Short Communication

J Bangladesh Agril Univ 17(3): 355–358, 2019 https://doi.org/10.3329/jbau.v17i3.43211

ISSN 1810-3030 (Print) 2408-8684 (Online)

Journal of Bangladesh Agricultural University

Journal home page: http://baures.bau.edu.bd/jbau, www.banglajol.info/index.php/JBAU

The relationship between SPAD chlorophyll and disease severity index in *Ganoderma*-infected oil palm seedlings

M.R.M. Rakib[1,2] ✉, A.H. Borhan[2], A.N. Jawahir[3]

[1]Sustainable Palm Oil Research Unit, Universiti Malaysia Sabah, 90000 Sandakan, Sabah, Malaysia
[2]Faculty of Sustainable Agriculture, Universiti Malaysia Sabah, 90000 Sandakan, Sabah, Malaysia
[3]Department of Biology, Faculty of Science, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

ARTICLE INFO

Article history:
Received: 01 June 2019
Accepted: 31 August 2019
Published: 30 September 2019

Keywords:
Disease severity,
Ganoderma,
Oil palm,
SPAD chlorophyll

Correspondence:
M.R.M. Rakib
✉: rakibmrm@ums.edu.my

ABSTRACT

Establishment of disease in oil palm seedlings through artificial inoculation of *Ganoderma* are widely used for studies of various aspects of plant pathology, including epidemiology, etiology, disease resistance, host-parasite interaction and disease control. The estimation of chlorophyll content in the infected seedlings possibly could provide a good indicator for degree of disease or infection, and changes during pathogenesis. Thus, the objective of this study was to evaluate the relationship between disease severity index (DSI) and chlorophyll content in *Ganoderma* infected oil palm seedlings. Three-month-old oil palm seedlings were infected with *Ganoderma* inoculum on rubber wood block (RWB), where 44 isolates of *Ganoderma* were tested. Disease severity index (DSI) and chlorophyll content using a single-photon avalanche diode (SPAD) meter were recorded at 4 weeks interval for a period of 24 weeks after inoculation (WAI). Pearson's correlation analysis and regression analysis were performed to evaluate the relationship between the variables. It was found that the relationship between DSI and SPAD chlorophyll value was inversely proportional (R = -0.92) in a linear trend (R^2 = 0.85). Furthermore, the increasing trend of the DSI across the weeks were fitted in a quadratic model (R^2 = 0.99). In contrast, the SPAD chlorophyll value declined in a linear trend (R^2 = 0.98). The SPAD chlorophyll value could be considered as a better alternative over the DSI as the SPAD chlorophyll value was strongly related to DSI, as well as able to detect physiological changes in the infected oil palm seedlings at the early stages of pathogenesis.

Introduction

Oil palm (*Elaeis guineensis* Jacq.) is an important plantation crop in the Southeast Asia, especially in Indonesia, Malaysia, and Thailand (USDA, 2018). However, the sustainability of oil palm in that region is threaten by *Ganoderma boninense*, a basidiomycete fungus causing stem rot disease in oil palm (Rees *et al.*, 2012; Rakib *et al.*, 2014). Establishment of disease by artificial inoculation is essential for studies of various aspects of plant pathology, including epidemiology, etiology, disease resistance, host-parasite interaction and disease control. Internal and external disease signs and symptoms are the common indicator used to evaluate the establishment of disease in plants. However, there are other alternative physiological characteristics of plant that could be used to evaluate the disease establishment, such as estimation of the chlorophyll content in the leaf of a plant measured using a SPAD chlorophyll meter (Uddling *et al.*, 2007). Based on a study by Goh *et al.* (2016), the chlorophyll content in *Ganoderma*-infected oil palm seedlings declined as the infection progress or during the pathogenesis of *Ganoderma*. Chang *et al.* (2015) also reported similar findings, where the chlorophyll content reduced as the disease progressed in different stages of cucumber growth.

Evaluation of disease severity based on the external disease signs and symptoms which correspond to disease scales require tedious work and careful observation for data recording, as well as time consuming. The use of the SPAD chlorophyll meter device could provide better alternative to evaluate disease severity in a plant (Chang *et al.*, 2015). Thus, the objective of this study was to evaluate the relationship between the values of disease severity index and SPAD chlorophyll in *Ganoderma* infected oil palm seedlings.

Cite this article

Rakib, M.R.M., Borhan, A.H. and Jawahir, A.N. 2019. The relationship between SPAD chlorophyll and disease severity index in *Ganoderma*-infected oil palm seedlings. *Journal of Bangladesh Agricultural University*, 17(3): 355–358. https://doi.org/10.3329/jbau.v17i3.43211

Materials and Methods

Ganoderma isolates and planting materials

A total of 44 isolates of *Ganoderma* from stem rot infected oil palm were obtained from estates located in Sarawak, Malaysia (Rakib *et al.*, 2014). Planting materials used in this study were 3-month-old oil palm (*Dura* x *Pisifera*) seedlings planted in mixed topsoil and sand medium in polyethylene bags.

Inoculation of Ganoderma on oil palm seedlings

Rubber wood block (RWB) sitting technique was used for inoculation of *Ganoderma* on the oil palm seedling as described in Rakib *et al.* (2015). Each *Ganoderma* isolate was tested with three seedlings per replicate in four blocks for a total of 12 seedlings per isolate. The total of 528 experimental units were arranged in a randomized complete block design (RCBD) under a rain-shelter facility with temperature and relative humidity ranging from 25-35 ºC and 60-80%, respectively.

Collection and analysis of data

Data were recorded at 4 weeks interval for a period of 24 weeks after inoculation (WAI). The disease severity index (DSI) was assessed according to Ilias (2000), where it was calculated based on the numerical values of disease scale correspond to external disease signs and symptoms of infected seedlings (Table 1). Disease severity index (%) = [Σ (A x B) / Σ n x 5] x 100, where 'A' is the disease scale (0 to 5), 'B' is the number of seedling showing the disease scale per treatment, 'n' is the total number of replication, and 5 is the constant representing the highest scale of assessment. SPAD chlorophyll values were measured using a SPAD chlorophyll meter (SPAD-502 meter, Konica Minolta, Japan) (Chang *et al.*, 2015; Goh *et al.*, 2016) at the third lanceolate or bifurcate leaf from the top leaf of the oil palm seedling. The data were subjected to Pearson's correlation analysis and regression analysis to evaluate the relationship between the variables using the Statistical Analysis System (SAS v 9.2) program.

Table 1. Numerical disease scales and their corresponding disease signs and symptoms

Scale	Disease signs and symptoms on oil palm seedling
0	Healthy plant with green leaves and no fungal mass development on any part of the plant
1	Appearance of 1-3 chlorotic leaves with no fungal mass development on any part of the plant
2	Appearance of fungal mass with or without chlorotic leaves
3	Appearance of >3 chlorotic leaves, necrotic leaves (dead leaves) with or without fungal mass development on any part of the plant
4	At least 50% of total leaf number showing severe chlorosis or necrosis with or without fungal mass
5	Dead plants with or without fungal mass

Results and Discussion

All 44 *Ganoderma* isolates tested showed positive signs and symptoms of disease infection on the oil palm seedlings. Leaf symptoms were observed as the infection progressed over time and eventually the seedlings died. Generally, the external disease signs and symptoms appeared at 12 weeks after inoculation (WAI) and in several seedlings, the symptoms appeared as early as 8 WAI. The external infection symptoms observed include leaf chlorosis (yellowing leaf) which eventually became necrotic (brown or dead leaf). Mycelial mass of *Ganoderma* appeared at the base of the seedlings which progressively develop into small white button that eventually formed bracket of *Ganoderma* (Figure 1). The progression of infection as observed in this study was similar to those reported by Sariah *et al.* (2007) and Kok *et al.* (2013).

The DSI and SPAD chlorophyll values of the oil palm seedlings inoculated with *Ganoderma* at the 24 WAI ranged between 10.77 - 68.33% and 12.10 - 30.80, respectively. Pearson's correlation analysis of the data (N = 44) recorded on the 24 WAI indicated that the DSI and SPAD chlorophyll values were highly correlated ($p < 0.0001$, R = -0.92). Further regression analysis of the data showed strong relationship ($R^2 = 0.85$) between DSI and SPAD chlorophyll values, where increase in the DSI caused linear declining trend in the chlorophyll value. The trend of the relationship can be written as SPAD chlorophyll value = -0.2893 DSI + 34.177 (Figure 2).

Figure 3 shows the trend of the average DSI and SPAD chlorophyll values of the *Ganoderma* infected oil palm seedlings (a total of 44 isolates of *Ganoderma* tested, or N = 44) at 4-weeks interval for a period of 24 WAI. The DSI was low for the first 8 WAI (0 - 0.76%), and the DSI values increased rapidly at the 12 WAI until the 24 WAI (4.31 - 35.14%). Meanwhile, the SPAD chlorophyll values decline constantly from 56.20 on the 0 WAI until 24.01 on the 24 WAI. This indicates that the SPAD chlorophyll value was more sensitive for detection of disease especially in the early stage when the external disease signs and symptoms are not obvious to the naked eyes. Pearson's correlation analysis showed that the DSI was positively correlated across the weeks (p = 0.01, R = 0.90), while the SPAD chlorophyll value was negatively correlated across the weeks ($p < 0.0001$, R = -0.99).

Fig. 1 Infection progression of *Ganoderma*-inoculated oil palm seedlings. (a) Healthy seedling with no sign of infection; (b) seedling started to show leaf chlorosis or yellowing of the leaf either with or without fungal mass; (c) more leaves became chlorotic and eventually became necrotic (dead leaves), and fungal mass appeared at the base of the seedling's bole (arrow); (d) severe leave chlorosis and necrosis; (e) basidiocarp of *Ganoderma* formed with severe leaf symptoms on a dying seedling; and (f) desiccated dead seedling with well-developed *Ganoderma* basidiocarp. Source: Rakib *et al.* (2015)

The increasing trend of the DSI across the weeks were fitted in a quadratic model (Average DSI = 0.0942 Week^2 - 0.8785 Week + 0.9804), with a R^2 value of 0.99. In contrast, the SPAD chlorophyll value declined in a linear trend (Average SPAD chlorophyll = -1.2955 Week + 53.537), with a R^2 value of 0.98.

Goh *et al.* (2016) reported that the SPAD chlorophyll values decline as the disease progress or during pathogenesis of *Ganoderma* in the oil palm seedlings. Similarly, Chang *et al.* (2015) also reported that increasing disease in different stages of plant growth caused reduction in the SPAD chlorophyll value. Significant reduction in the chlorophyll content could be related to the injury in the oil palm seedling's root and vascular system caused by the fungus infection (Shafri *et al.*, 2011; Goh *et al.*, 2016), which possibly caused reduction in nutrient adsorption, especially nitrogen (N) and magnesium (Mg). Nitrogen and magnesium are related to the biosynthesis of chlorophyll (Rissler *et al.*, 2002), and both nutrients have been positively correlated with the chlorophyll content in plants (Bojovic and Markovic, 2009; Alberto *et al.*, 2014). Furthermore, the shortage of N and Mg caused chlorosis, where the leaves appeared yellowish as observed in this study (Figure 1).

Conclusion

The relationship between DSI and SPAD chlorophyll value was inversely proportional in a linear trend with confidence level of 85%. The decline in the SPAD chlorophyll values were detected on the early stages of pathogenesis (0 to 8 WAI) although no obvious changes was recorded in the DSI values during that early stages. This conclude that SPAD chlorophyll value could be used as an alternative as well as better alternative indicator to evaluate the disease establishment in the oil palm seedlings due *Ganoderma* species.

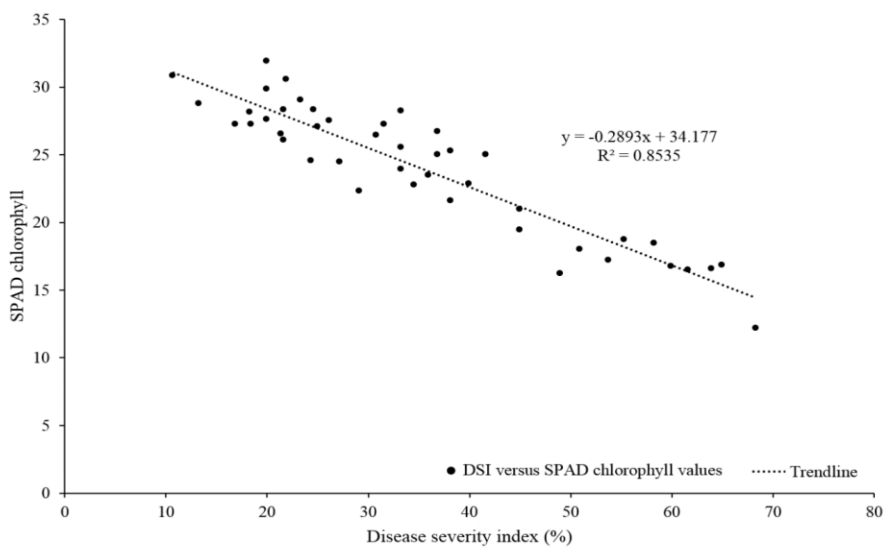

Fig. 2 Regression between disease severity index (DSI) and SPAD chlorophyll values on the 24week after inoculation with *Ganoderma* on the oil palm seedlings (N = 44)

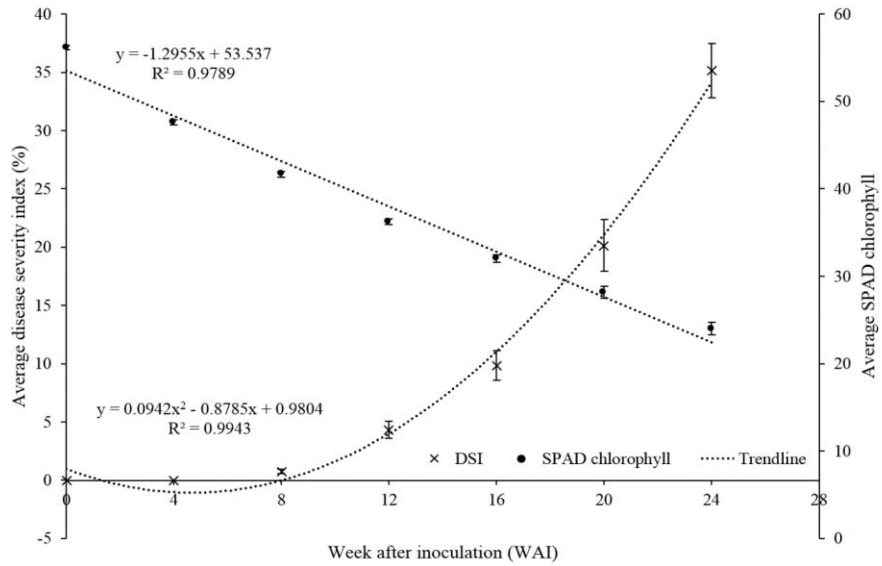

Fig. 3 Trends of average disease severity index (DSI) and SPAD chlorophyll values (± standard error) across 24 weeks after inoculation with *Ganoderma* on the oil palm seedlings

References

Alberto, S.M., Carlos, D.S.J., Pedro, D.F., Marcos, E.B.B., Lafayette, F.S., Janivan, F.S., Pedro, R.A.V. and Luciano, S.F., 2014. Chlorophyll and macronutrients content in leaf tissue of *Musa* sp. 'Prata-Ana' under fertigation. *African Journal of Agricultural Research*, 9: 1714–1720. https://doi.org/10.5897/AJAR2014.8683

Bojovic, B. and Markovic, A., 2009. Correlation between nitrogen and chlorophyll content in wheat (*Triticum aestivum* L.). *Kragujevac Journal of Science*, 31: 69–74.

Chang, R.K., Wang, Y.H., Zhang, X.T., Tang, G.C. and Wei, Y., 2015. The research of disease detection method of greenhouse cucumber leaf based on chlorophyll fluorescence analysis. *Universal Journal of Agricultural Research*, 3: 76–80. https://doi.org/10.13189/ujar.2015.030302

Goh, K.M., Dickinson, M., Alderson, P., Yap, L.V. and Supramaniam, C.V., 2016. Development of an in planta infection system for the early detection of *Ganoderma* spp. in oil palm. *Journal of Plant Pathology*, 98: 255–264.

Ilias, G.N.M. 2000. *Trichoderma* and its efficacy as a bio-control agent of basal stem rot of oil palm (*Elaeis guineensis*, Jacq.). PhD. Thesis, Universiti Putra Malaysia Selangor, Malaysia.

Kok, S.M., Goh, Y.K., Tung, H.J., Goh, K.J., Wong, W.C. and Goh, Y.K., 2013. *In vitro* growth of *Ganoderma boninense* isolates on novel palm extract medium and virulence on oil palm (*Elaeis guineensis*) seedlings. *Malaysian Journal of Microbiology*, 9: 33–42. https://doi.org/10.21161/mjm.45212

Rakib, M.R.M., Bong, C.F.J., Khairulmazmi, A. and Idris, A.S., 2014. Genetic and morphological diversity of *Ganoderma* species isolated from infected oil palms (*Elaeis guineensis*). *International Journal of Agriculture and Biology*, 16: 691–699.

Rakib, M.R.M., Bong, C.F.J., Khairulmazmi, A. and Idris, A.S., 2015. Aggressiveness of *Ganoderma boninense* and *G. zonatum* isolated from upper- and basal stem rot of oil palm (*Elaeis guineensis*) in Malaysia. *Journal of Oil Palm Research*, 27: 229–240.

Rees, R.W., Flood, J., Hasan, Y., Wills, M.A. and Cooper, R.M., 2012. *Ganoderma boninense* basidiospores in oil palm plantations: evaluation of their possible role in stem rots of *Elaeis guineensis*. *Plant Pathology*, 61: 567–578. https://doi.org/10.1111/j.1365-3059.2011.02533.x

Rissler, H.M., Collakova, E., DellaPenna, D., Whelan, J. and Pogson, B.J., 2002. Chlorophyll biosynthesis. expression of a second *Chl I* gene of magnesium chelatase in arabidopsis supports only limited chlorophyll synthesis. *Plant Physiology*, 128: 770–779. https://doi.org/10.1104/pp.010625

Sariah, M., Hussin, M.Z., Miller, R.N.G. and Holderness, M., 2007. Pathogenicity of *Ganoderma boninense* tested by inoculation of oil palm seedlings. *Plant Pathology*, 43: 507–510. https://doi.org/10.1111/j.1365-3059.1994.tb01584.x

Shafri, H.Z.M., Anuar, M.I., Seman, I.A. and Noor, N.M., 2011. Spectral discrimination of healthy and Ganoderma-infected oil palms from hyperspectral data. *International Journal of Remote Sensing*, 32:7111–7129. https://doi.org/10.1080/01431161.2010.519003

Uddling, J., Gelang-Alfredsson, J., Piikki, K. and Pleijel, H. 2007. Evaluating the relationship between leaf chlorophyll concentration and SPAD-502 chlorophyll meter readings. *Photosynthesis Research*, 91: 37–46. https://doi.org/10.1007/s11120-006-9077-5

USDA (United States Department of Agriculture). 2018. Oilseeds: World Markets and Trade. Foreign Agricultural Service, USDA. USA.