UNIVERSITI PUTRA MALAYSIA

NUTRITIONAL AND BIOCHEMICAL CHARACTERISTICS OF OIL PALM (Elaeis guineensis Jacq.) SEEDLINGS IN RELATION TO Ganoderma BASAL STEM ROT

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By

TENGOUA FABIEN FONGUIMGO

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

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DEDICATION

This Thesis is dedicated to
My understanding and lovely wife: Madame TENGOUA Josiane
My beloved kids:
SOBZE TENGOUA Melvis
NGUIMGO TENGOUA Ornella
MANEZEM TENGOUA Brynda
TEPIE TENGOUA Vivaldi Ryan
SONGFACK TENGOUA Hensla Warel,
for their love and patience.

Abstract of thesis submitted to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the degree of Doctor of Philosophy

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Basal stem rot (BSR) of oil palm caused by the fungus *Ganoderma boninense* is a highly damaging disease in South-east Asia. It is expanding gradually in some oil palm growing countries in Africa and South America. Up to date, available control measures have some limitations. Micronutrients known to have some beneficial effects on disease control have not been assessed on BSR yet. This study investigated the nutritional and biochemical characteristics of six oil palm progenies in relation to BSR. The optimum concentrations of boron (B), copper (Cu) and manganese (Mn) for the growth of oil palm seedlings was determined. Their subsequent effect on nutritional, biochemical and growth parameters of oil palm seedlings was tested prior to evaluating their effects on *Ganoderma* incidence and severity. The six oil palm progenies reported to respond differently to *Ganoderma* attack were found effectively different in many parameters. For instance, progenies were significantly different for their root nutrient content except for Zn. With the exception of leaf Cu, progenies also differed significantly in their leaf nutrient content. No significant difference was observed among progenies at 6-7 months for lignin in roots, but by 16-17 months, lignin content in roots of progenies significantly differed. All enzyme activities were significantly different in roots of oil palm progenies at 6-7 months. At 16-17 months, progenies significantly differed only for peroxidase activity. Two (2) mg B/mL and 2 mg Cu/mL of culture solution were identified as optimum concentrations for the growth of oil palm seedlings. All the tested concentrations of Mn (5, 10, 15 and 20 mg/mL) were phytotoxic, but 2 mg Mn/mL was maintained for subsequent studies to maintain nutrient balance. The single and combined concentrations of the selected micronutrients on oil palm seedlings generally increased SPAD chlorophyll value, plant height, and plant biomass compared with the control (no B, no Cu, and no Mn), suggesting the importance of B, Cu and Mn for the growth of oil palm seedlings. Apart from the control, no treatment was consistently higher or lower than the others for the studied

parameters. Hence, all the treatments were formulated in forms of fertilizers and tested on *Ganoderma* incidence and severity. Treatment T9 (B + Cu + Mn) in general gave the poorest performance for most growth and physiological parameters. Double combinations of treatments, T6 (B + Cu), T7 (B + Mn) and T8 (Cu + Mn) generally performed better than other inoculated treatments for nearly all the parameters assessed. In conclusion, a proper nutritional environment may effectively reduce *Ganoderma* incidence and severity; and the double combination of micronutrients may be more effective than individual nutrients or their triple combination.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

CIRI PEMAKANAN DAN BIOKIMIA ANAK KELAPA SAWIT (*Elaeis guineensis* Jacq.) BERKAITAN REPUT PANGKAL BATANG *Ganoderma*

Oleh

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Reput pangkal batang (BSR) kelapa sawit yang disebabkan oleh kulat *Ganoderma boninense* adalah penyakit sangat serius di Asia Tenggara. Ia berkembang secara beransur-ansur di beberapa negara yang ditanami kelapa sawit di Afrika dan Amerika Selatan. Sehingga kini, langkah-langkah kawalan yang ada sangat terhad dan tidak memberi kesan yang memuaskan. Unsur-unsur pemakanan mikro diketahui memberi kesan yang baik pada kawalan penyakit belum dinilai lagi pada penyakit BSR. Kajian ini ditumpukan kepada ciri-ciri pemakanan dan biokimia enam progeni kelapa sawit berkaitan dengan BSR. Kepekatan optimum boron (B), kuprum (Cu) dan mangan (Mn) untuk pertumbuhan anak kelapa sawit ditentukan. Kesan berikutnya terhadap pemakanan, biokimia dan pertumbuhan parameter anak kelapa sawit telah diuji sebelum menilai kesannya terhadap keterukan penyakit *Ganoderma*. Hasilnya, enam progeni kelapa sawit bertindak balas secara berbeza kepada serangan *Ganoderma* dalam banyak parameter yang disukat. Sebagai contoh, progeni berbeza secara ketara dalam kandungan nutrien akar kecuali Zn. Untuk daun kelapa sawit, kecuali Cu, semua progeni menunjukkan perbezaan yang ketara untuk semua kandungan nutrien. Tiada perbezaan yang ketara diperhatikan di kalangan progeni pada bulan ke 6-7 untuk lignin dalam akar, tetapi pada bulan ke 16-17, kandungan lignin dalam akar progeni berbeza dengan ketara. Semua aktiviti-aktiviti enzim berbeza secara ketara dalam akar progeni kelapa sawit pada bulan ke 6-7. Pada bulan ke 16-17, semua progeni ketara berbeza hanya untuk aktiviti peroksidase. Pada kepekatan 2 mg B/mL dan 2 mg Cu/mL telah dikenal pasti sebagai kepekatan optimum untuk pertumbuhan anak kelapa sawit. Kesemua kepekatan Mn diuji (5, 10, 15 dan 20 mg/mL) didapati fitotoksik, tetapi 2 mg Mn/mL dikekalkan untuk kajian seterusnya bagi keseimbangan nutrien. Ujian kepekatan yang telah dicampur satu mikronutrien dipilih pada anak kelapa sawit memberi hasil keseluruhan yang baik untuk nilai SPAD klorofil, ketinggian tumbuhan, dan biomas tumbuhan, kecuali kawalan (tiada B, Cu, dan Mn), menunjukkan kepentingan unsur-unsur tersebut bagi pertumbuhan anak kelapa sawit. Selain daripada kawalan, rawatan adalah lebih

tinggi secara konsisten atau lebih rendah daripada yang lain untuk parameter tersebut. Oleh itu, kesemua unsur tersebut telah dirumuskan dalam bentuk baja dan diuji ke atas anak kelapa sawit bagi menguji kejadian dan keterukan serangan *Ganoderma*. Rawatan T9 (B + Cu + Mn) secara umumnya memberikan nilai yang tidak memuaskan pada parameter pertumbuhan dan fisiologi. Secara keseluruhannya, gabungan dua rawatan, T6 (B + Cu), T7 (B + Mn) dan T8 (Cu + Mn) memberikan prestasi yang lebih baik daripada lain-lain rawatan yang diinokulat pada hampir semua parameter dinilai. Sebagai kesimpulannya, penambahan B, Cu dan Mn adalah berkesan bagi mengurangkan kejadian dan keterukan penyakit *Ganoderma* dan kombinasi dua antara nutrien lebih berkesan daripada nutrien individu dan/atau gabungan ketiga-ketiga nutrien.

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I certify that a Thesis Examination Committee has met on 4 July 2014 to conduct the final examination of Tengoua Fabien Fonguimgo on his thesis entitled "Nutritional and Biochemical Characteristics of Oil Palm (*Elaeis guineensis* Jacq.) Seedlings in Relation to Ganoderma Basal Stem Rot" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

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LIST OF ABBREVIATIONS

%	Percent
$^{\circ}$C	Degree Celcius
AA	Auto-analyzer
AAS	Atomic absorption spectrophotomer
ABTS	2, 2’-azino-bis (3-ethylbenzo-thiazoline-6-sulfonic acid)
ADP	Adenosine diphosphate
AIL	Acid insoluble lignin
Al	Aluminium
ANOVA	Analysis of variance
ASL	Acid soluble lignin
AUDPC	Area under the disease progress curve
B	Boron
BRIS	Beach ridges interspersed with swales
BSR	Basal stem rot
Ca	Calcium
$Ca(OH)_2$	Copper hydroxide (slaked lime)
CaO	Calcium oxide
CDC	Cameroon development corporation
cm	Centimetre
CPO	Crude palm oil
Cu	Copper
$CuCO_3$	Copper carbonate
$CuSO_4$	copper (II) sulphate
D × P	*Dura* × *Pisifera*
DAB	Diaminobenzidine
DI	Disease incidence
DMRT	Duncan’s Multiple Range Test
DOT	Disodium octaborate tetrahydrate
DR	Disease reduction
DSI	Disease severity index
DSIB	Disease severity index for bulb symptoms
DSIF	Disease severity index for foliar symptoms
DSIR	Disease severity index for root symptoms
EC	Enzyme code
EDTA	Ethylene diamine tetraacetic acid
ER	Epidemic rate
FAO	Food and Agricultural Organization of the United Nations
Fe	Iron
FELDA	Federal Land Development Authority
FFB	Fresh fruit bunches
g	Gram
g/L	Gram per litre
GSM	*Ganoderma*-selective medium
H_2O_2	Hydrogen peroxide
H_2SO_4	Sulphuric acid
ha	Hectare
HCl	Hydrochloric acid
hr	Hour

HRGP	hydroxyproline-rich glycoproteins
IAA	Indol acetic acid
K	Potassium
kg	Kilogram
LAC	Laccase
LSD	Least significant difference
M	Molar
MEA	Malt extract agar
mg	Milligram
Mg	Magnesium
mg/kg	Milligram per kilogram
mg/L	Milligram per litre
min	minute
mL	Millilitre
mM	Millimolar
mm	Millimetre
Mn	Manganese
$MnCl_2$	Manganese chloride
MnO(OH)	Manganite
MnO_2	Pyrolusite
$MnSO_4$	Manganese sulphate
Mo	Molybdenum
MPOB	Malaysian Palm Oil Board
N	Nitrogen
NaOH	Sodium hydroxide
NH_3	Ammonia
nm	Nanometre
O_2	Oxygen
ODW	Oven-dry weight
P	Phosphorus
PAL	Phenylalanine ammonium-lyase
PDA	Potato dextrose agar
pH	Hydrogen potential
PH	Plant height
P_N	Net photosynthetic rate
POX	Peroxidase
ppm	Part per million
PS I	Photosystem I
PS II	Photosystem II
PVP	polyvinyl pyrrolidone
PVPP	polyvinyl polypyrrolidone
RCBD	Randomized complete block design
RDW	dry weight
RFW	Root fresh weight
RNA	Ribonucleic acid
RS	Root surface
RT	Root tips
RV	Root volume
RWB	Rubber wood block
SDSAS	Sime Darby Seeds and Agricultural Services

SDW	Shoot dry weight
SFS	Severity of foliar symptoms
SFW	Shoot fresh weight
SPAD Chl	SPAD Chlorophyll
TDW	Total dry weight
TL	Total lignin
TLA	Total leaf area
TRL	Total root length
US$	United States dollars
UV	Ultra violet
Zn	Zinc
µL	Micro litre

CHAPTER 1

INTRODUCTION

1.1 Background Information

Oil palm (*Elaeis guineensis* Jacq.) is a perennial oil crop that exists in wild, semi-wild, and cultivated states in the equatorial tropics of Africa, South-East Asia, and the Americas (Hartley, 1988). The total area planted in oil palms estimated at 11×10^6 ha with 70% exploited by smallholders (Rival, 2007), has rapidly expanded. As a globally important crop, total land under oil palm cultivation has more than quadrupled, moving from less than 4×10^6 ha in 1961 to about 15×10^6 ha across the world (FAO, 2009; Turner et al., 2011; Anonymous; 2011). In many developing countries, oil palm is an alternative to cocoa, coffee and rubber, the traditional cash crops whose prices regularly fluctuate in the world market (Bakoume et al. 2002). In Africa, the oil palm grower is the first consumer of his palm oil or kernel oil and the excess is easily sold in the local market.

In 2008, the major vegetable oil production was 111.127 million tonnes. Palm oil contributed about 40% and ranked first just before soybean oil (33%), and accounted for about 67% of the world exports (Jackson et al., 2009). World palm oil production multiplied 15-fold since 1948 to reach 38×10^6 tonnes in 2007 (Rival, 2007). South-East Asia (Malaysia and Indonesia) contributed 86% of the global palm oil production. Malaysia, the second largest world's palm oil producer after Indonesia contributed 10.3% of the world oils and fats market with 15.82 million tonnes of the 154 million tonnes of oils and fats in 2007 (Global Oil and Fats, 2008). But oil palm is still an important source of income in Africa and Latin America (Billotte, 2004). An increase in world demand for edible palm oil is predicted as a result of future increases in the world population, the increase in per capita consumption of oils and fats, and development of the bio-diesel industry. Palm oil is poised to contribute significantly to meet this demand in view of its high yield of 4-5 tonnes per hectare per year (Barison and Ma, 2000); almost three times the yield of coconut and more than 10 times that of soybean (0.4 tonne per hectare) (Rajanaidu and Jalani, 1994). Furthermore, the production cost of palm oil in its ecosystem is the lowest compared to all other oil crops (Billotte, 2004).

Further improvement in palm oil production in the world is governed not only by the implementation of new plantations, the regeneration of old plantings, and the availability of high yielding planting materials, but also by good pest and disease control measures to fill the yield gap existing between field trials and plantations (Jalani et al., 2003). In South-East Asia, the major constraint to the oil palm industry is *Ganoderma*, a soil-borne fungus which leads to yield reduction to the affected oil palm and also to death. *Ganoderma* was also declared a serious pathogen in Cameroon (Tengoua and Bakoume, 2005) and was becoming serious in replanting areas (Tengoua, 2005).

1.2 Problem Statement

Basal stem rot due to *Ganoderma* spp is the major disease causing serious damage to oil palm in Papua New Guinea and the Pacific Islands (Flood and Hasan, 2004; Pilloti, 2005), and in Southeast Asia, namely in Malaysia and Indonesia (Chenon, 1975; Ariffin, 1990; Singh, 1991). In Africa, Cameroon is currently the only country where *Ganoderma* basal stem rot exists as a major disease. Dead palms due to *Ganoderma* were estimated at 53.22% in 25-year-old or older first generation plantations (Tengoua and Bakoume, 2005). It caused 6.4% of plant losses in 1995 and 1996 replantings in the Cameroon Development Corporation (CDC) plantations (Tengoua, 2005). In Malaysia, Singh (1991) reported plant losses as high as 85% on coastal soils by the time palms were replanted at 25 years. In Latin America, the existence of BSR has been confirmed (Martinez and Arango, 2013) even though the extent of damage is not yet determined. If no appropriate action is taken to control the disease, in the not-too-distant future, BSR will become a great concern in all the oil palm growing countries in the world. Unfortunately, to date, no definitive solution is available. The few control methods being implemented include: (i) Use of a balanced fertilizer, namely N, P, K (Turner and Poon, 1968; Mohd Tayeb et al., 2003), (ii) Manual application of calcium nitrate (Hendry, 1997; Sariah and Zakaria, 2000; Flood and Hasan, 2004), (iii) Excision of infected tissues, (iv) Mounding around the stem base to stimulate root production and provide additional support, the shredding of diseased palms into small fragments and spreading out instead of windrowing (Wan, 2007), (v) Digging of trenches to prevent mycelium spread of the pathogen (Flood and Hasan, 2004), (vi) Use of systemic fungicides (Ramasamy, 1972; Jollands, 1983; Khairudin, 1990a; Khairudin, 1990b; Lim et al., 1990) and natural fungicide (Nurfaezah et al., 2012), and (vii) Cultural techniques during replanting such as sanitation and clean clearing (Idris et al., 2004; Flood et al., 2005).

Biological control of the fungus is one of the pest management strategies with bright a prospect compared to chemical pesticides. It is also an environmentally safe alternative. Some microorganisms shown to have antagonistic action against *Ganoderma* include *Trichoderma* spp (Ilias, 2000; Sariah et al., 2005; Shamala, 2005; Izzati and Faridah, 2008; Siddiquee et al., 2009; Shamala et al., 2013; Susanto, 2013), *Aspergillus*, *Penicillium* spp, Arbuscular mycorrhiza (Idris and Ariffin, 2004; Shamala et al., 2013), *Gliocladium* (Flood and Hasan, 2004) and a non-pathogenic strain of *Ganoderma*, GanoEF1 (Idris et al., 2010). Bacteria, such as *Pseudomonas fluorescens* and *Bacillus* sp (Susanto et al., 2005; Susanto, 2013), *Pseudomonas aeruginosa* and *Burkholderia cepacia* (Zaiton et al., 2008; Bivi et al., 2010; Shamala et al., 2011) and actinomycetes (Lim et al., 2013) have also been involved in biological control of *Ganoderma* BSR. Plant extracts have also been tested against this pathogen (Noor Pahtiwi et al., 2013); but none of the above mentioned methods has yet been satisfactory in maintaining disease incidence at an acceptable threshold. Many of these methods efficiently work at laboratory and nursery levels, but face serious limitations for field application probably due to the variation in environmental conditions. This holds true especially for the use of *Trichoderma* as a bio-control agent whose population drastically drops in oil palm plantations from 10^6 to less than 10^3 cfu, rendering the application of the method ineffective or its maintenance at an efficient level uneconomical. Likewise, implementation of chemical control measures is not cost effective and is environmentally unfriendly with regard to the amount of chemicals needed to treat a few million hectares of oil palm plantations. Breeding for tolerance to *Ganoderma* is an optimistic perspect since there are putative resistant materials in African *Elaeis guineensis* collections (Idris *et al*., 2004; Durand-Gasselin et al., 2005), but much is still required before the release of *Ganoderma* tolerant planting materials. Diversity of strains due to mutations requires a wide range of putative tolerant oil palm materials. Unfortunately, current sources of partial tolerance are found only in limited oil palm materials to permit an effective breeding programme. Owing to these limitations, thinking about developing alternative cost effective and environmentally sound control measures such as improvement of the oil palm defence system through balanced fertilizer (with required quantity and quality of micronutrients) and lignification becomes an imperative.

Ganoderma is a white rot fungus that degrades lignin to water and carbon dioxide and uses cellulose as a nutrient (Siti el al., 2004; Paterson, 2007; Paterson et al., 2008). Hence, a comprehensive study of the mode of action of *Ganoderma* and setting up of new strategies would allow the development of an efficient integrative control measure. This includes the reinforcement of cell walls by improving the lignification process to create a stronger physical barrier against pathogen penetration. The differences in susceptibility observed in some oil palm progenies may be related to differences in their lignin content (Paterson et al., 2008). Lignification can be improved through the manipulation of certain nutritional factors directly or indirectly involved in the process. Boron, copper and manganese play an important role in lignin biosynthesis, in addition to their known biocidal effect on a number of plant pathogens and their other functions in the plant.

1.3 Research Objectives

The general objective was to examine nutritional status of oil palm seedlings with special reference to micronutrients B, Cu, and Mn to see whether their manipulation can reduce BSR disease. The specific objectives were: (i) to determine the nutrient status and biochemical characteristics of six oil palm progenies reported to behave differently toward *Ganoderma,* (ii) to determine the optimum concentrations of B, Cu and Mn for the growth of oil palm seedlings, (iii) to assess the effects of single and combined optimum concentrations of B, Cu and Mn on nutritional, biochemical and growth parameters of oil palm seedlings, and (iv) to test the effects of single and combined optimum concentrations of B, Cu and Mn on *Ganoderma* incidence and severity.

1.4 Outline of the Thesis

Since developing partially resistant material is a long term approach, it is imperative and essential that oil palm scientists continue to investigate other alternative management strategies. Over many years, micronutrient application has been totally overlooked in oil palm fertilization programmes. This appears to have weakened oil palms through exhaustion of soil reserves of these nutrients, and predisposed this crop to certain diseases that could be controlled by a balanced nutrient status. This study was carried out to identify the missing nutritional factors with emphasis on micronutrients that could be manipulated to control *Ganoderma* BSR in oil palm. With the nutrients identified, a genetic approach as stated by Amtmann et al. (2008), can be applied to establish a causal relationship between susceptibility/tolerance on the one hand, and nutrient assimilation capability on the other hand. When clearly identified, this information can be used to design agricultural strategies that support the nutritional status of the oil palm while exploiting their inherent potential for defence against BSR disease. After stating the problem with some background information in Chapter 1, a brief review on oil palm, *Ganoderma*, lignin, boron, copper, manganese, and their importance in plant disease is presented in Chapter 2. In Chapter 3, six oil palm progenies reported to behave differently towards *Ganoderma* BSR were examined to identify nutritional and biochemical characteristics that could explain differences observed when challenged with *Ganoderma boninense*. In view toward advising the incorporation of micronutrients in the oil palm fertilization programme with regard to their importance in growth and their potential role in plant defence against pests and diseases, different concentrations of boron, copper and manganese were tested in Chapter 4 to identify their optimum for the growth of oil palm seedlings. In Chapter 5, the optimum concentrations of selected micronutrients identified in Chapter 4 were tested singly and in different possible combinations on nutritional, biochemical and growth parameters of oil palm seedlings to select the best treatment (s) to be assessed with *Ganoderma*. With no treatment being distinctively and consistently better than others for the parameters tested, both single and combined optimum concentrations were formulated in forms of fertilizer treatments and examined for their effects on *Ganoderma* incidence and severity in Chapter 6. Chapter 7 summarizes the results obtained in this work, presents the conclusions and proposes some recommendations for further research.

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