DEVELOPMENT OF TECHNIQUE TO SCREEN COCOA FOR RESISTANCE AGAINST THE WHITE ROOT DISEASE CAUSED BY *RIGIDOPORUS LIGNOSUS* (Klot.) Bres.

By

HAJI AZMI BIN CHE AHMAD

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Master of Science

February 2005

To my wife, **Fatimah Ibrahim** and my children:

Ikhwan Budiman
Husnul Khatimah
Mohammad Sayuti
Ahmad Suhail
Nurul Basirah
Siti Asma
Ahmad Shauqi
Ahmad Suhaimi
Khairul Bariah

For their kindness, understandings, sacrifices, invaluable supports and encouragements that make it possible for me to complete this thesis works.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirements fot the degree of Master of Science

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Chairman: Associate Professor Zainal Abidin Meor Ahmad, PhD

Faculty: Agriculture

Rigidoporus lignosus, *Phellinus noxius* and *Ganoderma philippii*, Basidiomycetous fungi commonly associated with white, brown and red root diseases respectively, were isolated from infected cocoa roots. The disease symptoms and characteristics of each fungus were identified, and the prevalence of *R. lignosus* and white root disease over the others was confirmed in a study of three cocoa planting areas in Peninsular Malaysia. Growth of *R. lignosus* on potato dextrose agar medium was significantly influenced by factors of temperature and pH. The temperature range favourable for growth was 30ºC, while the optimum pH was 5. A study was undertaken to induce production of basidiocarps of *R. lignosus in vitro*. Basidiocarps were formed on autoclaved and non-autoclaved soil by 14 days after incubation. Ultrastructural studies of *R. lignosus* basidiocarp and basidiospore were done. The viability of basidiospores were confirmed by germination on glass slides coated with malt extract agar. Artificial inoculation of *R. lignosus* using mycelial mats and basidiospores

suspension failed to induce white root disease symptoms on cocoa seedlings. However, in this study a technique was successfully developed for screening cocoa seedlings for resistance against *R.lignosus*. The method involved the insertion of the tap root of 10-day old cocoa seedling into the hole of a *R. lignosus* colonized rubber wood block and subsequently covered in soil in a black polythene bag. Symptoms of white root disease were recorded within two weeks after inoculation and were measurable using a disease severity index. *R. lignosus* isolate CRD/LKM/10 was found to be the most virulent isolate as it had caused a significantly more severe white root disease infections in cocoa seedlings compared to six other *R. lignosus* isolates tested. The method of inoculation was subsequently used to screen cocoa seedlings of 15 selected cocoa clones (including the widely planted PBC 123 clone) for resistance against white root disease using *R. lignosus* isolate CRD/LKM/10 as the test fungal organism. The results revealed that cocoa seedlings of clones ICS 60 and PA300 were significantly less infected by white root disease thus indicating that they were more resistant to the disease compared to all other cocoa clones tested.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Master Sains

PERBENTUKAN TEKNIK BAGI MENYARING KERINTANGAN POKOK KOKO TERHADAP PENYAKIT AKAR PUTIH YANG DISEBABKAN OLEH *RIGIDOPORUS LIGNOSUS* (Klot.) Bres.

Oleh

Haji Azmi Bin Che Ahmad

Februari 2005

Pengerusi: Profesor Madya Zainal Abidin Meor Ahmad, PhD

Fakulti: Pertanian

Rigidoporus lignosus, Phellinus noxius dan *Ganoderma philippii* adalah kulat Basidiomycetes yang biasa dikaitkan dengan penyakit akar putih, perang dan merah telah dipencilkan dari akar koko yang berpenyakit. Simptom penyakit dan ciri-ciri setiap kulat tersebut dikenalpasti dan kekerapan jangkitan *R. lignosus* dan penyakit akar putih terhadap lain-lain penyakit akar disahkan dalam kajian di tiga kawasan tanaman koko di Semenanjung Malaysia. Pertumbuhan kulat *R. lignosus* di atas medium agar dekstrosa ubi kentang dipengaruhi oleh factor suhu dan pH. Julat suhu yang sesuai untuk pertumbuhan adalah 30^{o}C, manakala pH yang optimum ialah 5. Kajian dijalankan untuk menghasilkan basidiokarpa *R. lignosus in vitro*. Basidiokarpa terbentuk di atas substrat tanah yang diautoklaf dan tidak autoklaf selepas 14 hari pengeraman. Kajian ke atas struktur ultra basidiokarpa *R. lignosus* dan basidiospora juga dilakukan. Kebernasan basidiospora di pastikan dengan percambahannya di atas slaid kaca yang bersalutkan agar ekstrak malta. Inokulasi *R. lignosus* dengan menggunakan

miselium dan ampaian basidiospora gagal untuk menbentuk simptom penyakit akar putih ke atas anak pokok koko. Walau bagaimana dalam kajian ini satu teknik telah digunakan dengan jayanya untuk menyaring ketahanan anak benih koko terhadap *R. lignosus*. Kaedah ini adalah dengan memasukan akar tunjang anak benih koko yang berumur sepuluh hari ke dalam lubang blok kayu getah yang dikolonikan oleh *R. lignosus* dan ditutup dengan tanah dalam beg politen hitam. Tanda-tanda penyakit akar putih telah dicatatkan dalam tempoh dua minggu selepas inokulasi dan dapat diukur dengan menggunakan indeks keterukan penyakit. Pencilan *R. lignosus* CRD/LKM/10 didapati sangat virulen dan menyebabkan penyakit akar putih secara bererti ke atas anak pokok koko berbanding dengan enam pencilan *R. lignosus* yang lain yang diuji di dalam kajian ini. Kaedah inokulasi tersebut digunakan untuk menyaring kerintangan anak-anak benih koko daripada 15 klon koko yang terpilih (termasuk klon PBC 123 yang ditanam secara meluas) terhadap penyakit akar putih dengan menggunakan pencilan *R. lignosus* CRD/LKM/10 sebagai kulat pengguji. Keputusan menunjukkan anak benih dari klon ICS 60 dan PA 300 adalah kurang dijangkiti olah penyakit akar putih secara bererti. Ini menunjukkan bahawa klon-klon tersebut adalah lebih rintang terhadap penyakit akar berbanding dengan lain-lain klon koko yang diuji.

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I certify that an Examination Committee met on 28th February 2005 to conduct the final examination of Haji Azmi Bin Che Ahmad on his Master of Science thesis entitled "Development of Technique to Screen Cocoa for Resistance Against the White Root Disease Caused by *Rigidoporus lignosus* (Klot.) Bres. in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of Examination Committee are as follows:

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DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

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HAJI AZMI BIN CHE AHMAD

Date:

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