Chapter 2
Pathogenic Nature of *Ganoderma boninense* and Basal Stem Rot Disease

Abstract The oil palm industry is under threat of a prevailing incurable disease called Basal Root Stem (BSR), which is caused by a white rot fungi, known as *Ganoderma boninense*. With no current remedy at present, BSR is the major disease in oil palm plantations of SEA and, therefore, of great economic importance to the world oil palm industry, especially to Malaysia and Indonesia, which are oil palm major producers and exporters. The disease is highly associated with the decay of lower stem, leading to severe symptoms such as unopened and flattening spear leaves. There are numerous mode of infection associated with the epidemiology of *G. boninense* in oil palm plantation, including in-contact roots with nearby diseased palms and through airborne basidiospores. Deep insight on the route of infection and mycological pathogenicity behaviour of the pathogen is the greatest priority in order to successfully develop effective management practices for disease control.

2.1 Biology and Epidemiology of *G. boninense*

Ganoderma boninense is a polyporoid fungus which grows on wood. It belongs to the family of Ganodermataceae and Classed under Basidiomycetes (Idris 2009). It is a lignolitic fungus which commonly belongs to white rot fungi and known for their capability in degrading the lignin component of wood while leaving the white cellulose exposed (Paterson 2007). Therefore, this fungus is more active in degrading lignin compared to other groups.

In early reports, a number of *Ganoderma* species have been reported to be associated with the basal stem rot of oil palm (Turner 1981). Among them are *G. applanatum, G. boninense, G. chalceum*, *G. lucidum*, *G. miniatocinctum*, *G. pseudoferreu* and *G. tornatum*. Based on morphology of basidiomata and basidiospores collected from oil palm fields or induced in vitro, Khairudin (1990) concluded that two species namely *G. boninense* and *G. tornatum* are the causal pathogen of BSR. However, in later reports, after isolation of real pathogenic isolates of *Ganoderma*, it was identified that *G. boninense, G. zonatum* and

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G. miniatocinctum to be associated with BSR disease. After the general consensus, it was apparent that *G. boninense* is the main species pathogenic to the oil palm (Moncalvo 2000). In addition to this report, *G. boninense* was also found to be the most common and virulent species than the other two (*G. zonatum* and *G. miniatocinctum*) in several estates with high incidence of BSR disease (Idris 1999). While, *G. tornatum, G. applanatum, G. lucidum, G. pfeifferi* and *G. philippi* were not pathogenic (Idris et al. 2000).

Unfortunately, until today, studies have shown there are some uncertainties regarding the identity of the species of *Ganoderma* causing BSR in different countries leading to confusion in the identification process, thus, leading to inefficient disease management. Miller et al. (2000) also reported a great deal of variability between strains of *G. boninense*. This species also appears to have broad host specificity and there is conflicting information on its characteristics and also its relationship to species associated with previous cropping or vegetation. Higher incidences of BSR were observed in oil palm planted in ex coconut planting compared to ex rubber plantings (Turner 1981). This conflict has lead to the fungal variability related to the capacity of the species to adapt and survive in different substrate for a long time.

Over the last few decades, several studies have been conducted on the molecular aspect of these *Ganoderma* spp. Studies have shown high level of genetic variation among monokaryons of *G. boninense*, suggesting *G. boninense* is genetically heterogenous which could be caused by different geographical locations or outcrossing of the isolates over generations (Miller et al. 2000; Pilotti et al. 2003) whereby the pathogen could originated from the same species containing wide genetic variation or from closely related species (Zakaria et al. 2005). Pilotti (2005) has revealed *G. boninense* is heterothallic with a tetrapolar mating system and multiple alleles at both mating type loci; this favours out-breeding within a population (Sanderson and Pilloti 1997; Pilotti 2005). Thus, this out-crossing ability is responsible for the wide genetic variation found in the *G. boninense* population on oil palm.

Although the morphology of the genus *Ganoderma* remains confusing (Miller et al. 1999), it is now seems to be accepted (Ho and Nawawi 1985; Khairudin et al. 1991) that *G. boninense* is the predominant species responsible for BSR disease occurrence and development. Lim and Fong (2005) also suggested that BSR infection on oil palm tree could possibly be from different strains of the same *Ganoderma* species. Evidently, the identity of the pathogen is crucial in deciding the most efficient and economic disease management, hence, more studies on the identification of *Ganoderma* spp. should be conducted.

Based on in vitro study reported by Idris et al. (2000), the colonies of *G. boniense* was characterized morphologically as white in colour on the surface and the reverse was darkened (pigmented). Cultures of *G. boninense* had an undulating surface in the darkened regions that buckled the agar. *Ganoderma boninense* can grow at pH 3–8.5 at the optimum temperature of 30 °C, critically hindered at 15 and 35 °C, and unable to grow at 40 °C. Idris et al. (2000) also demonstrated when basidiomata of *G. boninense* was artificially induced on rubber

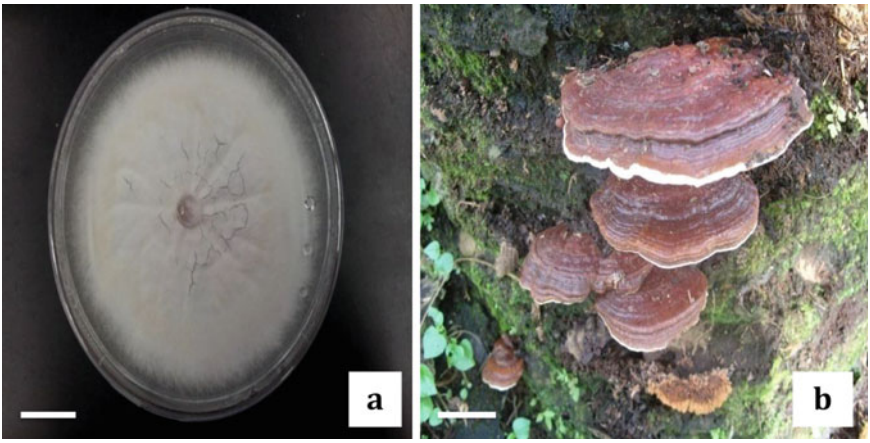

Fig. 2.1 Morphology of different stages of *G. boninense*. **a** Mycelial of *G. boninense* grown on potato dextrose agar (PDA), after 14 days of incubation at 28 °C. **b** Basidiocarps of *Ganoderma* on the trunk of infected oil palm tree. Bar scale: **a** 2 cm; **b** 5 cm

wood block (RWB), its formation was first identified by the appearance of a white mycelium after one to three weeks of incubation on RWB, which then developed into small, white, button-like structure. The apical end began expanding rapidly giving rise to bracket-like structures which were generally white when first formed, but as their length and width increased rapidly, the upper surface developed various yellowish-brown colour with concentric zonations (Idris 2009). *Ganoderma* are characterized by their large, perennial and woody basidiocarps (Fig. 2.1). Their fruiting bodies typically grow in a fan-like manner on the trunks of infected palms, with double-walled, truncate spores with yellow to brown ornamented inner layers.

2.2 Basal Stem Rot Disease

Oil palm is one of the most efficient and important crops in the world. Oil palm has contributed in uplifting the quality of life of people and has helped alleviate poverty among landless farmers. However, the never-ending problem of BSR disease has affected the production of oil palm and burden planters especially smallholders and farmers. BSR was once only found to be infecting older plants, but recently it has been found in seedlings (Sanderson 2005) and younger plants where symptoms appear earlier and are more severe, leading to greater replanting (Susanto et al. 2005). Losses begin to have a financial effect once the disease affects more than 10% of the stand (Hasan and Turner 1998). Losses due to BSR can occur not only through the direct reduction in oil palm numbers in the stand, but also through the reduction in the number and weight of fruit bunches from infected palms and those with subclinical infection (Flood et al. 2000). On average there is a decline of the

yield of the fresh fruit bunch (FFB) of 0.16 tonne per hectare for every palm lost, and when the stand had declined by 50% the average FFB yield reduction was 35% (Subagio and Foster 2003). Malaysia has recorded a yearly losses up to RM 1.5 billion (500 million USD) due to BSR.

In new oil palm planted area such as jungle or conversion from ex-rubber planting, BSR incidence of 25% has been recorded after 25 years of planting, while in ex-coconut planted area, an incidence of 60% occurred after 16 years (Singh 1991) whereas oil palm to oil palm under planting has resulted in 33% infection after 15 years. High incidence of BSR occurs in Malaysia and Indonesia with lower incidences recorded in Africa, Papua New Guinea and Thailand (Idris et al. 2004). A survey by Subagio and Foster (2003) on some oil palm plantations in Indonesia indicated the BSR infection rate was 70% in certain areas of a second planting cycle after 15 years of growing. Similar infection rates were also reported in Malaysia (Ariffin et al. 1996; Singh 1991). In Malaysia, high incidence of BSR disease was recorded when replanting to be placed in coastal clay soil area (Hasan and Turner 1998). This disease seemed to remain confined to the coastal areas, indicated the nature of the soil and its water retentions may have bearing on the disease development. Soils in coastal areas are mainly clays, silty clays or clay loams with poor internal drainage and high water retention capacity. However, BSR was also reported in peat soils, which were once thought to be non-conducive for the BSR disease (Cooper et al. 2011), serious incidences of the BSR disease have been also reported in these areas (Ariffin et al. 1989; Rao 1990).

2.3 Symptoms and Disease Development

Infection of *G. boninense* progresses slowly without any symptoms, thus, making it difficult to be recognized at the early stage. However, when the infection progresses to 60–70%, the symptoms begin to emerge. The earliest external symptoms of basal stem rot of oil palms occur in the foliage, generally after at least half of the cross-sectional area of the stem base has been destroyed. Decay leads to a restriction of water and nutrient supply to the aerial parts, causing symptoms resembling to those of water stress and malnutrition (Turner and Gillbanks 1974).

In young palms, the external symptoms of BSR usually comprise a one-sided yellowing or mottling of the lower frond, followed by necrosis (Singh 1991). Young unfolded leaves will become chlorotic and may be reduced in length, sometimes with necrotic on the tips. Similar symptoms also observed in mature palms, with multiple unopened spears, flattening of the crown, generally pale leaf canopy and production of basidiocarpsbasidiocarps (Turner 1981). Basidiomata may develops at the stem base, leaf base, or infected root; the location provides a guide to the diseases area inside the palms (Paterson 2007). In severe cases, affected palms will die and fall over. Severe infection by *G. boninense* may lead fracture at the base of oil palm and make it collapses, leaving diseased bole tissues on the

Fig. 2.2 Symptoms of Basal stem rot **a** Fallen oil palm tree due to rotten bole tissue, which weakens the tree making it susceptible to wind damage. **b** Typical basidiocarps of *G. boninense* on a BSR affected palm. **c** Unopened spears of oil palm tree. **d** Decaying in oil palm bole tissues leaving the trunk in hollow

ground. All of these symptoms can occur as combination and there is no fixed pattern or progression of symptoms (Fig. 2.2).

G. boninense is a soil-borne pathogen and there are three possible ways in which this fungi can spread directly to the host plants; root-to-root contact, basidiospore, and free secondary inoculum in the soil. Numerous infection trials using oil palm seedlings and often using large *Ganoderma*-colonized rubber-wood blocks have provided data supporting this view (Sariah et al. 1994; Breton et al. 2006). Rees et al. (2007) showed that by attaching infested wood blocks to roots, much smaller inoculum can be used, allowing infection to occur through unwounded roots and

progression and rate of invasion to be followed. Successful root infection was also reported by Chong et al. (2012) using direct spray of *Ganoderma* mycelia suspension onto seedling roots. *Ganoderma* species pathogenic to oil palm has wide host range (Turner 1981). The large inoculum left by coconut probably caused the high incidence of BSR on oil palm. It has been proven beyond doubt that root's contact with infected debris is an important method of infection and the dead diseased stumps were responsible for spread of BSR in the plantation.

Pilotti et al. (2003) and Sanderson (2005) have also reported basidiospores are implicated as the main mode of dispersal of *G. boninense* and grow in the non-living tissues. Mature basidiocarp produces thousands of basidiospores, and could well be good sources of inoculums for new infections, as dispersed by air movement and strong winds. These spores may colonise new substrate, enter wounds caused by shedding of branches and become new infection foci. However, Cooper et al. (2011), showed pruning can bring in spores as deep as 10 cm into the oil palm in which may accelerate further infection. However, very unlikely the basidiospore is the cause of the infection, as *Ganoderma* basidiocarps in field usually produce monokaryotic basidiospores. Monokaryotic mycelium from basidiospores can colonise palm wood but is non-infective (Hasan and Flood 2003; Rees et al. 2007); anastomosis with a compatible mating type is required to form the potentially invasive and faster growing heterokaryon. However, Hasan and Flood (2003) showed that single germinated basidiospores producing monokaryotic mycelia could colonise rubber wood and oil palm tissues.

Generally, initiation of BSR on oil palm by *G. boninense* established from infected debris that enters and gets in contact with roots and wounded part, which then progresses mainly through the inner, thin-walled cortex in plant. Rees et al. (2009) suggested that *Ganoderma* colonization appears to involve developmental switches which are biotrophic and necrotrphic phase. Initial infection starts with biotrophic phase where colonization in root cortex and stem base occur, involving largely intercellular colonization by wide hyphae of host cells with fully intact cell walls. This phase then followed by necrotrophic phase which associated extensive cell wall degradation. The formation of melanised mycelium might be considered as a third which is probably indicative of the oxidative breakdown of lignin and the white rot status ascribed to *G. boninense* (Adaskaveg et al. 1990).

Plant cell wall comprises of several components which are cellulose, hemicellulose, pectin and lignin. Thus, for successful colonization, production of an array of cell wall degrading enzymes (CWDE) (Cooper 1984) is likely to be required to penetrate and degrades these components in plant outermost tissues. *Ganoderma* produces several CWDEs such as amylase, oxidase, invertase, coagulase, protease, renetase, pectinase, and cellulosic enzyme. Based on Transmission Electron Microscopy (TEM) observation of infected roots and bole tissues, Rees et al. (2009) also suggested CWDEs are important in the extensive degradation of oil palm cell walls during the pathogenesis by *G. boninense* and are likely to be pathogenicity factors for this interaction. Development of holes through cell wall layers due to *G. boninense* attack is indication of simultaneous wood decay with the ability of *G. boninense* to produce enzymes that can attack all cells' wall layers.

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