RED-EDGE INDICES TO DIAGNOSE ORANGE SPOTTING DISEASE OF OIL PALM IN MALAYSIA

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ABSTRACTS: The use of hyperspectral remote sensing techniques for plant disease diagnosis is gaining much prominence due to its non-destructive feature. The steep gradient in reflectance between visible and near-infrared region is known as the red-edge, which is located between 680 and 780 nm. Red-edge is a stress diagnostic indicator that provides for non-destructive diagnosis of plant disease. This paper investigates the potential of two selected red-edge wavebands (680 nm and 754 nm) in diagnosing Orange Spotting (OS) disease of oil palm. OS is a fast emerging disease in Malaysian oil palm plantations. Coconut cadang-cadang viroid (CCCVd) is the causal agent of OS disease. Four well-known red-edge indices namely Ratio Vegetation Index (RVI), Red Edge Position (REP), Normalized Difference Red Edge Index (NDREI) and Chlorophyll Index (CI) were evaluated using selected wavebands. A spectroradiometer (Model: ASD FieldSpec® HandHeld 2), which operates in the spectral range of 325-1075 nm, was deployed to measure leaf reflectance of fifteen inoculated and five healthy oil palm seedlings grown under glasshouse conditions. A highly infective CCCVd variant, OP246, was used to inoculate the seedlings. Reflectance was measured at 15, 30, 45 and 60 days after inoculation. This work was aimed at investigation of variations on red-edge indices within the specific intervals of inoculation. Red-edge indices were calculated from inoculated and healthy seedlings and analyzed using one-way analysis of variance. Mean differences were interpreted at 0.05 significance level using Latin Square design. REP was selected as best red-edge index as multiple comparison of means values for healthy and inoculated seedlings were significantly different at 95% confidence level at all intervals.

Keywords: Orange spotting, Hyperspectral remote sensing, Red-edge indices

INTRODUCTION

Viroids are exogenous infectious subviral plant pathogens well known since many years. These single-stranded, circular, non-protein-coding RNAs of just 246-401 nucleotides (nt) are able to parasitize the host plant (Diener, 2003; Flores et al., 2005; Gago-Zachert, 2015; Horst, 2013). For an example, African oil palm (*Elaeis guineensis Jacq*; Arecaceae) is a host for Coconut cadang-cadang viroid (CCCVd; family *Cocadviroid*, genus *Pospiviroidae*). CCCVd is the causal agent of OS disease of oil palm (Vadamalai, 2005; Vadamalai et al., 2009, 2006; Wu et al., 2013). OS is an emerging disease in Malaysian oil palm plantation. It was earlier described as Genetic Orange Spotting (GOS) (Forde and Leyritz, 1968). CCCVd spreads naturally by unknown means. Its eradication has not yet been successful (Randles et al., 2009). Therefore, CCCVd-infected oil palm seedling can only be managed by removal from nursery stage and replanting. In addition, CCCVd develops the disease at three stages i.e. early, middle, and late (Hanold and Randles, 1991). Early stage of disease development that lasts for 2-4 years on average causes more damage severity than later stages (Rodriguez et al., 1995), as reported for a 296-

nt form of CCCVd in coconut palm. Three CCCVd variants (i.e. OP297, OP293, and OP270) have been characterized from an asymptomatic oil palm in Malaysia showed more than 90% sequence similarity with 296-nt form of CCCVd in coconut (Vadamalai et al., 2006). In a recent investigation, an oil palm variant (OP 293) also showed low accumulation of viroid load with no symptoms during one year after the inoculation (Thanarajoo, 2014).

For detection of CCCVd, the most popular molecular marker technique is Real-time Reverse Transcription Polymerase Chain Reaction (RT-PCR) (Wu et al., 2013; Vadamalai et al., 2006) that involves destructive leaf sampling and takes a longer time for laboratory analysis. RT-PCR requires skilled and experienced investigator to detect a CCCVd in oil palm. Moreover, a highly controlled and contamination-free environment has also been maintained in a laboratory. On the other hand, hyperspectral remote sensing can be employed for rapid and non-destructive diagnosis of OS disease. It could be a prompt tool for preliminary screening of CCCVd infected oil palm seedlings at nursery stage.

The recent published literature highlights the application of hyperspectral non-imaging sensors in diagnosing plant disease. High resolution hyperspectral non-imaging sensors (i.e., spectroradiometers) typically consist very narrow and contiguous spectral wavebands. Spectroradiometers are used to measure crop reflectance over a full range of electromagnetic spectrum up to 2500 nm. Plant health can be closely monitored on the basis of crop reflectance using spectroradiometer. Use of spectroradiometer is real-time, cost-effective and non-invasive, therefore, it generates considerable savings in time, cost and labor. In the current study, we observed important differences between healthy and inoculated oil palm seedling using a spectroradiometer in a red-edge range (680-780 nm). Red-edge is a steep gradient in reflectance between visible red and Near Infrared (NIR) wavebands which has been frequently shown to indicate plant stress. Two specific objectives of this research are:

1. To investigate the potential of red-edge wavebands in differentiating between CCCVd-inoculated and healthy oil palm seedlings
2. To investigate the variations on red-edge indices at different intervals after inoculation.

MATERIALS AND METHODS

An experiment was performed under glasshouse conditions (14-20ºC) for six months from 30th of January, 2015 to 31st of June, 2015 at Universiti Putra Malaysia, Serdang, Malaysia. A total of twenty 3-month old healthy oil palm seedlings were transported into a glasshouse. Twenty oil palm seedlings were included in this study. Fifteen oil palm seedlings were inoculated with a variant of CCCVd (i.e. OP_{246}) using razor blade slashing while five seedlings were grown uninoculated. Seedlings were inoculated on 15th of February, 2015. An Analytic Spectral Device (ASD) spectroradiometer, FieldSpec–II (325-1075 nm), was employed for leaf reflectance measurement through 30th of April, 2015. Spectra were measured at 15, 30, 45 and 60 days after the inoculation (dai).

For reflectance measurement, oil palm seedlings were taken outside of the glasshouse on clear sunny days between 1100 and 1300 h. Reflectance was measured using a fixed 25° FOV fibre-optic cable at an integration time of 17 ms. A 50 cm measuring distance between sensor and target was maintained during spectral measurement. A total of ten spectra were measured from each seedling at a one trigger followed by white referencing with the help of white reference panel (Specralon®,Labsphere Inc., North Dutton, NH, USA).

Reflectance spectra that measured at different intervals were averaged to develop spectral signatures for inoculated and healthy oil palm seedlings. These spectral signatures were plotted against the red-edge region. Two spectral wavebands were selected from red-edge range. Four red-edge indices i.e. Ratio vegetation index (RVI), Red-edge position (REP), Normalized difference red-edge index (NDREI) and Chlorophyll index (CI) were evaluated with selected red-edge wavebands (Table 1).

Table 1. Red-edge indices selected in this study.

Abbreviation	Algorithm	Formula	Reference
RVI	Ratio vegetation index	R_{red} / R_{NIR}	Pearson and Miller (1972)
REP	Red-edge position	$R_{red} + R_{NIR} / 2$	Guyot and Baret (1988)
NDREI	Normalized difference red-edge index	$R_{NIR} - R_{red} / R_{NIR} + R_{red}$	Barnes et al. (2000)
CI	Chlorophyll index	$R_{NIR} / R_{red} -1$	Gitelson et al. (2006)

A one-way Analysis of Variance (ANOVA) was performed in order to investigate the variation on red-edge indices at different intervals. During statistical analysis, ANOVA with Latin Square design (LSD) was performed to compare the treatment means at 0.05% level of significance using a statistical program, SAS 9.2 (SAS Institute Inc., Cary, NC, USA).

RESULTS AND DISCUSSION

Automatic differentiation between healthy and unhealthy plants on the basis of hyperspectral reflectance has a vital role in precision plant protection. We have successfully differentiated oil palm seedlings that were inoculated with CCCVd from healthy seedlings from spectral signatures. There was small difference interpreted in spectral patterns between inoculated and healthy spectral signatures (Figure 1). Healthy spectral signature showed higher reflectance in NIR region than inoculated spectral signature due to leaf scattering and no absorption. First and last point of steep slop were selected as red-edge wavebands for further studies. These selected points were 680 nm (a red wavebands) and 754 nm (an NIR waveband).

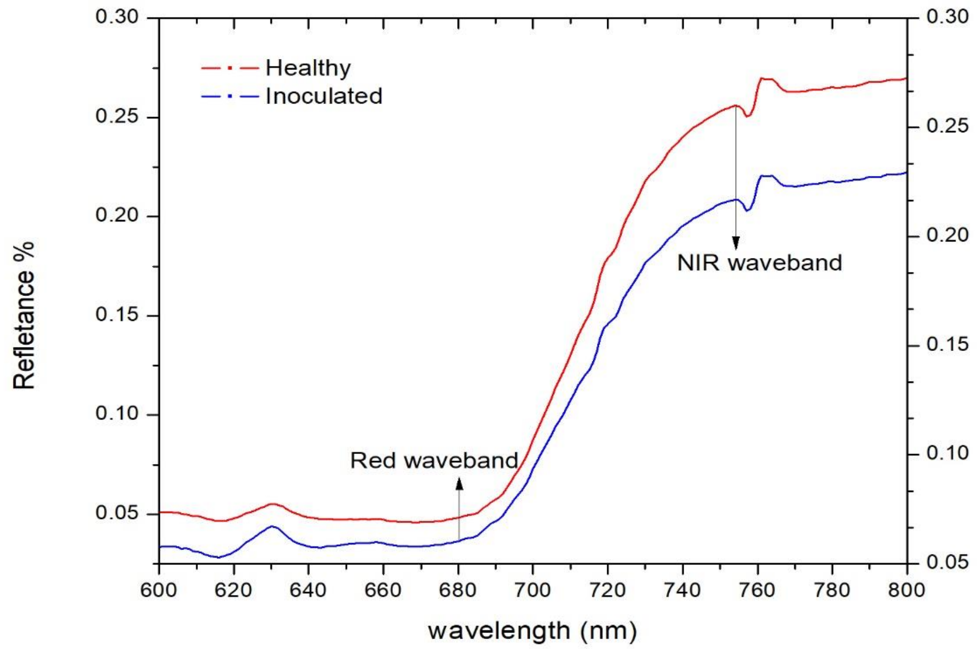

Figure 1. Mean spectra of healthy and inoculated oil palm seedlings.

Since spectral signatures showed very little spectral variation between inoculated and healthy seedling, selected red-edge wavebands (680 nm and 754 nm) were used to calculate red-edge indices for studying variation within the specific intervals of inoculation. In this study, REP was selected as the best red-edge index as per its performance within different intervals. Multiple comparison of means values were significantly different at 95% confidence level at all the intervals (Table 2). REP has proved as a best indicator of crop stress.

Table 2. Multiple comparisons of mean values of red-edge indices at 95 % confidence level calculated on 15, 30, 45 and 65 dai from healthy and inoculated leaf spectral signatures.

Index	Treatment	Means			
		15 dai	30 dai	45 dai	60 dai
RVI	Healthy	0.26b	0.21b	0.26a	0.25b
	Inoculated	0.33a	0.33a	0.23a	0.33a
REP	Healthy	1.02b	1.02b	1.02b	1.02b
	Inoculated	2.03a	2.03a	2.03a	2.03a
NDREI	Healthy	- 0.07b	0.13a	0.19a	0.09a
	Inoculated	0.01a	0.05b	0.14a	0.06a
CI	Healthy	3.06a	4.18a	3.45a	7.00a
	Inoculated	2.06b	2.08b	3.44a	6.25a

RVI was also found significantly different at 15, 30 and 60 dai. It has been observed that RVI was most similar to REP that could also be used as a good stress indicator. This result opens up a new possibility to evaluate red-edge indices (such as REP and RVI) for OS diagnosis at leaf scale. Moreover, there is still need of statistical classifiers for classification of red-edge indices to improve their accuracy. Application of red-edge indices for spectral differentiation would make OS diagnosis more attainable. The hyperspectral red-edge will be a useful diagnostic tool for OS diagnosis, especially when OS has been associated with asymptomatic oil palm.

CONCLUSION

Advance information about infection of OS disease could assist with red-edge wavebands and red-edge indices. In future, selected red-edge indices (REP and RVI) will play a vital role in screening oil palm seedlings at the leaf scale. This study provides an important insight for applying spectroradiometer and red-edge indices in diagnosing of OS disease.

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