Differences Between Healthy and *Ganoderma boninense* Infected Oil Palm Seedlings Using Spectral Reflectance of Young Leaf Data

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Abstract: *Ganoderma boninense* (*G.boninense*) is the causal agent of basal stem rot (BSR) which significantly reduced the productivity of oil palm plantations in Southeast Asia. At early stage, the disease did not show any physical symptoms that could be seen with naked eyes resulted in detection difficulties. To date, there was no effective detection for this disease, and conventional methods such as manual and laboratory-based required trained specialists as well as time-consuming. Therefore, this study was conducted using hyperspectral remote sensing to investigate the differences in spectral reflectance of young leaf (frond one (F1) of healthy and *G. boninense* infected oil palm seedlings. The seedlings were inoculated with *G. boninense* pathogen at five months old. At five months after inoculation, 558 spectral signatures of F1 were extracted from acquired hyperspectral images. Noise removal was done to the extracted spectral signatures to remove outliers in the data. Then, the spectral signatures were averaged and plotted to observe the differences. Differences in reflectance of healthy and *G. boninense* infected seedlings were seen evidently in the near-infrared (NIR) region. Thus, this study showed evidence that F1 spectral reflectance has the ability to detect early stage of *G. boninense* infection at oil palm seedlings.

Keywords: *Ganoderma boninense*, BSR disease, Hyperspectral imaging, Oil palm seedlings, Spectral reflectance.

Introduction

Malaysia at present monopolises 28% of world palm oil production and currently the second world's largest exporter of palm oil after Indonesia, with 33% of world exports. Many commercial plantation companies cultivated oil palms on a vast scale because of the high

yield and low production cost. Nevertheless, the production of oil palm in Malaysia has exposed to BSR disease, which caused by _G. boninense_ pathogen. _G. boninense_ is a white-rot fungus that able to degrade lignin component and damage the xylem pathway causing a vital problem in water and nutrient transportation to the aerial part of oil palm (Paterson, 2007; Shu'ud _et al_., 2007). As a result, the symptoms appear similar to water and nutrient deficiency. Besides mature trees, oil palm seedlings are also prone to the infection where the symptoms develop earlier and dreadful (Sanderson, 2005).

The earliest visual symptoms of _G. boninense_ infection at oil palm seedling is the development of fruiting body at bole, yellowing of older leaves as well as necrosis of basal frond, reflecting over 50% internal damage of stem base. However, early detection based on these symptoms is complicated and confounding because the fruiting body may appear unpredictably before or after leaf symptom development (Sariah _et al_., 1994; Idris _et al_., 2006). Consequently, healthy and mild infected seedlings are hard to differentiate and easily be overlooked. In addition, there is growth inhibition in terms of production of new leaves, height and girth as the infection progresses, due to low photosynthesis rate. Besides the visual symptoms, _G. boninense_ infection can also be identified from brown discolouration of the inner longitudinal section of bole. Nevertheless, this type of detection is unfeasible for commercial plantation as the process is tedious, labour extensive and might lead to tree destruction.

Various techniques have been developed to detect BSR disease at early stage namely Ganoderma Selective Medium (GSM) (Darus & Seman, 1993), polymerase chain reaction-DNA (PCR-DNA) technique (Idris _et al_.,

2003), enzyme-linked immunosorbent assay-polyclonal antibody (ELISA-PAb) (Idris & Rafidah, 2008), GanoSken Tomography (Idris _et al_., 2010), Field Spectroscopy (Izzuddin _et al.,_ 2013), Mid-infrared spectroscopy (Liaghat _et al.,_ 2014b) and Terrestrial Laser Scanning (Khairunniza-Bejo & Vong, 2014; Azuan _et al_., 2019; Husin _et al.,_ 2020). However, limitations of these techniques are cost-time efficiency, impractical for large plantation area, and need experts to execute the procedures. Nowadays, the standard practice to detect _G. boninense_ in a plantation is by visual examination, which is labour intensive, time-consuming and human dependent.

Different studies have shown the capability of hyperspectral imaging in detecting symptomatic and asymptomatic plant diseases. For example, Bravo _et al._ (2003) and Moshou _et al._ (2005) have utilised visible-NIR hyperspectral imaging for the early detection of yellow rust disease in winter wheat. Susič _et al._ (2018) and Lu _et al._ (2018) utilised hyperspectral technology to detect and distinguish different causes of tomato stress and different stages of multi-tomato leaf disease, respectively. Kumar _et al._ (2012) investigated the applicability of aerial hyperspectral imaging for detection of greening in a citrus plantation. Similarly, Shafri & Hamdan (2009), Shafri _et al._ (2012) and Izzuddin _et al._ (2015) used airborne hyperspectral imaging to detect BSR disease in oil palm plantation. Meanwhile, Liaghat _et al._ (2014a) and Ahmadi _et al_. (2017) collected spectral signatures from young leaves from matured oil palms using spectroradiometer and developed classification models to separate the healthy, mild, medium and severe _G. boninense_ infected oil palms. For oil palm seedlings, Shafri _et al._ (2011) and Izzuddin _et al._ (2017) have utilised APOGEE spectroradiometer to collect reflectance

172

spectra of young leaves after six months of inoculation with *G. boninense* pathogen. However, the foliar symptom had appeared after two months of inoculation. The result showed that there were difficulties to distinguish the healthy and mild infected seedlings due to overlapped reflectance spectra.

As mentioned earlier, previous studies were unable to differentiate the healthy and mild *G. boninense* infected seedlings. According to Zwiggelaar (1998), spectral reflectance of plant was affected by physical structure, chemical composition, and spectral properties of leaves. Meanwhile, changes in pigment levels of leaves commonly associated with physiological reactions, environmental, stresses and diseases reactions (Chang, 1998; Gamon & Surfus, 1999; Gitelson *et al*., 2001, Gitelson *et al.,* 2002). Furthermore, there is a need to fill the knowledge gap in BSR detection, particularly in oil palm seedling with inoculation period of fewer than six months. Therefore, this study was conducted to focus on detection of BSR at young seedling with the age of five months old, prior to symptoms expression. The analysis was done based on reflectance spectra taken from frond 1 (F1) of healthy and *G. boninense* infected seedlings. Besides, this study also conducted to determine the capability of hyperspectral imaging to detect *G. boninense* infection in oil palm seedlings prior to symptoms expression.

Materials & Methods

Experimental design

The study was carried out in a controlled environment at Universiti Putra Malaysia (UPM) Transgenic Greenhouse (2°59'33.10"N, 101°43'19.16"E), Serdang, Malaysia. The samples consisted of 28 oil palm seedlings (commercial standard crosses of Dura × Pisifera, DxP) at four months old

obtained from Sime Darby Plantation, Banting, Malaysia. The seedlings were placed inside the greenhouse before inoculation for a month to acclimate the condition. The temperature inside the greenhouse was set at 27°C, referring to Kamil & Omar (2016). All seedlings were watered and fertilised regularly at the same rate.

The acclimatised five months old seedling was transplanted into a polybag containing 90% topsoil, 10% sand and 6 cm × 6 cm × 6 cm rubberwood block (RWB) that colonised with *G. boninense* pathogen. The roots of the seedlings were placed on top direct contact with the inoculated RWB (Fig. 1) and covered with soil; this method was called sitting technique (Naidu *et al*., 2018). Seedlings transplanted with uninoculated RWB acted as the control in this study. Polymerase chain reaction (PCR) test was conducted using two of the inoculated (U) seedlings after two months of inoculation to confirm the *G. boninense* infection.

Hyperspectral image acquisition was conducted at five months after inoculation using FireflEYE S185 snapshot camera (Cubert GmbH, Ulm, Germany) with a spectral range of 450 nm- 950 nm (125 bands) and spectral sampling of 4 nm. The camera was mounted horizontally on a custom tripod that positioned 2.6 m from the ground level (Fig. 2). The system was controlled by data acquisition software, Cube-Pilot supplemented by the manufacturer. An image of a seedling was taken at a time against a black background board on a sunny clear day from 11:00 a.m. to 2:00 p.m. local time to get a natural illumination.

The camera was calibrated with white and dark references before each image acquisition so that the integration time is almost the same, which could reduce the effects of illumination

173

and detector sensitivity. A dark reference was done by closing the lens while the white reference was carried out using a white rectangular board (99% light reflection) positioned flat and close to the lens. Each collected spectrum was calibrated as:

$$Reflectance = \frac{(Image - Dark)}{(White - Dark)} \qquad (1)$$

Fig (1): Sitting technique where RWB was placed in direct contact with the roots of an oil palm seedling.
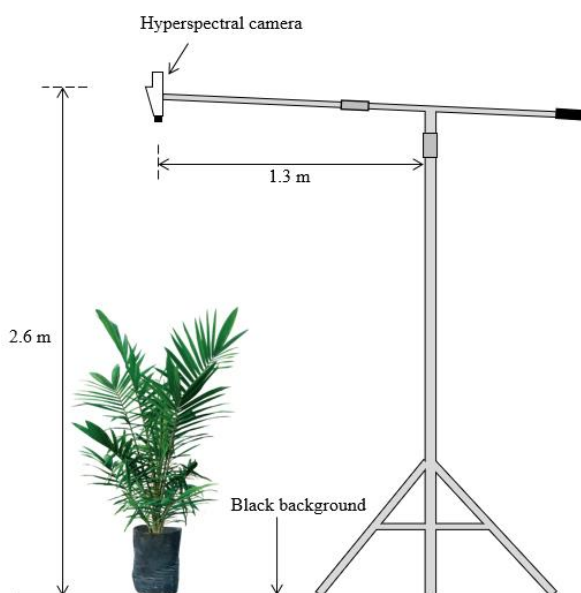

Fig. (2): Illustration of hyperspectral image acquisition setup inside the greenhouse.

Data pre-processing

Cube-Pilot software was used to extract the spectral signatures of F1 from the hyperspectral images. Since F1 was at the top of the oil palm crown, it was clearly visible in the images (Fig. 3). An average of 20 spectral signatures was collected randomly from the right and left leaflets of F1 for each seedling.

Fig. (3) Spectral extraction from the right and left leaflets of frond 1 (F1).

Thereafter, outliers in the spectral signatures were removed using the box plot method. Box plot statistically examined the data in a graphical way to describe the distribution of the data. The outliers were categorised as the spectral signature that has infrequent values from the rest of the spectral signatures.

Data analysis

Reflectance analysis was popularly used in previous studies to analyse the spectral signatures of plants. Plant stress level resulted from abiotic or biotic factors usually declines the concentration of leaves chlorophyll (as well as carotenoids) which affect the spectral reflectance of leaves and provide the opportunity to determine the plant health status remotely (Zarco-Tejada *et al*., 2004; Malenovský *et al.,* 2006).

The reflectance analysis was conducted by calculating the average spectral signatures of healthy and *G. boninense* infected seedlings. The averages were then plotted against reflectance to observe the patterns and differences between healthy and *G. boninense* infected seedlings were determined.

Results & Discussion

Fig. (4) shows the result of reflectance analysis of F1 for healthy (H) and *G. boninense* infected (U) seedlings. The U seedlings demonstrated low reflectance in the NIR range (750 – 950 nm) while the H produced high reflectance. The pattern produced by U seedlings in NIR range was normal for diseased plants as a result of xylem destruction, which thus caused chlorophyll pigments reduction and water deficiency.

Furthermore, NIR wavelength could penetrate deeper through leaf pigments compared to the visible wavelength; therefore, changes of reflectance in the NIR range were more evident compared to changes in the visible range during the stress period (Liaghat *et al.,* 2014a). The changes were caused by the rupture of mesophyll cell wall of leaves (Gausman, 1977; Chappelle *et al.,* 1992; Slaton *et al.,* 2001; Ahmadi *et al.,* 2017) resulting in lower reflectance and higher absorptance of NIR. In addition, the results obtained in this study have coincided with Liaghat *et al.* (2014a), where healthy oil palms reflected higher light than *G. boninense* infected oil palms, primarily in the NIR range.

In this analysis, the H seedlings reflected a slightly higher visible light than U seedlings, which was contrary with theory studied by other researchers. Healthy plants typically generated lower visible reflectance than diseased plants, particularly in the green wavelengths which represent higher chlorophyll content of leaves. Nonetheless, Shafri *et al.* (2011) had obtained a similar outcome as this study in which healthy seedlings produced higher reflectance in the green wavelength compared to *G. boninense* infected seedlings. This reflectance pattern might therefore be a specific spectral signature for oil palm seedlings since each plant has a specific spectral signature in various spectral regions as claimed by Schmidt & Skidmore (2003).

In this study, there were no physical symptoms of *G. boninense* infection appeared at the seedlings after five months of inoculation; however, the PCR test had generated positive results which indicated the presence of the infection. The late appearance of visual symptoms may vary due to the age of seedlings during inoculation, as younger palms potentially be affected by the *G. boninense* pathogen quicker than matured palms because of a not well-developed rooting system (Rees *et al.,* 2009). Thus, the physical symptoms of younger seedlings will appear early compared to older seedlings. Besides, Shafri *et al.* (2011) claimed that the differences in disease infection were also due to the resistance of the oil palm seedlings towards *G. boninense* pathogen.

The results have successfully filled the gap on the study of BSR detection at the very young age of seedlings (less than six months old) which thus could provide us with an idea of infection progress. Furthermore, the successfulness of the proposed method in detecting BSR at the very early stage (no symptoms) could prevent the disease widespread.
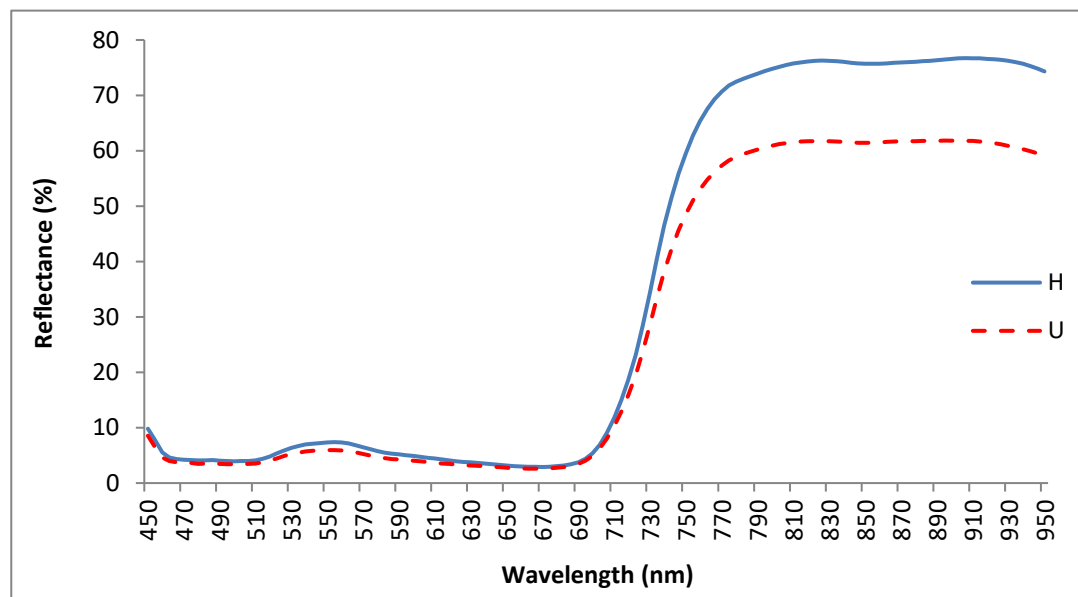
Fig. (4): Average reflectance of healthy (H) and *G. boninense* infected (U) leaves of F1.

It is also very useful in the real application at the nursery level to test products and screen for tolerant materials.

Conclusion

The differences between healthy and *G. boninense* infected oil palm seedlings were clearly observed in the NIR spectrum where the healthy young leaves gave higher reflectance compared to *G. boninense* infected leaves, although there were no physical symptoms developed. Furthermore, the reflectance at the green spectrum was slightly higher for the healthy leaves. Therefore, it is verified that the usage of F1 was feasible to detect the *G. boninense* disease. This information is useful because this approach can be expended to the more practical application using unmanned aerial vehicle (UAV), where the identification of F1 is easier since it is located at the top of oil palm crown.

Acknowledgement

Conflicts of interest

The authors declare that they have no conflict of interests.

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176

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177

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