BASAL STEM ROT OF OIL PALM DISEASE DEVELOPMENT PLANTED ON MINERAL AND PEAT SOILS AND PATHOGENICITY OF *Ganoderma*

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2024

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by

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Thesis submitted in fulfilment of the requirements
for the degree of
Master of Science

June 2024

ACKNOWLEDGEMENT

In the name of Allah, the Most Gracious and the Most Merciful.

First of all, I would like to thank my first supervisor, Prof. Dr. Latiffah binti Zakaria for all her sacrifices, guidance, advice, great effort, support, encouragement and sharing her expertise to complete my study. Secondly, I would like to express heartfelt gratitude to my second supervisor, Dr. Brian P. Forster for all his astounding support, his guidance, encouragement and advice throughout this project. I am also grateful to the Verdant Biosciences's management (Dr. Stephen Nelson and Bapak Ahmad Subagio) who provides various support for the experiments and also study.

My special and sincere appreciation goes to my families and in laws especially my beloved husband, son, my twins for their endless support, prayers, encouragement, and sacrifices in ensuring the success of my study. My Father and siblings who always support and pray for me to gain success during my study period.

My gratitude goes to Pathology team and all staff of Verdant Bioscience for their cooperation and technical support. I would also like to thank all the staff of the School of Biological Sciences for their technical support. Besides, I would like to thank Dr. Nurul Farizah Azuddin, Postdoctoral researcher in School of Biological Sciences for her endless support, advice, assistance throughout my study period. I would also like to thank my laboratory colleagues in Lab 117, Aisha Mohammad, Asmaul Husna and Musa Hamidu for their support. Finally, I express my heartiest thanks to all those who helped me directly or indirectly to complete my M.Sc degree successfully.

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LIST OF SYMBOLS AND ABBREVATIONS

%	Percentage
<	Less-than
°C	Degree Celsius
€	Euro
µl	Microlitre
µm	Micrometre
Cm	Centimetre
ANOVA	Analysis of Variance
Bp	Base pair
BLAST	Basic Local Alignment Search Tool
BSR	Basal Stem Rot
CPO	Crude palm oil
CPKO	Crude palm kernel oil
cm	Centimeter
CRISPR	Clustered Regularly Interspaced Short Palindromic Repeats
Cu	Copper
D	Dura
DI	Disease incidence
DS	Disease severity
DNA	Deoxyribonucleic acid
rDNA	Ribosomal DNA
FRIM690	*Ganoderma boninense* Forest Research Institute Malaysia 690
G	Gramme
GPS	Global positioning system
H	Hour

Ha	Hectare
ITS	Internal transcribed spacer
Kb	Kilobase
Kg	Kilogramme
L	Litre
M	Metre
mA	Milliampere
MD66	*Ganoderma* boninense isolate MD66
MEGA	Molecular Evolutionary Genetics Analysis
Min	Minute
Ml	Milli litre
ML	Maximum Likelihood
Mm	Milli metre
MPa	MegaPascal
Mt	Metric ton
mtDNA	Mitochondrial DNA
NCBI	National Centre for Biotechnology Information
P	Pisifera
PA	Potato Agar
PCR	Polymerase Chain Reaction
PNG	Papua New Guinea
PSA	Potato Sucrose Agar (PSA)
pH	Potential of hydrogen
PKO	Palm Kernel Oil
rDNA	Ribosomal DNA
RAPD	Random Amplification of Polymorphic DNA
RFLP	Restriction Fragment Length Polymorphism

RWB	Rubber Wood Block
S	Second
sp.	Species (Singular)
spp.	Species (Plural)
SNP	Single nucleotide polymorphism
SPSS	Statistical Package for Social Sciences
SSR	Simple Sequence Repeat
TBE	Tris-Borate-EDTA
UPMGB001	Universiti Putra Malaysia *Ganoderma boninense* 001
US	United States
USD	United States Dollar
V	Volt
YR	Yellow-Red
Zn	Zinc

LIST OF APPENDICES

PERKEMBANGAN PENYAKIT REPUT PANGKAL BATANG KELAPA SAWIT DITANAM PADA TANAH MINERAL DAN TANAH GAMBUT, DAN KEPATOGENAN *Ganoderma*

ABSTRAK

Kelapa sawit (*Elaeis guineensis* Jacq.) mempunyai nilai ekonomi yang tinggi dan menyumbang kepada ekonomi di banyak negara pengeluar terutamanya Indonesia dan Malaysia, sebagai pengeluar utama. Walau bagaimanapun, kelapa sawit dijangkiti penyakit reput pangkal batang (RPB) yang disebabkan oleh kulat basidiomiset, menjadi ancaman utama kepada industri kelapa sawit. RPB menyebabkan jangkitan serius di Indonesia dan Malaysia, yang membawa kepada kerugian serius apabila menjangkiti kelapa sawit yang lebih muda. Di Indonesia, insidens RPB yang tinggi telah dilaporkan pada kelapa sawit yang ditanam di tanah mineral dan tanah gambut terutamanya pada penanaman generasi kedua. Oleh itu, objektif kajian ini adalah untuk mengenal pasti pencilan *Ganoderma* yang dikaitkan dengan RPB dalam kedua-dua tanah mineral dan tanah gambut dengan menjalankan ujian kepatogenan bagi pencilan *Ganoderma* daripada tanah mineral dan tanah gambut dan untuk memerhati dan memantau perkembangan penyakit RPB pada pohon kelapa sawit yang ditanam di tanah gambut dan tanah mineral. Enam belas basidiokarpa *Ganoderma* telah berjaya diperolehi daripada kelapa sawit yang dijangkiti di ladang Simalungun (tanah mineral) dan Labuhan Batu Selatan (tanah gambut) di Sumatera Utara, Indonesia. Ciri-ciri morfologi basidiokarpa merangkumi huraian *Ganoderma boninense*. Untuk mengenal pasti pencilan kulat dengan lebih tepat, penjujukan DNA kawasan transkripsi penjarak dalaman (ITS) digunakan dan jalur 600 bp dihasilkan. Carian BLAST pangkalan data Genbank menunjukkan semua pencilan menunjukkan 99 – 100% persamaan dengan

jujukan *G. boninense* yang di deposit dalam Genbank. Analisis filogenetik menunjukkan pencilan yang dipencilkan daripada ladang tanah mineral dan tanah gambut berkelompok bersama dengan *G. boninense* rujukan, oleh itu mengesahkan pencilan daripada kedua-dua ladang adalah *G. boninense*. Ujian kepatogenan telah dijalankan ke atas anak benih kelapa sawit menggunakan blok kayu getah (BKG) yang di kolonisasi oleh miselia *Ganoderma* sebagai sumber inokulum. Ujian kepatogenan dilakukan di tapak semaian berasingan di Simalungun (tanah mineral) dan Labuhan Batu Selatan (tanah gambut). Enam belas pencilan terdiri daripada lapan pencilan dari tanah mineral dan lapan pencilan dari tanah gambut telah diuji dalam ujian kepatogenan. Keputusan ujian kepatogenan menunjukkan semua pencilan dari tanah mineral adalah patogenik dengan kevirulenan rendah hingga sederhana dan keterukan penyakit 22.8% hingga 73.6%. Bagi pencilan dari Labuhan Batu Selatan (tanah gambut), tujuh pencilan bersifat patogenik dan satu pencilan tidak patogenik. Pencilan patogenik dari Labuhan Batu Selatan (tanah gambut) juga menunjukkan kevirulenan rendah hingga sederhana dengan keterukan penyakit 2.8% hingga 56.2%. Pencilan tidak patogenik tidak menghasilkan sebarang gejala RPB semasa tempoh percubaan. Ujian kepatogenan juga menunjukkan pencilan *G. boninense* dari tanah mineral adalah lebih virulen dan menghasilkan gejala lebih awal daripada pencilan dari tanah gambut. Perkembangan penyakit lebih perlahan pada anak benih kelapa sawit yang diinokulasi dengan pencilan dari tanah gambut berbanding anak benih kelapa sawit yang ditanam di dalam tanah mineral. Pemerhatian ini mungkin berkaitan dengan sifat tanah kerana tanah mineral yang digunakan dalam ujian kepatogenan terdiri daripada kira-kira 67% pasir yang menyumbang kepada kelonggaran sifat fizikal tanah dan keliangan yang tinggi yang menyokong pertumbuhan akar yang lebih cepat ke sumber inokulum. Perkembangan penyakit RPB di ladang dijalankan selama 37 bulan di kedua-dua

ladang Simalungun (generasi kedua kepala sawit tanah mineral) dan Labuhan Batu Selatan (generasi pertama kelapa sawit tanah gambut, ditanam semula dari hutan). Simptom pada kelapa sawit yang dijangkiti di kedua-dua ladang adalah serupa di mana pelepah dan daun jelas kelihatan pucat hijau dan kekuningan serta kelihatan daun lembing yang belum dibuka. Perkembangan penyakit diperhatikan di ladang tanah gambut dan tanah mineral menunjukkan dalam tempoh 37 bulan pemerhatian, simptom RPB berkembang lebih cepat pada kelapa sawit yang ditanam di tanah gambut berbanding di tanah mineral, dengan bilangan pokok kelapa sawit yang ditanam di tanah gambut mati atau hampir mati. Ketersediaan bahan organik lebih tinggi dalam tanah gambut menjadi sumber nutrien penting untuk *G. boninense*. Sisa baki batang dan akar dari hutan boleh didapati di tanah gambut yang juga boleh menjadi sumber inokulum. Di samping itu, paras air dalam tanah gambut juga boleh menjejaskan jangkitan *Ganoderma*. Maklumat perkembangan penyakit RPB melalui ujian kepatogenan dan pemerhatian di lapangan boleh digunakan sebagai anggaran kemandirian kelapa sawit yang dijangkiti dalam kedua-dua tanah mineral dan tanah gambut. Pemahaman keseluruhan tentang semua faktor yang berkemungkinan menyumbang kepada jangkitan dan perkembangan RPB adalah penting untuk mengurus jangkitan dan dalam membangunkan sistem perlindungan tanaman bersepadu, terutamanya di ladang penanaman semula.

BASAL STEM ROT OF OIL PALM DISEASE DEVELOPMENT PLANTED ON MINERAL AND PEAT SOILS AND PATHOGENICITY OF *Ganoderma*

ABSTRACT

Oil palm (*Elaeis guineensis* Jacq.) has high economic value and contributes to the economy of many producing countries, especially Indonesia and Malaysia as the leading producers in the world. Oil palm is infected by *Ganoderma* fungus causing Basal Stem Rot (BSR) disease, a serious disease affecting oil palm productivity in Indonesia and Malaysia. In Indonesia, a high incidence of BSR has been reported in oil palm planted in mineral and peat soils particularly in second generation plantings. The objectives of the present study were to identify *Ganoderma* isolates associated with BSR in both mineral and peat soils, to conduct pathogenicity tests of *Ganoderma* isolates from mineral and peat soils and to observe and monitor BSR disease development in infected areas in peat and mineral soil plantings. Sixteen *Ganoderma* basidiocarps were successfully collected and isolated from infected oil palm in both Simalungun (mineral soil) and South Labuhan Batu (peat soil) plantations in North Sumatra, Indonesia. The morphological characteristics of basidiocarps fall within the description for *Ganoderma boninense.* In order to accurately identify the fungal isolates, DNA sequencing of Internal Transcribed Spacer (ITS) region was applied and 600 bp band were produced. Based on BLAST search, the isolates showed 99 – 100% similarity with the *G. boninense* sequences deposited in the Genbank. Phylogenetic analysis indicated the isolates from mineral soil and peat soil were grouped together along with *G. boninense* reference which confirmed the isolates from both soil types as *G. boninense*. Pathogenicity test was conducted on oil palm germinated seeds using rubber wood block (RWB) colonized by *Ganoderma* mycelia as a source of inoculum.

The pathogenicity tests were performed in separate nurseries at Simalungun (mineral soil) and South Labuhan Batu (peat soil). Sixteen isolates, comprising eight isolates from mineral soil and eight isolates from peat soil were tested for their pathogenicity. The results of pathogenicity trials indicated that all the isolates from mineral soil were pathogenic with low to moderate virulence and disease severity of 22.8% to 73.6%. For isolates from peat soil, seven isolates were pathogenic, and one isolate was found to be avirulent (non-pathogenic). Pathogenic isolates from peat soil also showed low to moderate virulence with disease severity of 2.8% to 56.2%. The avirulent isolate did not produce any BSR symptoms during the period of the trial. The pathogenicity test also indicated *G. boninense* isolates from mineral soil were more virulent and produced disease symptoms earlier than isolates from peat soil. Disease development was slower on oil palm seedlings inoculated with isolates from peat soil compared to those from mineral soil. This observation may be related to the soil properties as the mineral soil used in the pathogenicity tests consisted of about 67% sand which contributes to loose physical properties of the soil and high porosity which supports faster root grow to the inoculum source. Basal stem rot development in the field was conducted for 37 months in both Simalungun (second generation mineral soil oil palm planting) and South Labuhan Batu (first generation peat soil oil palm, replanted from forest) plantations. Symptoms of the infected oil palm in both plantations were similar with obvious pale green and yellowing of the fronds and leaves as well as unopened spear leaves. Disease development was observed in both peat and mineral soil plantations over 37 months. BSR symptoms developed faster in oil palms planted in peat soil than those in mineral soil, with a higher number of dead and moribund palms planted in peat. The availability of organic matter is higher in peat soils which are a source of nutrients for *G. boninense.* Stem and root residues from forest are available

in the peat soil which could also become sources of inoculum. In addition, the water table in peat soils can affect *Ganoderma* infection. An overall understanding of all possible factors that contribute to infection and development of BSR is important in managing BSR and in developing an integrated crop protection system, especially for replanting plantations.

CHAPTER 1

INTRODUCTION

The centre of origin of oil palm (*Elaeis guineensis Jacq.*) is in West Africa and was given its botanical name by Jacquin in1763, the species identifier is based on its origin, Guinea. The oil palm taxonomic clade is Order Arecales and Family Arecaceae (Henderson, 1986; Rajanaidu et al., 2017). Oil palm's introduction to Southeast Asia involved the transfer of four seedlings by the Dutch to the Botanical Garden at Bogor, Java, Indonesia in 1848. The seedlings arrived via West Africa and The Netherlands. Thereafter the Dutch East Indies distributed progenies from 1853 onwards and formed the basis of the oil palm industry in Indonesia (Rajanaidu, 2017).

The first oil palm introductions to Malaysia were initiated by the British in the 1870s. In 1912, Henri Fauconnier (a writer from France) imported seeds from Sumatra, Indonesia into Malaysia, with support from a Belgian agronomist who had begun establishment of the first oil palm plantations in Indonesia (M. Adrien Hallet). From these seeds, Malaysia's developed the first commercial oil palm plantations in 1917 at Tennamaram Estate, Selangor where oil palm replaced coffee (Rasiah and Shahrin, 2005; Reuters, 2009).

Southeast Asia is the main production region of palm oil with Indonesia and Malaysia being the main producers. Oil palm has now become an important plantation crop in Pacific-Rim countries such as: Thailand, Columbia, Nigeria, Ecuador, Honduras, Papua New Guinea, Guatemala and, Costa Rica (Pacheco et al., 2017). Malaysia was the leading producer from 1970 to 2006, but was overtaken by Indonesia in 2007, which now records 57% of global palm oil production (Gro-Intelligence, 2019; Shahbandeh, 2020a).

In recent years, during the period of 2008-2019 the export of palm oil from Indonesia increased from 15.1 metric tonnes to 29.5 metric tons with an export value of USD15.6 million. The export volume is quite large due to the land area (Hirschmann, 2020) planted with oil palm which is a major factor contributing to increased production. Oil palm plantations in Indonesia are mainly located on the island of Sumatra (with plantations covering 9,487,200 ha). However, there are significant plantation in Kalimantan (4,805,400 ha), Sulawesi (471.300 ha), Papua (223.100 ha) and other small areas in other regions (Direktorat Jenderal Perkebunan, 2020).

The development of oil palm cultivation has stimulated the threat of diseases and pests. Among the diseases, basal stem rot (BSR) caused by *Ganoderma* spp. is the most serious and prominent oil palm disease. In several old oil palm plantations in Indonesia, the disease incidence has reached 50% and caused a major loss in production and profit (Susanto and Sudharto, 2003). In Malaysia and Papua New Guinea, the main species associated with BSR in Indonesia is *G. boninese* (Pilotti, 2001).

In Indonesia, the causal organism, *G. boninense,* has been reported in North Sumatra, West Sumatra, Riau and Pontianak (Purnamasari et al., 2012; Susanto et al., 2013b). *Ganoderma boninense* has also been reported in Malaysia, and in addition to other *Ganoderma* species, *G. miniatocintum*, and *G. zonatum* were also reported as being pathogenic and causing BSR of oil palm (Idris et al., 2000). In the present study, *Ganoderma* isolated from infected oil palms were identified to determine their species identity as well as to understand whether other species of *Ganoderma* are associated with oil palm BSR in Sumatra.

BSR can cause 80% of stand loss (dead palms) through their economic life span (Turner, 1981; Turner, 2003). At replanting (normally after about 25 years), some oil palm areas could have 40–50% BSR infection, with most standing palms showing disease symptoms (Flood et al., 2000a). Soil type has been linked to disease spread; and disease incidence was reported to increase at a higher rate on inland lateritic soils and peat soils oil palm area, compared to mineral soils, irrespective of the cropping history (Ariffin et al., 2000).

In Indonesia, BSR has been reported in both mineral and peat soils. In one situation, a mineral soils plantation in North Sumatra the BSR incidence was reported to be approximately 35-63%. Observations by Verdant Bioscience, Indonesia showed BSR to be spread rapidly not only in mineral soils, but also peat soils (Susanto et al., 2013a; Virdiana et al., 2017). Alarmingly, after 10 years of planting, 80 out of 156 oil palm blocks of first generation oil palm in peat soil in North Sumatra, were infected by *Ganoderma* but with low level of disease incidence, <1% (Virdiana, 2017).

On mineral soil oil palm fields in North Sumatra, 0-1.5% of *Ganoderma* infections were recorded on palms <6 years old. The percentage of infection increased dramatically to 13-87% in palms >16 years old with stand palm per hectare were 35 to 119 palms per hectare (Virdiana et al., 2012b). *Ganoderma* starts to infect young palms (2-3 years old) with infection rates in third and fourth generations of oil palm being <1% (Priwiratama et al., 2014a; Prasetyo & Susanto, 2016; Prawiratama and Susanto, 2020). The evidence indicates that the percentage of oil palms infected by *Ganoderma* in mineral soils is higher than peat soils. This could be due to oil palm replantings in mineral soil (second, third or fourth generations), but in peat soils the first generation of oil palm is badly affected (Susanto and Huan, 2010; Virdiana, 2017). Thus, oil palm in peat soils is more susceptible to BSR infection.

Currently there are no resistant oil palm varieties and breeding efforts are hampered by a lack of good sources for resistance. Finding the most suitable method to control BSR in oil palm plantation is not easy. No single control method can give 100% success rate and therefore, integrated plant disease management comprising of cultural practice, biological and chemical controls are recommended. In order to control BSR in peat and mineral soils, it is important to understand the pathogenicity of the causal pathogen, *Ganoderma* sp. and to observe disease development. Information on the pathogenicity of *Ganoderma* sp. from mineral and peat soils is needed to determine whether disease severity is affected by variation in pathotype, environment (soil type) or both.

Disease symptoms in the field include three or more un-open spear leaves (newly emerged fronds) and the appearance of basidiocarps in basal positions on the trunk. Foliar symptoms might not be visible in some infected palms, but basidiocarps do appear. In some replanting systems in Sumatra where old palms were pushed over and palms infected by BSR removed, young replanted palms became infected by the disease and died from as early as the second year onwards after re-planting. Within 10 years, an economic loss is encountered, which may become severe after 15 years (Turner, 1981). In the present study, observations of disease development were made on infected oil palms of the same age (planted in 2006) in both peat and mineral soils. At the beginning of the study, in 2020 the incidence in peat soil was low (<2% infected oil palm). In mineral soil the disease incidence was much higher (about 15% to 52%) in older palms of more than 10 years old of third generation oil palm. Observations of disease development based on disease severity on infected oil palm is important as it allows prediction on the survival of the infected palms and there by crop yield potential.

The main aims of the present study are to determine the pathogenicity of *Ganoderma* isolates from mineral and peat soils and to observe BSR disease development in the field. The specific objectives are:

i. to identify *Ganoderma* isolates associated with BSR in both mineral and peat soils.

ii. to evaluate seedling pathogenicity test of *Ganoderma* isolates from mineral and peat soils.

iii. to observe and monitor disease development in peat and mineral soil plantings.

CHAPTER 2

LITERATURE REVIEW

2.1 Oil palm taxonomy and origins

Oil palm is classified belonging to the genera Palmarum, a monocotyledon in the Aracaceae (previously known as Palmae) family. The Aracaceae has six sub-familes, Arecoideae, Calamoideae, Ceroxyloideae, Coryphoideae, Nypoideae, and Phytelephantoideae of which oil palm is a member of the Arecoidease (GRIN [Global Resources Information Network], 2011). The sub-family Arecoideae is divided into tribes and sub-tribes. The genera *Elaeis* and *Barcella* fall into the tribe Cocoaeae and sub-tribe Eleaidinae. The genus *Elaeis* consist of two oil palm species, *Elaeis guineensis* Jacq. and *Elaeis oleifera* Kunth, which are characterised by morphological traits such as trunk growth, root development, leaf structure, inflorescence type, fruit type; total lipid content; and fatty acid profiles (Henderson, 1986; Corley and Tinker, 2015). The two species are separated geographically, of which *E. guineensis* is an African species and *E. oleifera* is a South American species (Rajanaidu et al., 2017).

Zeven (1965) and Moore & Uhl (1982) hyphothesized that oil palm ancestor originated from Gondwanaland. In the prehistoric era, Gondwanaland or Gondwana, included modern South America, Africa, Arabian Peninsula, Antartica, Australia and India. Continental drift provided two geographically separated regions and species evolution with *E. guinensis* in Africa and *E. oleifera* in South America. It is estimated that *E. oleifera* and *E. guinensis* diverged about 51 million years ago (Singh et al., 2013). At present, these oil palm species exist in the wild and semi-wild states in Africa (*E. guinensis*), and Southeast Asia; and South and Central America (*E. oleifera*), but in recent times plant breeding has brought both species together (Corley and Tinker,

2015; Sitepu et al., 2018). *Elaies guinensis* and *E. oleifera* have the same number of chromosomes (2n = 32) and interspecific hybrids can be produced although these are generally sterile (Rajanaidu et al., 2017).

2.2 Oil palm cultivation

The inhabitants of West Africa traditionally exploited natural and semi-wild palm groves for oil. When European explorers/traders reached West African in the 15th century, palm oil was used extensively by local people for cooking and soap making (Crone, 1937; Hartley, 1988, Agu and Okagu, 2013). There is evidence that the first overseas shipment was 32 barrels of oil in 1590 (from West Africa to Britain), and soap was made from it as early as 1589 (Corley and Tinker, 2003). However, the potential of palm oil was not realized and was over-shadowed in Africa by trade in ivory, timber, gold, spices and slavery (Corley and Tinker, 2003).

In the mid-1800s (coinciding with the collapse of the slave trade), it was realized that oil palm had value as an oil-yielding crop, trade and exported began from the Guinea coast. Exports of African palm oil increased steadily from 1860s to the early 20th century to meet European demand for industrial oil. In 1911, export of 87,000 tonnes of palm oil with a value of £2 million was conducted from British Colonial Territories in West Africa. This was hampered by civil unrest in Africa and It resulted in lowering production from most African nations during the 20th century. Today only the Democratic Republic of Congo and the Ivory Coast report significant palm oil production (Hartley, 1988; Corley and Tinker, 2015).

Oil palm is grown in the humid tropics between latitudes 20° north and 20° south of the equator and covers over 8.5 million hectares world-wide. It is grown mainly from seed although clonal plantings of tissue culture produced ramets is also

practiced (Sayer et al., 2012). Field ready seedlings are produced in the nursery with the aim of producing healthy, vigorous and uniform plants (Sitepu et al., 2018).

There are normally two stages in the nursery for preparation of planting materials, a pre-nursery (for germinated seeds planted in small polybags or pot-trays) and a main nursery (to grow young oil palm in large polybags) (Sitepu et al., 2018). Small polybags are convenient for sowing germinated seeds and planting tissue culture produced ramets, which are transferred to larger polybags in the main nursery, which is also cheaper than field nursery practices. The pre-nursery stage involves sowing germinated seed and growing these for two to three months. The palms are then potted into large polybags in the main nursery where they remain for a further 7-10 months before field planting. In some cases, ramets are used instead of germinated seeds (Sitepu et al., 2018). Young palms can be planted in the field after 9-12 months pre-nursery stage; and 6-9 months in the main nursery stage. Oil palm is a crop that matures at about 20-25 months after field planting (Laksono et al., 2018; Sitepu et al., 2018).

Harvesting of oil palms begins 2.5 years after field planting (Corely and Tinker, 2015). Bunch weight increases as palms age, in 3-year-old palms, the mean bunch weight is less than 5 kg, while at 15 years, this increases to over 25 kg and can occasionally reach over 50 kg (Corley and Gray, 1976; Verheye, 2010, Corley and Tinker, 2015). The combination of declining yields and increasing height means that replanting is usually considered necessary at about 25 years after the original planting, though this will vary depending on the vigour of vegetative growth and other factors (Tinker, 2000; Jalani et al., 2001).

2.3 Oil palm industry and economic value

Oil palm delivers the highest yield per hectare of any oil crop (3–4 tonnes average of mesocarp oil per hectare per year), (Wahid et al., 2005). The oil is produced

in the fruits of the palm. The fruit is made up of a central hard-shelled nut surrounded by an outer fruit flesh (mesocarp) which contains oil, and the kernel also contains oil.

Oil palm has three fruit types of which Dura has thick-shelled endocarp, Pisifera is shell-less or no shell but with traces of a fibre ring around the kernel, and Tenera (a cross between Dura and Pisifera) has thin-shelled nuts with fibre ring around the shell and a thick fleshy mesocarp (Figure 2.1). The Tenera type is therefore the basis for commercial palm oil production worldwide, including in Indonesia (Wening et al., 2012; Corley and Tinker, 2015; Widodo et al., 2019). Worldwide, Tenera is the recommended commercial variety as it has the highest amount of oil-bearing mesocarp per fruit.

The first plantation in Africa was planted with Dura palms which have thick-shelled fruits (Figure 2.1). In the 1920s, plant breeding of oil palm was initiated and in the 1950s–60s the more productive Tenera type was planted as the preferred commercial variety in Africa and Southeast Asia. The Tenera (thin-shelled) type has 30% greater oil yield compared to thick-shelled Dura (Widodo et al., 2019).

Figure 2.1 Fruit types of oil palm: (A) Dura (thick shell); (B) Tenera (thin shell); (C) Pisifera (shell-less). Source: Widodo et al. (2019).

The oil palm fruit consists of an outer skin (exocarp), an oily flesh (mesocarp) and a shell (endocarp) which protects the kernel (endosperm and embryo) (Figure 2.2). The three pericarp organs are maternal. It derived from the female flower carpel. The

kernel consists of endosperm which supports embryo growth during early seed development, germination and seedling growth. The endosperm and embryo are the products of double-fertisation. The fleshy mesocarp of the fruit provides crude palm oil (CPO), whereas the kermeil provides crude palm kernel oil (CPKO).

Figure 2.2 Palm oil fruit morphology of Tenera. Source : Widodo et al. (2019)

The main fatty acid in CPO is palmitic acid (44%), followed by oleic acid (39%), linoleic acid (11%), stearic acid (5%) and other fatty acids (Siew, 2002). The CPO is also a major source of pro-vitamin A and vitamin E (Barcelos et al., 2015). Palm kernel oil (PKO) has an oil quality similar to coconut, the main fatty acid is lauric acid (up to 50%), followed by myristic (15%) and other essential fatty acids (Sambanthamurthi et al., 2000).

The oil percentages of mesocarp for Tenera is 56%, whereas for Dura it is 49%, Pisifera fruits have 61% oil in their mesocarp, but this is not economic due to the low female fertility of Pisifera palms (Basyuni et al., 2017). A major advantage of the oil palm crop is it is harvested continually, thus providing a relatively stable and reliable commodity compared to annual oil crops. Palm oil is primarily a food crop (used in cooking), but it is also very versatile and has a number of uses: cosmetics, tooth paste, shampoo, concrete, pest control formulations, pharmaceuticals, plasticides, detergents, as well as in industrial processes (textiles, aluminium, etc.).

Palm oil provides over 30% of all the worldwide oil production, in 2019/2020 this amounted to 75.7 million metric tonnes. Oil palm yields 11 times more oil per land area than its closest rival, soybean, indeed oil palm only takes up 7% of the total world hectarage of oil crops (Sitepu, 2018; Shahbandeh, 2020b), it is thus the most efficient oil crop (Corley and Tinker, 2003). The cost of palm oil production is currently lower compare to other vegetable oils, a major factor here is relatively cheap labour costs (Tan et al., 2009).

Eighty five percent of the world's palm oil is produce from Malaysia and Indonesia (Southeast Asian countries). Malaysia was the leading producer from 1970 to 2006, but was overtaken by Indonesia in 2007, which now supplies 57% of global palm oil. Indonesia is the leading palm oil exporting country with an export volume of about 43.5 million metric tonnes in 2020 (Figure 2.3) (data from Sambanthamurthi et al., 2000; Shahbandeh, 2020b; Gro-Intelligence, 2019).

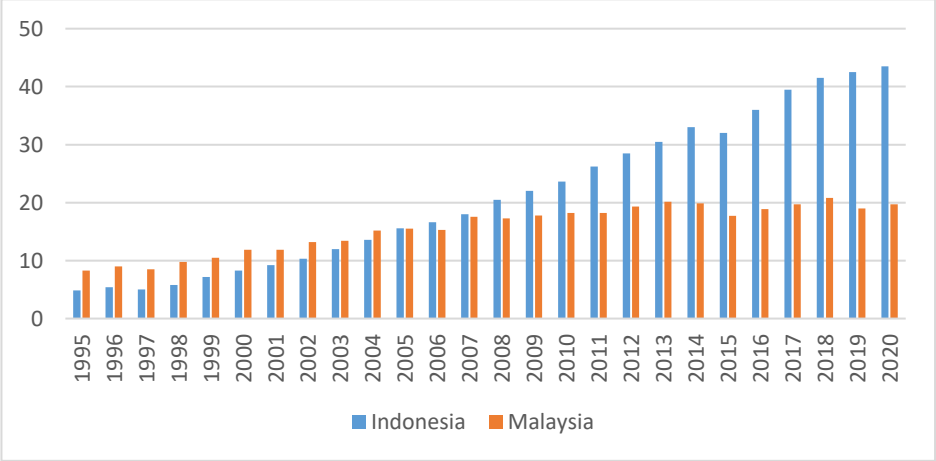

Figure 2.3 Oil palm production in Malaysia and Indonesia from 1995 – 2020. (Based on the data from USDA PS&D, Gro Intelligence (2019).

Import oil palm products were estimated at US$33.8 billion in 2020 (Workman, 2020). Asian countries imported the most palm oil with the highest purchase of US$17.7 billion in 2020 which is over half (52.3%) of world-wide

purchases, European imports accounted of 24.8%, while 15.7% of palm oil was imported into Africa. Lower percentages of imported palm oil were: North America (4.3%), Latin America (2.5%), the Caribbean, and Oceania (0.3%) and New Zealand and Australia (Workman, 2020).

India and China were the largest importers of palm oil in 2020, with a value of US$5.4 billion (21.9% of total palm oil imports) and US$4.1 billion (16.7% of total palm oil imports). The Netherlands (US$1.7 billion), Spain (US$1.2 billion) and Italy (US$1.1 billion) are the second largest importers with 6.9%, 4.8% and 4.3% of total palm oil imports, respectively while United States purchased US$1 billion with 4.1% of total palm oil import. The largest importers of palm oil in the world for 2020 were India, China and The Netherlands (Workman, 2020).

2.4 History of oil palm cultivation in Indonesia

In 1848, four oil palm seedlings arrived in Southeast Asia. These were introduced by the Dutch and planted in the botanical gardens located at Bogor, Java, Indonesia. The seedlings arrived via West Africa and The Netherlands. From 1853 onwards the progenies of these four founding palms were distributed were the foundation of Indonesian oil palm industry, started by the Dutch East Indies company. A major influencer was M.A. Hallet, a Belgian with African oil palm connections and knowledge, who planted palms at Sungei Liput, Aceh, Indonesia in 1911 and also in Pulu Radja in Sumatra, Indonesia in 1912 (Zeven, 1967; Hartley, 1988; Rajanaidu, 2017; Durrand-Gasselin, 2021).

Figure 2.4 shows the growth of the Indonesian oil palm industry with respect to land area of smallholders, state-owned companies and the private sector from 1999 until 2018. Four million ha land area were planted with oil palm in 1999 which

increased to 12,760,000 ha in 2018 (Statistik Perkebunan, Indonesia 2011; Jelsma et al., 2017; Sitepu et al., 2018).

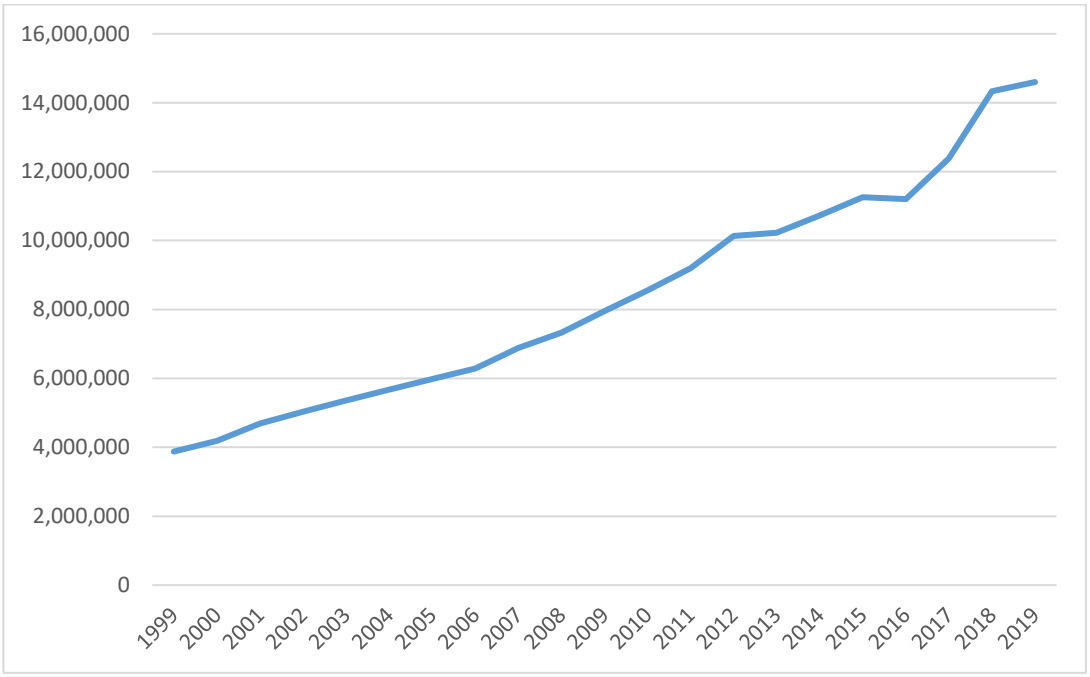

Figure 2.4 Expansion of Indonesian oil palm plantation area from 1999 to 2019 (data generated based on information from Badan Pusat Statistik Indonesia 2018b and 2019).

In Indonesia, oil palm was cultivated commercially in 1911 under Dutch administration, and the first plantation was located on the east coast area of Sumatra (Corley and Tinker, 2003). The east coast area of Sumatra with a high rainfall (minimum 1,600 mm/year) and a tropical climate (within 10° of the equator) and fertile soil was suited for oil palm. Moreover, the most important inputs, land and labour were available and relatively cheap at the time (Stoler, 1985; Breman, 1989). An area of 110,000 ha was planted with oil palm in 1940, but following World War II and Indonesia independence struggles, the plantations in Sumatra dramaticaly decreased and further planting progress was severely retarded. By 1956, only a 15% increase in the oil palm planting area was recorded (over the pre-war era) with low yields per ha (Hartley, 1988; Rajanaidu et al., 2017).

It was not until the 1970s that oil palm plantations began to expand on a substantial scale (Budidarsono et al., 2013). During the 1967 - 1997 period, the oil palm planting area increased slowly about 20-fold. The slow rate of growth was due to several factors such as, El Niño related drought, decreasing global crude palm oil prices, global economics and social and political crises in 1997. Later, in 1999 where economic, social, and climatic conditions become more favorable, the oil palm industry picked up again (Casson, 2000).

Oil palm plantation development in Indonesia was supported by a rise in global demand. The 1990 - 2010 period, produced a 300% increase in oil palm production, while world production of soybean increased by 220%. There was a rise in demand for crude and kernel oil from 2 Mt to over 50 Mt in the 50 years (from 1970 – 2020). The expansion of the Indonesian palm oil industry (Figure 2.5) resulted in the country becoming the world's leading oil palm producer in 2007, followed by Malaysia (Byerlee et al. 2016, Sitepu et al., 2018).

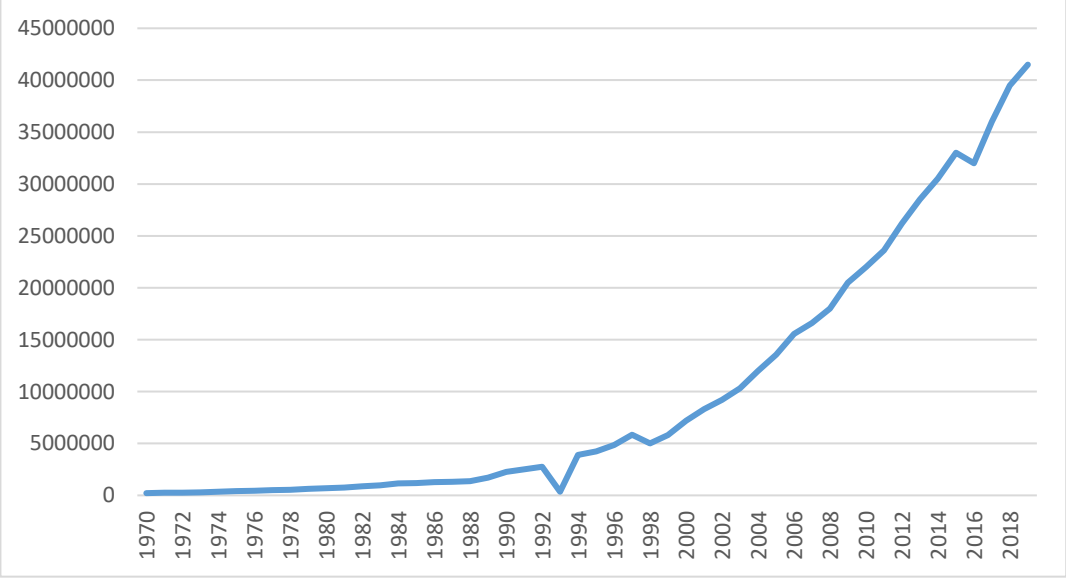

Figure 2.5 Indonesian oil palm production since 1970 (Based on the data from Gro-Intelligence, 2019).

Independent small-holders account for about 40% of oil palm production in Indonesia, the remaining 60% is managed by large-scale plantations (state-owned = or private corporations). The largest oil palm area in Indonesia is on the island of Sumatra (64%), followed by Kalimantan (31%) which showed a major expansion during 2005 – 2013, with 1.5 million ha of addition area in large scale plantations and 228,000 ha for small-holders. Recent oil palm planting developments in Sumatra were in Riau with 2.21 million hectares in 2017 (17.8 % of the total Indonesian oil palm planting area), where there is a focus on small-holders (Obidzinski et al., 2012; Badan Pusat Statistik Indonesia, 2018b; Workman, 2019).

2.5 BSR of oil palm

2.5.1 Occurrence of BSR

BSR of oil palm was first detected in the Republic of Congo, West Africa in 1915 (Wakefield, 1920). In early observation, BSR of oil palm was not considered a serious disease (Thompson 1931; Turner 1981). In early studies, BSR was found to infect older palm, over 30 years of age being most frequently affected (Turner and Bull, 1967). Later BSR was detected in much younger palms of 15 – 20 years old, the disease then began to infect large numbers of palms 10-15 years after planting, and the symptoms could often be found in palms of 5 years of age onwards. After that, infected oil palms as young as one year old were found (Turner, 1981; Azahar et al., 2008; Wong et al., 2012). In general, infected young palms require about 6 to 24 months to die after initial symptoms of disease appear, whereas for mature palms this normally takes 2–3 years (Miller et al., 1999; Corley and Tinker, 2003).

In early studies on BSR disease incidence, high disease incidence was observed on oil palm fields replanted from coconut. According to Turner (1981) & Singh (1991),

BSR incidence on coconut replanting field, was much higher (35 – 39%) than field replanting from rubber (2- 4%). The greatest losses were in oil palm fields in which old coconut trunks from the previous crop had been buried to minimise *Oryctes rhinoceros* infestation (Turner, 1981; Singh, 1991). Later, BSR infection was recorded on oil palm around 12-24 months after planting, with a higher incidence on 4–5-year-old palms, especially where, the previous crop was also oil palm (Singh, 1991) or under planting area (Ariffin et al., 1996).

In new oil palm fields where the previous vegetation was jungle or conversion from rubber plantation, 25% disease was observed after 25 years of planting oil palm, while if the previously crop was coconut up to 60% BSR was found after 16 years. In the first generation of oil palm replanted from oil palm, 33% infection occurred after 15 years (Singh, 1991).

BSR of oil palm was recorded in Southeast Asia particularly in Malaysia and Indonesia. Other countries where BSR was reported include Angola, Cameroon, DR Congo, Ghana, Nigeria, San Tome, Tanzania, Zambia and Zimbabwe in Africa; Honduras in Central America, and Papua New Guinea in Oceania (Turner, 1981; Ariffin et al., 2000; Paterson, 2020), Colombia (Nieto, 1995) and Thailand (Tummakate and Likhitekaraj, 1998).

BSR incidence is higher in Malaysia and Indonesia compared to BSR incidence in other countries such as Africa, Thailand, Pakistan and Papua New Guinea (Arifin et al., 2000; Obidzinski et al., 2012; Pornsuriya et al. 2013; Workman, 2019). The higher incidence of BSR occurrence in Malaysia and Indonesia might be related to the greater development of oil palm cultivation in these countries which began earlier than in Thailand, Pakistan and Papua New Guinea. It appears that the BSR pathogen,

particularly *G. boninense* has adapted to the environment quickly and sources of inoculum have become available especially in replanting oil palm fields (Paterson, 2019; Lam et al., 2019; Paterson et al., 2020). Lower BSR incidence in the Phillipines and Thailand may be due to the recently established oil palm plantations in these two countries and their distance from Malaysia and Indonesia (Corley and Tinker, 2015; Woods, 2015).

2.5.2 Causal pathogens

Several *Ganoderma* species have been observed to be related to oil palm BSR. The first identification of BSR disease in Malaysia was reported in 1930 with *G. lucidum* (W. Curt.) Karst as the causal fungus (Turner 1981). Later in 1985, *G. boninense* was identified as the most common species infecting oil palm in Malaysia (Ho and Nawawi, 1985). In 2000, *G. boninense*, *G. zonatum, G. miniatocinctum* and *G. tornatum* were found to be associated with BSR in Malaysia (Idris et al., 2000, Idris, 2023). However, only *G. boninense, G. zonatum, G. miniatocinctum* were observed to be pathogenic to oil palm; *G. tornatum* was considered to be non-pathogenic as it grew on dead palms. Samples of *Ganoderma* were collected randomly from Sarawak, Malaysia and identified and DNA amplified using multiplex polymerase chain reaction (multiplex PCR). The amplicon analysis showed *G. boninense, G. zonatum* and *G. miniatocinctum* to be associated with BSR disease (Rakib et al., 2014). The three species were also reported by Idris et al. (2000) as causal pathogens of BSR.

The uncertainty of the causal pathogen of BSR resulted in some authors referring to the pathogen simply as *Ganoderma* rot of oil palm (Corley and Tinker, 2003). However, with comprehensive studies on taxonomy of *Ganoderma*, and application of molecular methods, *G. boninense* is identifed as the major causal

pathogen of BSR in Southeast Asia and Papua New Guinea (Moncalvo, 2000; Ariffin et al., 2000; Pilotti, 2005). In Sumatra, Indonesia, *G. boninense* was the only species found to infect living oil palm (Rees et al., 2012). *Ganoderma boninense* was also associated with BSR in Papua New Guinea and Solomon Island (Pilotti et al. 2001; Gorea et al. 2019).

2.5.3 BSR of oil palm in Indonesia

Ganoderma infection of oil palm was first reported in Indonesia in 1931 (Turner, 1981). Incidence of BSR in Indonesia was originally linked to old senescencing palms (25 to 30 years old) and did not become prevalent until coconut plantations were replanted with oil palm. However, BSR infection on old oil palms was low at 1 – 10%, and therefore did not have a significant impact on the yield, as the yield was compensated for by surrounding healthy palms. Turner (1981), reported that 1% disease incidence does not reduce oil palm yields appreciably.

Mono-culture led to high disease incidence of BSR which has risen sharply in the last three decades. Increased disease incidence has affected yields and BSR has become a major devastating disease of oil palm. Where the previous crop was generally forest, the disease only infected older palms. However, after successive re-planting, from one cycle into the next cycle, BSR incidence increased and appeared much earlier with symptoms observed in younger palms, and this caused major reductions in yield (Breton et al., 2010; Purba et al., 2012).

In several old oil palm plantations in Indonesia, disease incidence has reached 80% and has caused major losses in production (Susanto, 2002; Susanto et al., 2003). In one oil palm estate in North Sumatra, a census was conducted in 2011 using Global Positioning System (GPS) which recorded 0 - 1.5% of BSR infection in oil palms less

than 6 years old. The percentage infection increased dramatically to 13 - 87% in oil palms more than16 years old with a stand per hectare ranging from 35 - 119 palms (Virdiana et al., 2012a).

2.5.3(a) BSR disease of oil palm in peat soil

In Indonesia peat lands cover approximately 4.9 million hectares, mainly located in Sumatra, Kalimantan and Papua. In Kalimantan, almost half (42%) of oil palm plantation are on peatlands, mainly in the province of West Kalimantan, which covers 309.3 ha. The majority of the plantations in peatlands are large-scale operations (Miettinen et al., 2012). Peatland soil or peat soil is largely an accumulation of plant debris from trunk and roots in water logged conditions (Adon et al., 2012; Mohamed et al., 2014a). Peat soil has a high organic matter content, very acidic with light brown or dark brown colour (Ling et al., 2013; Miettinen et al., 2016; Pradipta, 2017; Badan Pusat Statistik, 2018a).

Although peat soil has low pH, low micro and macro nutrients content with high organic, acidic and low nutrient, some peatlands are considered suitable for oil palm planting as they have homogeneous soil features with a constant water supply and a flat topography. Deep peat soils have now been developed for oil palm. The utility of modern ‘heavy’ equipment has facilitated this expansion aloing with new agronomic practices in oil palm nutrition in deep peat soil plantations. However, costs of production are high due to costs forroad construction, drainage, soil preparation and compaction, mineral fertilizer applications, and other practicaities in the management of peat soils (Mutert et al., 1999).

At one time it was thought that peat soils were non-condusive for BSR infection (Turner, 1981; Cooper et al., 2011), however, serious BSR incidences have been reported (Ariffin et al., 1989; Rao, 1990). Ariffin et al. (1989) and Singh (1991) cautioned that high *Ganoderma* incidence has been identifed at a relatively young age. BSR disease has become a major issue because of a higher disease incidence in peat soils (Ariffin et al., 2000), the disease incidence occurs earlier (8 years) than on mineral land (Susanto and Huan, 2010). BSR was also reported to spread rapidly in oil palm planted in peatland but with low infection rates: after 10 years of planting, 80 out of 156 oil palm blocks were infected at very low level (<1%) of infection (Virdiana, 2017).

Figure 2.6 shows the percentage of BSR infection in an oil palm estate, planted on peat land in North Sumatra. The infection is generally low (<1.3%), and infection occurs after 3 years of planting with <0.09% infection. Generally, BSR infection increased with age, however, some older oil palms have lower incidence of infection. From these observations, sanitation was recommended by destroying infected oil palm trees which can become sources of inoculum or disease infection.

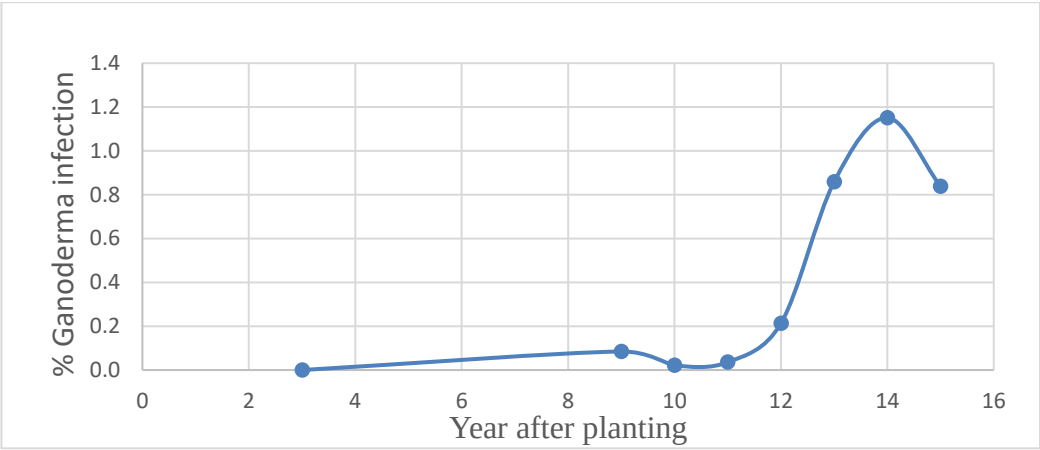

Figure 2.6 Percentage of BSR infection in an oil palm estate planted on peat soil in North Sumatra

Using nearest neighbour analysis, Supriyanto et al. (2020) showed that the spreading pattern of BSR on a peatland plantation in Indonesia tended to from clusters. However, the patterns observed by Supriyanto et al. (2020) contrasted with the findings by Azahar et al. (2011) who showed that the distribution patterns of BSR occured randomly. The spread of BSR from one area to another is thought to occur primarily through wind-borne basidiospores. Thus, there may be two modes of BSR spread:1) wind-borne spores over large distances and 2) root contact within a plantation (Supriyanto et al., 2020).

There are two peat soil characteristics that affect the infection by *Ganoderma*: 1) water table, and 2) pH at a depth of 0 - 15 cm. The average level of the water table in peat soil is positively correlated with the occurrence of BSR. In a study by Susanto et al. (2008) on peat soils located in North Sumatra, it was reported that oil plam fields that flooded frequnetly often tend to have lower BSR occurrence which is might be related to the influence of water table level supporting anaerobic conditions in peat. The lower water table level will support greater soil aeration, which will enhance growth of *Ganoderma*.

Soil pH from pH 4 - 5 is considered favourable for oil palm planting in Southeast Asia (Goh, 1995; Corley and Tinker, 2003; Paramananthan, 2003). However, pH of peat soil at 0 - 15 cm depth is acidic, ranging from pH 2.4 - 3.4, and therefore not suitable for *Ganoderma* growth. Optimal growth of *Ganoderma* occurs at pH 3.7 – 5.0 (Nawawi and Ho, 1990) as well as at pH 4 – 5 (Peng et al., 2019). However, BSR occurs in peat environments which might be due to several factors including adaption of the causal pathogen to the peat environment and the availability of nutrients.

A study by Supriyanto et al. (2020) in PT. Bumi Pratama Khatulistiwa (PT. BPK) in Kuburaya Regency, West Kalimantan Province, showed that the high incidence of BSR in peatland was related to three contributing factors. The first factor is the causal pathogen, i.e. *Ganoderma* has adapted to the peatland environment and the inoculum can survive in the peat soil as well as on the infected oil palms. The second factor is related to copper (Cu) and zinc (Zn) deficiencies in peatland where uptake of these micronutrients by oil palm is limited making oil palms more susceptible to *Ganoderma* infection (see also Huan and Wahidin, 2010). The third factor is related to *Ganoderma* as a facultative parasite that can survive as a saprophyte and turn pathogenic when a suitable host or condicions becomes available (Turner, 1965; Ariffin et al., 2000).

2.5.3(b) BSR disease of oil palm in mineral soil

Physically, mineral soils are porous with a mixture of inorganic particles, decaying organic matter, air and water (Brady, 1984). Although *Ganoderma* infection is widespread in mineral soil areas (Idris et al., 2001; Susanto et al., 2013a), limited information is available on BSR incidence in oil palm growing on peat soil.

Figure 2.7 shows BSR incidence in one of the oil palm plantations in North Sumatera recorded by Verdant Bioscience Indonesia. The figure shows BSR disease incidence from first, second and third generations of the oil palm planting. Symptoms of BSR started 5 years after planting, increased with the age of the oil palm, and reached 52% in 24 years after planting.

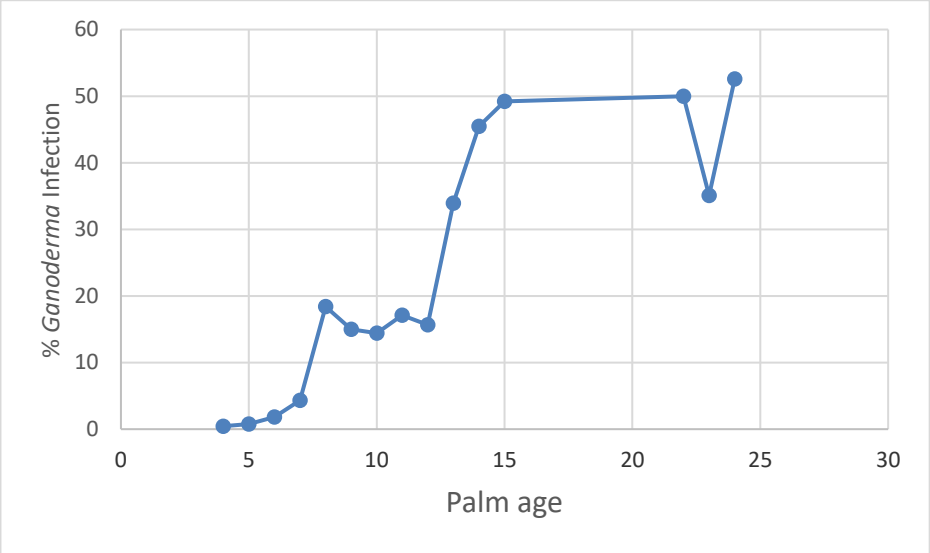

Figure 2.7 Percentage of BSR infection in an oil palm estate planted on mineral soil in North Sumatra. Source: Verdant Bioscience Indonesia

2.6 Taxonomy of *Ganoderma*

The genus *Ganoderma* was established in 1881 by a Finnish mycologist, Karsten which at the time consisted of one species, *Polyporus lucidus*, (= *G. lucidum* (Curtis: Fr.) P. Karst). Subsequitenly, Patouillard (1889) and Costa-Rezende et al. (2017) amended the classification to include all polypores with double-walled basidiospores and the revision included 48 species worldwide (Moncalvo et al., 1995a; Seo and Kirk, 2000).

Ganoderma is a large genus with more than 300 species and one of the most taxonomically scrutinized genera (Richter et al., 2015). Previously, *Ganoderma* was classified in the Order Aphyllophorales and Family *Ganodermataceae*. The latest classification of *Ganoderma* based on NCBI database (https://www.ncbi.nlm.nih.gov/taxonomy) is as follows: Eukaryota; Opisthokonta; Fungi; Dikarya; Basidiomycota; Agaricomycotina; Agaricomycetes incertae sedis; Polyporales; Polyporaceae; *Ganoderma.*

Ganoderma is a cosmopolitan genus, distributed worldwide in tropical and temperate regions, growing on numerous coniferous, deciduous, and palmaceous hosts. Many species are commonly found in sub-tropical and tropical regions as they can survive in hot and humid conditions (Pilotti et al., 2004). Many species are saprophytes, as decomposers of organic matter in the soil, but some are pathogens of hardwood trees (Hepting, 1971; Mizuno et al., 1995; Bhosle et al., 2010). Pathogenic species include BSR pathogen of oil palm caused by *G. boninense, G. miniatocintum* and *G. zonatum* (Idris et al., 2000; Pilotti, 2005; Susanto et al., 2005), and root-rot disease of *Acacia* trees caused by *G. steyaertanum, G. philippii,* and *G. mastoporum* (Glen et al., 2009). Some species have medicinal properties such as *G. lucidum* (Bhosle et al., 2010; Bishop et al., 2015).

Taxonomic studies of the genus *Ganoderma* have traditionally used several characteristics of macro-morphology of the basidiocarp or fruiting body (thickness and colour of the basidiocarp, pore numbers per mm, cuticle thickness, pore tubes, angle and diameter of the cap margin, stipitate or sessile attachment to the substrates) and microscopic characteristcis of the basidiocarp including context colour, shape of the margin as well as the basidiospores features (spore size, spore shape, ornamentation and wall layers).

The bracket-shaped basidiocarps of *Ganoderma*, grow from a living or dead trunk or branch. In *Ganaoderma* taxonomy, two types of basidiocarps are commonly observed, laccate or shiny upper surface, or a non - laccate with dull upper surface (Figure 2.8). Laccate or shiny basidiocarp commonly have a yellowish, deep red or reddish-brown surface. Non-laccate basidiocarps have a dull appearance with grey-brown to black surface. In general, species within *G. lucidum* complex have a laccate surface whereas species with a non-laccate surface are placed in the *G. applanatum*