UNIVERSITI PUTRA MALAYSIA

DETERMINATION OF BIOLOGICAL SPECIES OF *GANODERMA BONINENSE* (PAT.) AND THEIR PATHOGENIC POTENTIAL ON OIL PALM (*ELAEIS GUINEENSIS* JACQ.) SEEDLINGS

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By

N E L S O N

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

February 2002

DEDICATION

This thesis is dedicated to my beloved parents

Malik Habib and Asma

who have always supported and encouraged me to do the best.

And to my wife

Dian Fiantis

and our children

Tito Muhajir and Muhammad Irfan

for their love.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

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Chairman : Associate Prof. Dr. Faridah Abdullah

Faculty : Science and Environmental Studies

A total of 5 *Ganoderma* isolates from oil palm (*Elaeis guineensis* Jacq.) and coconut (*Cocos nucifera* L.) hosts were used in the determination of *Ganoderma boninense* based on monokaryotic mating compatibilities. *Ganoderma* specimens collected from both oil palm and coconut hosts fell into 2 types; one was reddish brown with a varnished dorsal surface ('laccate') represented by isolates EGB-01, CN-L1 and CN-L2 and the other a dull brown non-varnished dorsal surface ('non laccate') represented by EG-NL and CN-NL. The isolate WD814 identified as *G. boninense* by the Forestry and Forest Products Research Institute (FFRRI) at Tsukuba, Japan, was included as an outgroup sample.

The present study showed that based on sexual compatibilities of monosporus cultures obtained from a single fruitbody, the mating pattern of *G.*

boninense was heterothallic and tetrapolar. The cultures fell into 4 distinct sex groups from which 4 monokaryon testers were successfully obtained. When tested for intergroup compatibilities, isolates CN-L1 and WD814 were compatible with EGB-01, whereas CN-L2, EG-NL and CN-NL were not. Thus, monokaryon compatibility crosses confirmed that although EGB-01, CN-L1 and WD814 were from oil palm, coconut and *Livistona* palm hosts respectively, they all belonged to the same biological species.

The pathogenic potential of isolates from oil palm and coconut hosts were tested on oil palm seedlings over a 12-month period in glasshouse trials. Only EGB-01 and CN-L1 isolates were found to be pathogenic on oil palm seedlings, with both showing 100% infection. Totally no infection was established in palms inoculated with the non-*G. boninense* isolates EG-NL, CN-NL and CN-L2. When infected seedlings were uprooted, the point of entry of *G. boninenese* was found to be the larger primary root of the plant. A scanning electron micrograph (SEM) of a newly infected root showed that the fungi colonised the epidermis, cortex, endodermis, lacunae, as well as plugged the xylem and phloem tissues. This indicates that fungal colonisation was not restricted to any particular tissue. This study concluded firstly, more than one species of laccate *Ganoderma* can be found on coconut stumps. Secondly, *G. boninense* isolated from oil palm and coconut stumps were pathogenic on oil palms. Thirdly, the non *G. boninense* specimens (EG-NL, CN-NL and CN-L2) were not pathogenic to oil palms.

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

PENENTUAN SPESIES BIOLOGI *GANODERMA BONINENSE* (PAT.) DAN KEUPAYAAN JANGKITANNYA KE ATAS ANAK POKOK KELAPA SAWIT *(ELAEIS GUINEENSIS* JACQ.*)*

Oleh

N E L S O N

Februari 2002

Pengerusi : **Prof. Madya Dr. Faridah Abdullah**

Fakulti : **Sains dan Pengajian Alam Sekitar**

Sejumlah 5 isolat *Ganoderma* daripada perumah-perumah kelapa sawit (*Elaeis guineensis* Jacq.) dan kelapa (*Cocos nucifera* L.) telah digunakan dalam penentuan spesis untuk *Ganoderma boninense* berasaskan keserasian pengawanan monokarion. Spesimen *Ganoderma* yang dikutip daripada perumah kelapa sawit dan kelapa tergolong kepada 2 jenis utama; pertama yang berwarna merah keperangan dengan permukaan dorsal berkilat dan diwakili oleh isolat-isolat EGB-01, CN-L1 dan CN-L2 sementara jenis kedua pula berwarna perang pucat dengan permukaan dorsal yang tidak berkilat, diwakili oleh EG-NL dan CN-NL. Isolat WD814 yang dikenalpasti sebagai *G. boninense* oleh Forestry and Forest Products Research Institute (FFRRI) di Tsukuba, Jepun, digunakan dalam kajian sebagai sampel kelompok luar.

Kajian ini menunjukan system pengawanan *G. boninense* adalah heterotalik dan tetrapolar berasaskan keserasian diantara kultur-kultur spora tunggal yang

diperolehi dari satu jasad buah. Kultur-kultur terbahagi kepada 4 kumpulan kelamin yang terpisah dimana daripadanya, 4 monokarion penguji telah berjaya didapatkan. Apabila diuji keserasian antara kelompok, isolat CN-L1 dan WD814 didapati serasi dengan EGB-01, sedangkan dengan isolat CN-L2, EG-NL dan CN-NL tidak. Dengan itu, hasil persilangan keserasian monokarion telah mengesahkan bahwa walaupun EGB-01, CN-L1 dan WD814 masing-masing didapati daripada perumah-perumah kelapa sawit, kelapa dan palma L*ivistona*, kesemuanya adalah anggota kumpulan spesies biologi yang sama.

Kajian keupayaan jangkitan bagi isolat daripada perumah-perumah kelapa sawit dan kelapa telah diuji keatas anak pokok kelapa sawit selama 12 bulan melalui percubaan rumah kaca. Hanya isolat EGB-01 dan CN-L1 sahaja didapati menyebabkan penyakit keatas anak-anak pokok kelapa sawit dengan kedua-duanya menunjukkan 100% jangkitan. Tidak berlaku langsung jangkitan keatas anak-anak pokok kelapa sawit yang diinokulasi dengan isolat-isolat EG-NL, CN-NL dan CN-L2, jenis bukan *G. boninense*. Apabila anak-anak pokok yang terjangkit dikeluarkan dari tanah, titik awal kemasukan *G. boninense* didapati berlaku pada akar primer tanaman. Gambar akar yang baru terjangkit yang diambil melalui mikroskop elektron pengimbas (SEM) menunjukkan bahwa koloni kulat telah bertapak pada bahagian epidermis, korteks, endodermis, lakuna dan juga telah menyumbat ruang xilem dan floem. Ini menunjukkan bahwa pengkolonisasi kulat tidak terhad pada mana-mana tisu tertentu sahaja. Sebagai rumusan, kajian ini mendapati, pertama, lebih dari satu spesies *Ganoderma* berkilat terdapat pada tunggul-tunggul kelapa. Kedua, *G. boninense* yang diasingkan daripada kelapa sawit dan kelapa adalah

bersifat patogen pada kelapa sawit dan yang ketiga, spesimen yang bukan *G. boninense* (EG-NL, CN-NL dan CN-L2) tidak bersifat patogen pada kelapa sawit.

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CHAPTER 1

INTRODUCTION

Oil palm cultivation spreads across the equatorial region over 3 continents. It is planted as a cash crop in Southeast Asia to replace stands of rubber, coconut or primary forest. However, the intensive monoculture of this crop has brought with it an environmental imbalance which resulted in the appearance of many diseases, pest and nutritional or physiological disorders (Turner, 1981).

Ganoderma basal stem rot (BSR) of oil palm is of considerable economic importance particularly in Southeast Asia. The disease shortens the productive life of plantations and caused significant early losses in subsequent plantings. The genus *Ganoderma* are common saprophytes within an oil palm cropping system, but a numbers of species have been reported to be in direct association with oil palms, ranging from 6 (Steyaert, 1967b) viz. *G. boninense, G. miniatocinctum, G chalceum, G. tornatum, G. zonatum* and *G. xylonoides* to at least 15 species (Turner, 1981). From studies on 5 oil palms estates in Malaysia, Ho & Nawawi (1985) considered *Ganoderma boninense* as the causal agent of BSR. However, as with the earlier authors (Steyaert, 1967b; Turner, 1981) no taxonomic key was developed, making the field identification of the fungus still unresolved. Thus the name has been frequently used "indiscriminately as a convenient tag for *Ganoderma* basidiomata on oil palm but the authenticity of the species could not

be verified" (Miller *et al.*, 1994). Originally, the species name of *G. boninense* was given by Patouillard in 1887 when describing collections of *Ganoderma* from the Bonin, Nouka-hiva and Marquesas Islands in the Pacific Oceans. He described them without establishing formal taxonomic distinctions (Commonwealth Agricultural Bureaux, 1975), which later was the cause of many taxonomic confusions.

Currently, the taxonomy of species of *Ganoderma* that specifically caused BSR on oil palms is still unclear. Currently, taxonomic divisions within the genus *Ganoderma* in general is still chaotic. This is due to the presence of heterogenic forms, dubious nomenclature and inconsistencies in application of the numerous criteria by which the genus has been subdivided (Bazzalo and Wright, 1982). Past taxonomic criteria based on macro morphological characters of the basidiome have not been able to differentiate the relationships between pathogenic *Ganoderma* of different hosts. This is because some features can vary due to environmental conditions (Steyaert, 1980). In addition, basidiomes normally appear only towards the last stages of the disease and so diagnosis of BSR on the basis of basidiome characters is not practical (Miller *et al.*, 1994).

The traditional classification and identification of *Ganoderma* was based upon morphological characteristics of basidiocarps, basidiospore, and mycelium. Zhao (1989) used the morphological-species concept based on spore size, context colour, cutis layer, laccate or non-laccate pileus, stipitate or sessile condition and sporophore pore size to classify the various *Ganoderma* specimens from China.

His research encompassed both anatomical and morphological characteristics. Pegler and Young (1973) and Steyaert (1980) reported that basidiospore characteristics can vary according to environmental conditions and therefore was not a credible taxonomic criterion.

The use of other methods like molecular studies (Miller, 1995; Abdullah, 1996; Idris, 1999; Latiffah, 2002) have not resolved the identification problems associated with *Ganoderma* species. The present study is focused on the use of the biological concept in determining conspecificity of specimens to *Ganoderma boninense* from Malaysia, the causal pathogen of basal stem rot. The principle of the method is as outlined in Raper (1966), where individuals belonging to the same species are ones that are able to interbreed and form a population. Adaskaveg and Gilbertson (1986) suggested that interfetility tests be used as an aid to determine the identity of species. Since *Ganoderma* is a sexual fungus, homokaryons of *Ganoderma* species must first be obtained.

Many previous studies link disease development to palms planted on ex-coconut plantations (Navaratnam, 1964, Turner, 1965a). The latter author reported that the incidence was highest when the oil palms were grown on lands that were formerly coconut plantations. Coconut stumps that were left in between rows of new oil palm plantings were believed to form the infection foci for disease development. This implied that *Ganoderma* on coconut stumps and those on oil palms were identical. However, no scientific investigations have yet been done to support this contention. Likewise, no studies have been conducted to

establish their differences, i.e. to indicate that they were taxonomically and pathologically different. Hence, a major part of the present studies was to establish whether *Ganoderma* from oil palm and coconut hosts were all *G. boninense* and whether they can all infect oil palm seedlings. This study chose isolate EGB01 to represent *G. boninense* based on its closest conformity to morphological descriptions of that species by Steyaert as outlined in IMI paper (Commonwealth Agricultural Bureaux, 1975). Tester strains will be developed from EGB-01 and then tested with the outgroup sample, isolate WD-814 from Japan and identified by the Forestry and Forest Products Research Institute (FFPRI) of Japan as *G. boninense*. Once the conspecificity was established, this study investigates whether *Ganoderma* sp. from oil palm and coconut stumps found locally were also *G. boninense*.

The objectives of this study were:

1. To determine the mating system of *G.boninense* and to develop tester strains for the local *G. boninense* isolate represented by EGB-01 based on compatibility tests between sibling monokaryons.
2. To investigate mating compatibilities between tester strains of the local *G. boninense* isolate EGB-01 with WD-814 which was *G. boninense* from Japan. The testers were the used on 4 other *Ganoderma* specimens isolated from both oil palm and coconut stumps.
3. To establish the pathogenic potential of the same 5 *Ganoderma* specimens isolated from oil palm and coconut stumps from (2) above, on oil palm seedlings.