Pak. J. Bot., 44(6): 2001-2004, 2012.

THE SUSCEPTIBILITY OF DIFFERENT VARIETIES OF OIL PALM SEEDLINGS TO *GANODERMA BONINENSE* INFECTION

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Abstract

Optimal yield production of oil palm in Malaysia and other countries of South East Asia are hampered by the presence of devastating Basal Stem Rot (BSR) disease caused by *Ganoderma boninense*. For more than 40 years, *Ganoderma* remained to be the most serious problem in many areas in Malaysia and Indonesia. But unfortunately, there is no single reliable application in suppressing or controlling either the disease severity or a promising resistant variety of oil palm to this pathogen. AVROS is the most commonly planted oil palm variety in Sabah derived from the *Dura* x *Psifera* (D x P) and claimed to be more resistant to *G. boninense* in comparison to other commercial varieties. In this paper the susceptibility of AVROS to *G. boninense* is compared to two other varieties; Calabar and Ekona which have the same *Dura* but with African origin *Psifera*. In general, the accumulation of the ergosterol, a specific indicator associated with fungi, increased significantly throughout the study in all the three varieties indicating increasing of infection. Varieties of Ekona and Calabar were found to be more susceptible to *G. boninense* based on the higher content of ergosterol found in root compared to AVROS at week four, five and six. The ergosterol results are also supported by the disease severity score as described by Horsfall and Barratt, 1945 which shown Calabar and Ekona scored higher than AVROS in the infection.

Introductıon

The oil palm (*Elaeis guineensis*) industry in Malaysia is big and diversified. Malaysia has the second largest oil palm plantation areas in the world after Indonesia and oil palm industry has been reported by the World Bank as one of the very important tool in eradicating poverty in developing countries such as Malaysia and Indonesia. Crops have been challenged with various pests and diseases such as *Fusarium* root rot in okra (Ahmad *et al*., 2012), *Fusarium* in Gladiolus (Nasir *et al*., 2012) and many others but in oil palm the greatest threat to its production in South East Asia is Basal Stem Rot, caused by the white-rot fungus *Ganoderma boninense* (Chong *et al*., 2011 b). The most common manifestation of the disease is basal stem rot (BSR), where progressive decay of the root system and ultimately of the lower stem induces disease symptoms (Rees, *et. al*., 2009). BSR can kill more than 80% of stands by the time they are half-way through normal economic life (Abdul Razak *et al*., 2004). Some of the previous treatments that claim to be effective in managing *G. boninense* are preventive treatments such as soil drenching, clean clearing, crop rotation, fallow period, burning and windrowing (Susanto *et al*., 2005) and ameliorative treatments by using different types of fungicide and application methods, surgery, biological control and attempt to develop resistance genotypes (Sariah & Zakaria, 2000). The recent studies on the possibility to manage *G. boninense* effectively include alteration of lignin content in oil palm root (Paterson, 2007; Paterson, 2008) and the effect of phenolics in oil palm root to *G. boninense* (Chong *et al*., 2012; Arif, *et al*., 2007). *In vitro* studies on the effect of syringic acid, caffeic acid and 4-hydroxybenzoic acid also show that these compounds have significant fungitoxic effect towards *G. boninense* (Chong *et al*., 2009 a and b). There is currently no proven cure for *G. boninense* infection. Numerous treatments and control show various degrees of effectiveness. To compliment the on going research involving *G. boninense*- oil palm, we have studied the susceptibility of 3 different varieties of oil palm available in Sabah namely; the most common planted commercial variety AVROS and another two other varieties; Calabar and Ekona which have the same *Dura* with AVROS but with an African origin *Psifera* to provide a more scientific suggestion for planters in selecting varieties for their estates.

Materıals and Methods

Seed and plant materials: Certified disease free seeds, varieties of Ekona, Calabar and AVROS were provided by Borneo Samudera Sdn Bhd, Sabah Malaysia and grown to one year old in Peat Vriezenveen Substrate, Product of Holland.

***Ganoderma boninense*:** Fruiting bodies of *G. boninense* were collected from oil palm trees in Borneo Samudera Langkon Estate in Sabah, Malaysia. Internal tissues of fruiting bodies was excised and cultured on *Ganoderma* Selective Medium (GSM). GSM was prepared as described by Ariffin & Idris, 1991. GSM provides a useful tool for isolating *Ganoderma* free from other contaminants. The contents of fungicides and antibiotics are optimal to control growth of bacteria and other contaminating fungi while allowing *Ganoderma* to thrive. Once the pure culture of *Ganoderma* was isolated, it was transferred and maintained at 25^{o}C on Potato Dextrose Agar (PDA) for normal growth, re-inoculated into oil palm seedlings after 12 days fully grown on PDA. The pathogen was later re-isolated from infected oil palm seedlings to GSM. Procedures were repeated throughout the project to maintain the pathogenicity of *G. boninense.*

Identification of *G. boninense* using molecular technique: Identification of the pathogen was as described by Chong *et al*., (2011 a). DNA of *G. boninense* was isolated using the modified mini protocol for purification of total DNA from plant tissues by Qiagen. PCR amplification of the fungal was on ITS 1 and ITS 2 regions, and 5.8S gene. Size of the PCR amplicon was approximately 650 bp and trimmed with BioEdit software to 295bp before verification was conducted using the Basic Local Alignment Search Tool (BLAST). The isolate was later confirmed as *Ganoderma boninense* strain FA5017 with accession EU841913.1

Development of reliable artificial direct inoculation of oil palm seedlings with *G. boninense*: The oil palm roots were inoculated as described by Chong *et al*., 2012. In brief, oil palm roots were sprayed with a suspension of *G. boninense* fragments, incubated at 25°C before in darkness for 12 days. The finely blended suspension of 17.5mL of mycelia mixed with 50 µL of Tween 20®, scraped from the entire surface of a nine cm diameter petri dish was taken up by 20mL of Potato Dextrose Broth (PDB).

Extraction of ergosterol from infected oil palm roots: All infected roots and uninfected roots (as control) were extracted as described by Genney (2000). Roots with 100 mg of weight were extracted in methanol using bead beating to physically crush the sample at the same time. Polyvinylpyrrolidone (PVPP) was added (10% w/v) to the methanol to precipitate phenolic compounds. The extract was centrifuged and the supernatant was made up to 1.5mL before being filtered through a 0.45µm acetate syringe tip filter.

Ergosterol analysis and quantification: The Eclipse XDB-C_{18} 4.6 mm x 150 mm x 5 µm column was utilized with an Agilent Series 1200 HPLC system. The wavelength of UV detector was set at 282 nm, and the isolated peak elution at about 5.5-5.8 min retention time was identified as ergosterol based on its co-chromatography and identical absorption spectrum with pure standard (20 µg mL^{-1}) from Sigma at the flow rate of 1.5 mL min^{-1}. The system was run isocratically with 100% methanol.

Disease Severity Scale: The disease severity of the oil palm roots artificially infected with *G. boninense* was assessed based on the Disease Severity Scale as described by Horsfall & Barratt, 1945.

Results and Dıscussıon

In general, the accumulation of the ergosterol increased significantly from week three to five in different varieties indicating the increase of infection throughout the study (Fig. 1). Varieties of Ekona and Calabar were found to be more susceptible to *G. boninense* based on the higher content of ergosterol found in root compared to AVROS at week 4, 5 and 6. The membrane lipid ergosterol is found almost exclusively in fungi, and is frequently used by microbiologists as an indicator of living fungal biomass, based on the assumption that ergosterol is labile, and therefore rapidly degraded after the death of fungal hyphae (Mille-Lindblom *et. al.*, 2004). The main advantage with ergosterol compared to other biomarkers, such as chitin and ATP, is its specific association with fungi. Low amount of ergosterol can be found in algae and protozoa (Raederstorff & Rohmer, 1987; Peeler *et al*., 1989), but generally it is safe to use it as a specific biomarker for fungi (Newell, 1992).

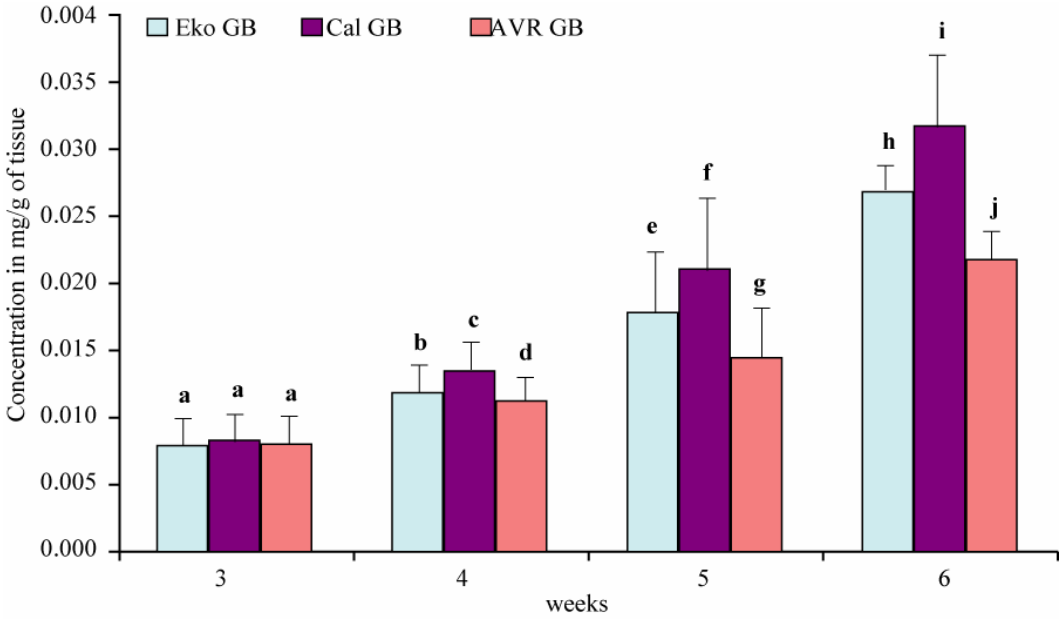

Fig. 1. Accumulation of ergosterol content in different varieties of oil palm seedlings at different weeks after infected by *Ganoderma boninense*. Eko denotes Ekona, Cal: Calabar, AVR: AVROS, GB: *Ganoderma boninense*. Bars represent standard deviation. Means tagged with the same letter are not significantly different using Tukey test (P= 0.05).

The result based on the disease severity score (Table 1) as described by Horsfall & Barratt, 1945 was accordance to the finding from ergosterol analysis (Fig. 1) for all of the treatments. All control plants scored less than 2.0, indicating the plants were not infected by *G. boninense* or other fungi. Some control plants scored slightly more than 1.0 may due to natural lignifications on the oil palm root. In contrast to the ergosterol analysis, the disease severity score suggest a short recovery at week 4 for the *G. boninense* infected plants. The scores significantly lower in week four compared to week three for all treatments, except controls but higher in week 5 and 6. Among the varieties, Ekona and Calabar were found to be more susceptible to invasion of *G. boninense* compared to AVROS, but some of the differences may not significant.

Table 1. Disease severity score of oil palm root as described by Horsfall and Barratt 1945 scale on week three to six after different treatment. GB denotes *Ganoderma boninense*.

Treatments	Score at Week 3	Score at Week 4	Score at Week 5	Score at Week 6
Ekona + GB	9.0 ±0.00	7.4 ± 1.67	11.2 ± 0.45	12.2 ± 0.45
Ekona Control	1.2 ± 0.45	1.0 ± 0.00	1.0 ± 0.00	1.0 ± 0.00
Calabar + GB	8.8 ± 0.45	6.6 ± 1.14	10.2 ± 1.48	12.4 ± 0.55
Calabar Control	1.0 ± 0.00	1.2 ± 0.45	1.4 ± 0.55	1.2 ± 0.45
AVROS + GB	9.0 ± 0.71	6.2 ± 1.10	9.8 ± 2.17	11.4 ± 0.55
AVROS Control	1.4 ± 0.55	1.0 ± 0.00	1.0 ± 0.00	1.0 ± 0.00

Recently, there is an indication that there are differences in susceptibility to BSR between germplasm materials from different genetic origins (Arif *et al*., 2007). This indication provides hope in generating oil palm varieties with reduced level of susceptibility using existing genetic materials. The differences that contributed to different susceptibility may be associated with cell maintenance and development, genes involved in the biosynthesis of lignin and phenolics and genes implicated in oxidative burst, programmed cell death or hypersensitive responses (Schenk *et al*., 2000).

Conclusion

Varieties of Ekona and Calabar are found to be more susceptible to *G. boninense* based on the higher content of ergosterol found in root and higher disease severity score compared to AVROS at week 4, 5 and 6.

Acknowledgments

The authors are very grateful for the financial support from Ministry of Science, Technology and Innovation, Malaysia and Ministry of Higher Education, Malaysia. We acknowledged the contribution of plant materials from Borneo Samudera (M) Sdn Bhd. The authors are indebted to Foong Choon Pin for his assistance in molecular identification of *Ganoderma boninense*.

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(Received for publication 3 October 2011)