basidiospores which were ellipsoid in shape (*Figure 6*) and some were ovoid. The mean length of basidiospores varied between isolates, ranging from 7.1 to 13.8mm and width varied ranging from 4.8 to 8.3mm.

COMPARISON OF THE MORPHOLOGICAL CHARACTERISTICS *IN VITRO* OF ISOLATES OF GANODERMA

In an attempt to obtain further resolution of the morphological characteristics of each type, the data was subjected to Canonical Discriminant Analysis (CDA) and analysis of variance (ANOVA). The result of the plot of canonical discriminant functions showed a dispersion but groupings of isolates according to the types of Ganoderma could be identified. The type A isolates were characterised by narrow basidiospores (5.4mm) and a faster growth diameter at 5 days (41.7mm). The type C isolates were

Figure 6. Basidiospores of Ganoderma viewed under light microscope (magnification 40X).

characterised by a slow growth diameter at 5 days (34.2mm) with short basidiospores (8.2mm). Within the B type isolates, there appeared to be two levels (namely, types B1 and B2), which corresponded with differences in mycelial growth and basidiospore size. On the basis of earlier descriptions species as reported by Steyaert (Bulletin du Jardin Botanique National de Belgique, 1967), they were identified as G. boninense (type A), G. zonatum (type B1), G. miniatocinctum (type B2), and G. tornatum (type C). Analysis of variance indicated that G. boninense isolates had significantly ($p<0.001$) faster growth of colonies on PDA than G. zonatum, G. miniatocinctum, or G. tornatum. G. boninense isolates also produced significantly ($p<0.001$) larger, wider and thicker basidiomata *in vitro* than other species of Ganoderma. Significant differences ($p<0.001$) were found between all species of Ganoderma for basidiospore length and the ratio of length to width.

CONCLUSION

The isolates of Ganoderma could be grouped into one of the three main types (designated as types A, B or C), based on the characteristics of the observed basidiomata and basidiomata formed on the RWB incubated in the glasshouse. Further analysis on the basis of morphological data *in vitro*, the existence of four species of Ganoderma was confirmed to be associated with oil palm in Malaysia. This was two more than was reported by Khairudin (Master of Agriculture Science Thesis, 1990), namely G. boninense and G. zonatum, and in contrast to the conclusion drawn by Ho and Nawawi (Pertanika, 1985) that only one species was present, namely G. boninense. In this study, four species of Ganoderma were identified, they are G. boninense (type A), G. zonatum (type B1), G. miniatocinctum (type B2) and G. tornatum (type C). Their potential pathogenicity to oil palm need to be investigated.

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