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

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**ARTICLE INFO**

**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 threatened 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

Chlorophyll and disease severity index in oil palm seedlings

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 °C and 60-80%, respectively.

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

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

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 (R² = 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 leaf 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 24-week after inoculation with *Ganoderma* on the oil palm seedlings (N = 44)
Chlorophyll and disease severity index in oil palm seedlings

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


