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OIL PALM ROOTS COLONISATION BY *Ganoderma boninense*: AN INSIGHT STUDY USING SCANNING ELECTRON MICROSCOPY

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ABSTRACT

Oil palm (Elaeis guineensis *Jacq.*) *is an oil crop widely cultivated throughout South-east Asia countries. Unfortunately, the industry is badly affected by Basal Stem Rot (BSR) disease, caused by soil-borne pathogen,* Ganoderma boninense*, which is the most devastating and widespread disease in oil palm. Although some investigations have been conducted, complete understanding on BSR infection is yet to be achieved. In this article, observation on roots of inoculated oil palm seedlings under scanning electron microscopy showed* G. boninense *colonisation predominantly formed on root surface, proving endophytic colonisation primarily through the hair base, crossing epidermal cells, and followed by spreading into cortical cells which a possible pathway leading to vascular system. It also showed the formation of highly dense hypal network within the colonised area, mainly in the epidermal and cortical cells. By six months of inoculation, cell compartment of infected roots was lost and noticeable changes in the root cells structure were observed. The present study provides additional insights in the infection process and pathogen route inside the host tissue and may be useful for development of disease control strategies.*

Keywords: *Ganoderma boninense*, oil palm, scanning electrom microscopy.

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INTRODUCTION

Basal Stem Rot (BSR) disease, caused by the basidiomycete fungal pathogen *Ganoderma boninense*, is known as one of the most devastating diseases in oil palm. The disease causes great economic losses to oil palm industry with no chance of healing after establishing a rapid pathogenesis. Although, BSR has been jeopardising the oil palm industry for decades, efforts to control the disease have been hampered due to lack of knowledge on the dynamics of the pathogen (Sanderson, 2005). BSR is a soil-borne disease and there are three possible ways which this fungus can spread directly to the host plants; root-to-root contact, basidiospores, and free secondary inoculum in the soil. Numerous infection trials using oil palm seedlings and often using large *Ganoderma*-colonised rubber wood blocks (RWB) have provided data to support these views (Sariah *et al*., 1994; Breton *et al*., 2006). Rees *et al*. (2007) demonstrated that by attaching infected rubber-wood blocks to roots, much smaller inoculum can be used, allowing infection to occur through unwounded roots, progression and rate

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of invasion can be monitored. Colonisation on root surface of oil palm seedlings by *G. boninense* has been described in limited number of studies. In the current study, the use of electron microscope has provided topogrophical and morphological images on the colonisation features of *G. boninense*. In this study, mode of invasion by *G. boninense* in oil palm seedling was investigated using scanning electron microscopy (SEM) to provide information on the establishment and nature of infection of *G. boninense* in oil palm seedling.

MATERIALS AND METHODS

Preparation of *Ganoderma* Inoculum

Ganoderma boninense isolate (Chong *et al*., 2011) was used for infection of oil palm seedlings in the nursery trial. Method for preparation of *Ganoderma* inoculum on 6 x 6 x 12 cm RWB was adopted from Breton *et al*. (2006) with slight modification. RWB were initially cleaned with running tap water and then autoclaved for 2 hr at 121°C in autoclavable polypropylene plastic bags individually. After that, 100 ml of sterile molten malt extract agar (MEA) were added into each plastic bag and were left to solidify. The RWB were inoculated with mycelial plugs (6 mm) from a 14-day old *G. boninense* culture. The inoculated RWB were sealed and incubated for eight weeks in dark and at room temperature of 25±2°C to allow the fungal mycelia to fully colonise the blocks.

Inoculation of Oil Palm Seedlings

The RWB inocula were introduced to eight-month old oil palm seedlings (Deli *dura* x AVROS *pisifera* crosses) grown in polybags containing soil and sand mixture (2:1). The seedlings were first uprooted carefully and later kept seated on the *G. boninense*-inoculated RWB to ensure the roots were in direct contact with the inoculum. Ten oil palm seedlings were subjected for *Ganoderma* inoculation, meanwhile another 10 oil palm seedlings served as negative controls. The seedlings were placed in a netted plant house and watered daily.

SEM Preparation and Observation

After six months of inoculation, destructive root samplings were done and the root samples were cleaned with tap water and air dried. Roots were excised from healthy (uninoculated) and diseased tissue of inoculated oil palm roots and fixed in 4% solution of gultaraldehyde with sodium buffer 0.2 M (pH 7.2-7.4%) and incubated for 24 hr at 4°C. The specimens were then cut into 1 cm^3 size using scalpel and soaked in 0.1 M phosphate buffer three times for about 30 s with 10 min interval. The specimens were then dehydrated in solutions of methanol at 30%, 50%, 70%, 90% and finally 100%, 30 min in each solution. After dehydration, the specimens were dried in the carbon dioxide in critical point dryer (CPD) for 90 min, attached to SEM stubs and coated with gold using sputter coater (Emitech K550X) for 60 s. Prepared specimens were then examined under a SEM (Carl Zeiss, MA10) with three replicates for each sample.

RESULTS

SEM made it possible to reveal the presence and localisation of fungal hyphae inside the root tissues of oil palm seedlings infected by *G. boninense*. *Figure 1a, 1b* and *1c* show the typical topography of oil palm root seedlings where intact cells of the epidermal layer formed a series of parallel ridges and valleys. No detectable fungal tissue was observed on the root tissues of uninoculated seedlings (*Figures 1a, 1b* and *1c*). Meanwhile root tissues from inoculated seedlings were severely damaged with complete breakdown of the oil palm root cells. Observation on cross-section of inoculated oil palm root shows that *G. boninense* mycelia colonised the root surface directly and progressed through the inner, thin-walled cortex, as revealed by SEM (*Figures 1d, 1e* and *1f*). The SEM observation also revealed some alterations of root cells structure including tissue degradation and cortex disorganisation. All infected oil palm root tissues show similar physical damage evidence under SEM observation. Evidence of highly densed fungal mycelia growth along the cortical cells were also observed under SEM indicated as ph in *Figure 1f* with detail view in *Figure* 2.

DISCUSSION

The current study shows that *G. boninense* successfully infected the oil palm seedlings initiated from the point of contact between the host roots and *Ganoderma* inoculum after six months inoculation. Process of colonisation on the root surface by *G. boninense* is an essential step to subsequent penetration of the pathogen between host cells and to ultimately induce host damage. *G. boninense* exploits a sequential biotrophic- and necrotropic-infection switch strategy to colonise its host plant, known as hemibiotrophs (Ho *et al*., 2016). During the initial infection of biotrophic phase, colonisation of *Ganoderma* occurs in root cortex with numerous hyphae occupying the entire host cells that have fully intact cell walls (Rees *et al*., 2009). Once biotrophic growth has been successfully established, the fungus switches to nectrotropic phase associated with extensive cell wall degradation, that kill and

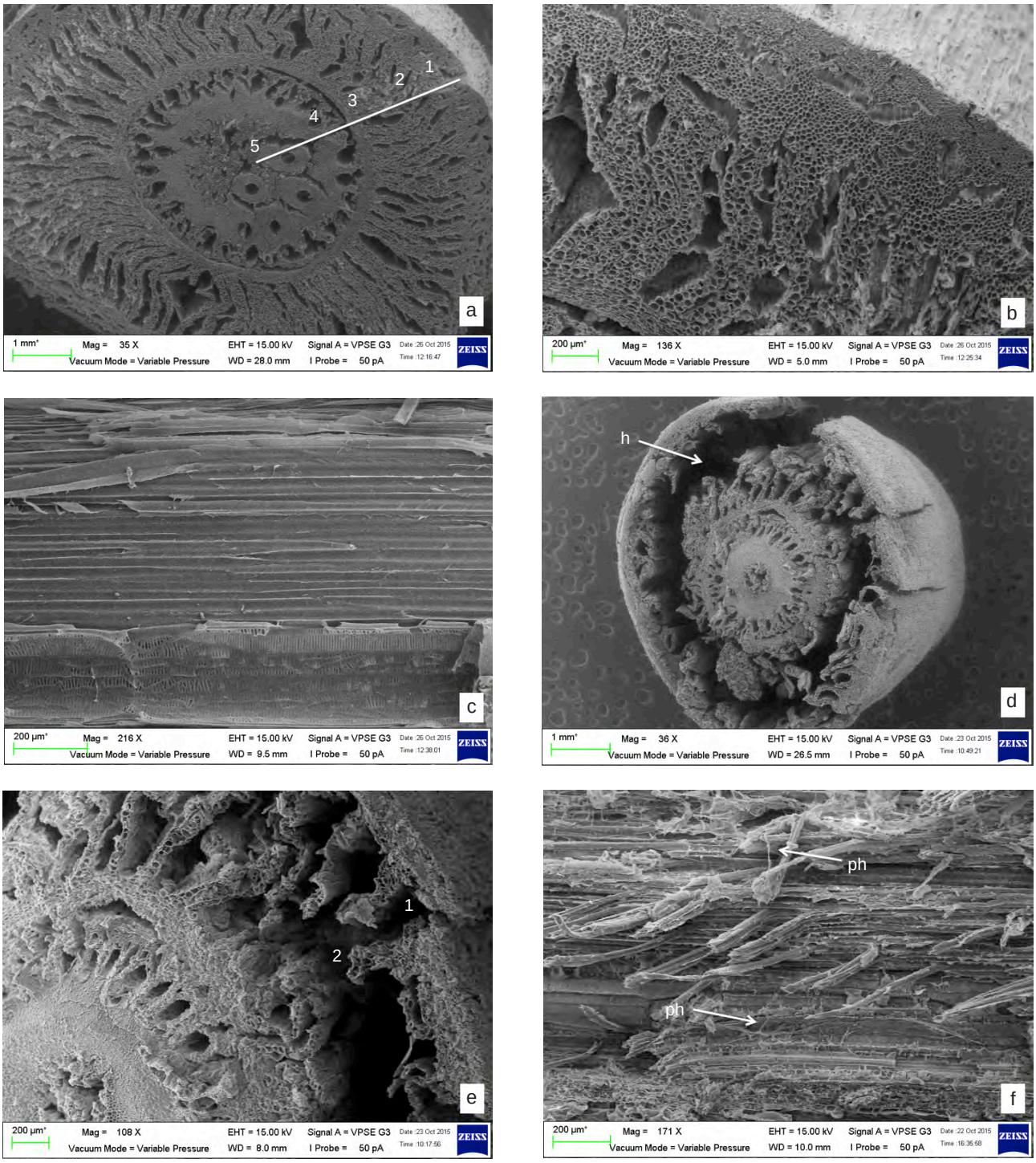

Figure 1. (a - c) Scanning electron microscopy (SEM) images of root tissue of oil palm seedlings free of inoculant fungus, G. boninense. *(a) View of cross-section of uninoculated oil palm seedling revealing an intact and undamaged cell structure, where five zones 1-5 are generally distinguished; 1- epidermis; 2 - cortex; 3 - vascular bundles; 4 - medullary vessels; 5 - pith. (b) Close-up view of zone 1 and 2 of* Figure 1a *showing a healthy-looking crotical cells. (c) Transversal section view of uninoculated cortex tissue showing a smooth and undamaged structure. (d) SEM of root tissue of oil palm seedling 6 months after inoculation with* G. boninense *showing root cortex section collapsed and formed a hollow indicated as 'h' in between the epidermis and endodermis cells as due to cell death, however the vascular system remain intact but distorted. (e) A disintegrated and disorganised structure of the epidermal indicated as '1' and cortical cells indicated as '2'. (f) Detailed view of zone 2 (refer to* Figures 1a *and* 1e *of infected root showing intense damages and highly colonised with* G. boninense *mycelia indicated as 'ph'.*

macerate host tissues. The formation of melanised mycelium might be considered as a third phase which is probably indicative of the oxidative breakdown of lignin and the white rot status ascribed to *G. boninense* (Adaskaveg *et al.*, 1990; Paterson, 2007).

This article shows the route and progress of *G. boninense* colonisation after six months of

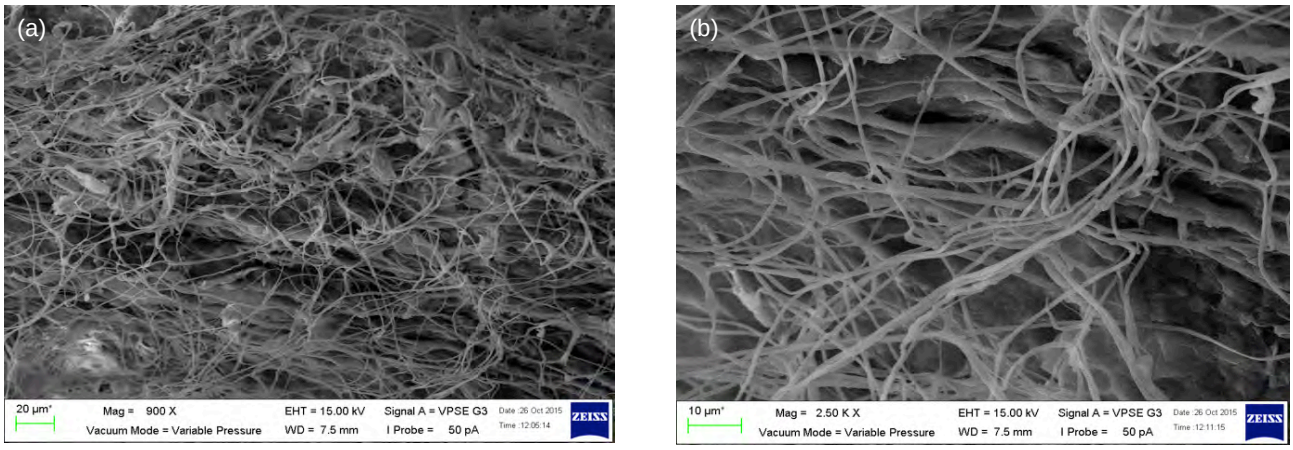

Figure 2. (a - b) Further detailed view of zone (2) in Figure 1e *of infected root tissues examined using scanning electron microscopy (SEM) showing an extensive colonisation of* G. boninense *and highly densed growth of the pathogen in the epidermal and crotical cells of 15-month old (six months after inoculation) oil palm seedling.*

artificial inoculation. By this time, colonisation of *G. boninense* had reached the cortical cells. SEM observation at the most seriously infected area showed the pathogen had invaded the cortex with highly densed mycelia growth observed along the tissue. Evidence on the extensive growth of *G. boninense* in the inner cortical cells and breakdown of the tissues as shown in *Figures 1* and *2* complimented the study by Rees *et al*. (2009). The growth habit of *G. boninense* hyphae observed along the walls of epidermal and cortex cells of oil palm roots is also similar to the study reported by Lopez-Llorca *et al*. (2002) on colonisation of barley by Verticulum chlamydosporium. SEM observation shows the infection route of *G. boninense* initiated from the epidermal surface of oil palm root tissue towards the xylem vessels. No evidence on the progression of fungal growth through the vascular system has been observed in the present study. However, distortion of the latter cells as observed under SEM may be due to the plant defense responses toward pathogen attack. Invasion of *G. boninense* in the root cortex as observed via SEM provides crucial evidence of cell wall degrading enzymes (CWDE) production by the fungi, which created a penetration holes through the cell wall layers allowing the entrance of *G. boninense* (Cooper, 1984). This finding is also supported by previous work by Rees *et al*. (2009) which discovered penetration holes on the outer cell of oil palm root tissue through transmission electron microscopy (TEM). The invasion process of cell wall layer of oil palm root cells resulted from the development of multiple holes through localised area causing a complete breakdown of cell wall. Hemibiotrophic behaviour of *G. boninense* with abundant hyphae propagate soon after invading the host tissue lead to the formation of an infection cushion consisting of a bundle of hyphae on the root surface which then directly penetrate the host tissue using small infection holes. Successful penetration is followed by proliferation of young hyphae along the root host tissue. Finally, tissues of the oil palm roots lost their integrity and looked like rotten mass covered with dense mycelium of the fungus. As the roots decay, the pathogen form source of inoculum in the soil, where they remain viable for many years (Hasan and Turner, 1998). This inoculum then actively proliferate once it found suitable host with favourable environment.

CONCLUSION

The colonisation of *G. boninense* in oil palm is mainly through the infection cushions, which usually resulted from repeated branching and development of infection holes. Enzymes produced by the fungus may be involved in weakening and loosening the wall structure of host cell enabling the fungus to spread within the tissue in order for complete breakdown of root cells. Overall, this study provides evidence of the route and nature of infection by *G. boninense*.

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