Use of Spectral Reflectance to Discriminate between Potassium Deficiency and Orange Spotting Symptoms in Oil Palm (*Elaeis guineensis*)

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Abstract: Potassium (K) deficiency and Orange Spotting (OS) disease exhibit similar symptom via visual assessment. This work investigates the separability of K deficiency and OS disease symptoms using spectral reflectance. This assessment was conducted at a commercial oil palm plantation located in Sungai Buloh, Selangor. Leaves from K-deficient trees, OS-infected trees and nonsymptomatic trees (control) were sampled for spectral reflectance acquisition. Leaf spectral reflectance was acquired under constant halogen lighting. All leaf samples exhibited a green peak at 555 nm wavelength, with an average reflectance value of 0.15. Reflectance between OS-infected and K-deficient leaves showed significant separability at the 400-538 nm and 667-688 nm wavelength regions. Reflectance of K-deficient leaves was significantly different than that of OS-infected leaves across all severity classes.

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1. Introduction

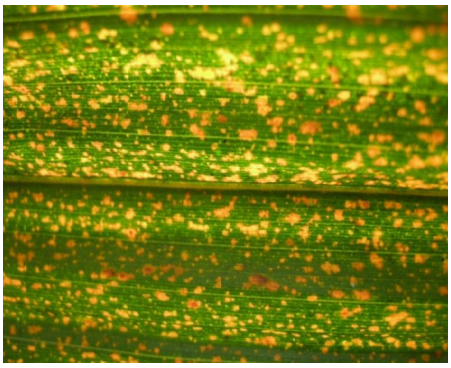
Potassium (K) is one of sixteen essential nutrients required for oil palm (*Elaeis guinensis*) growth and reproduction. K is a highly mobile element in plants and is translocated from older to younger tissue. K deficiency symptoms in oil palm usually appear first on the lower (older) leaves and progresses toward the upper (younger) leaves as K deficiency becomes more severe. The most common symptom of K deficiency in oil palm is the yellow-orange spotting (chlorosis) along the leaf margin. In severe cases of K deficiency, the yellow-orange spots turn into necrotic spots making it vulnerable to pathogenic infection.

Orange Spotting (OS) disease in oil palm, which is associated with Coconut Cadang-Cadang Viroid (CCCVd), has similar foliar symptoms to that of K deficiency (Figure 1). In some cases, OS disease and K deficiency symptoms co-occur within the same oil palm tree. This causes difficulty in identification and evaluation of OS and K disorders at field scale. As such, discrimination of OS disease and K deficiency symptoms via visual assessment can be a problem, especially in a mature oil palm stand due to canopy architecture and tree height.

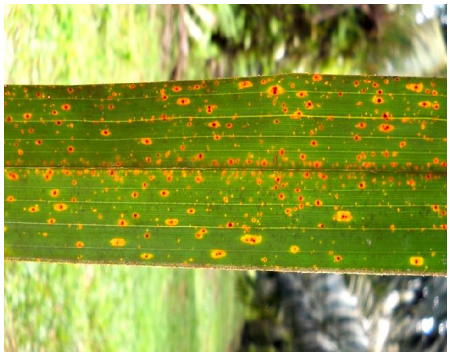
Poor separability between OS disease and K deficiency symptoms via visual assessment makes it difficult for these disorders to be detected and managed in a timely manner. At present, assessment of OS disease incidence and K deficiency occurrence.

Spectral reflectance from plant leaves could serve as a means to identify and quantify plant diseases (Sankaran, 2010). The precision of plant disease identification is typically higher when using spectral reflectance techniques as compared to visual assessment (Steddom *et al.*, 2005). The Photosynthetic Active Radiation (PAR) region (400-700 nm) and the Near Infra Red (NIR) region (700-1200 nm) of the electromagnetic spectrum contain information on physiological stress levels in plants (Xu *et al.*, 2007). As such, these disease-specific spectral wavelengths can be used to detect plant diseases non-destructively (West et al., 2003). Generally, plant leaves exhibit lower spectral reflectance at the PAR region compared to reflectance at the NIR region (Araus *et al.*, 2001). Belasque *et al.* (2008) identified citrus canker disease in citrus shrubs based on reflectance at 452, 685 and 735 nm. Similarly, Naidu *et al.* (2009) identified grapevine leafroll disease based on reflectance at 684, 752 and 970 nm.

Spectral reflectance has also been used to quantify plant macronutrient levels. Albayrak (2008) estimated nitrogen, phosphorus and potassium contents in sainfoin pasture using the reflectance ratio of 650 to 780 nm. Basayigit and Senol (2009) found that reflectance at 440, 520, 600 and 720 nm had a strong relationship with K content in several deciduous orchard plants.



(a)



(b)

Figure 1: Comparison of symptoms between (a) OS-infected and (b) K-deficient leaves in mature (> 8 years old) oil palm trees are based on highly laborious and time- consuming protocols. OS disease in oil palm is confirmed by the presence of CCCVd-like molecules using ribonuclease protection assay (Vadamalai *et al.*, 2009) while K deficiency is determined based on the critical level of leaf K. Leaf K content (commonly expressed as percent dry matter) is analyzed using the dry ashing method. Usually, mature trees with K deficiency symptom have leaf K values of lesser than 1 % (Joo, 1994).

Studies employing spectral reflectance techniques for plant disease identification caused by a single pathogen are well documented (Moshou *et al.*, 2004; Qin and Zhang, 2005; Sankaran, 2010). However, only few studies have looked into the use of spectral reflectance to discriminate multiple plant diseases within the same plant host. Graeff *et al.* (2006) identified and discriminated powdery mildew disease from take-all disease in wheat based on reflectance at 490, 510, 516 and 540 nm. Liu *et al.* (2010) used hyperspectral analysis to discriminate empty panicles caused by *Nilaparvata lugens* and panicles infected with *Ustilaginoidea virens* in rice plants. Mahlein *et al.* (2012) discriminated Cercospora leaf spot, powdery mildew and leaf rust symptoms on sugar beet leaves using hyperspectral imaging.

However, there is no literature on the use of spectral reflectance techniques to discriminate between plant disease and plant nutrient deficiency with similar symptoms. This work was aimed at investigating the use of spectral reflectance to discriminate between OS disease and K deficiency symptoms in oil palm.

2. Material and Methods

This work was conducted in two commercial oil palm plantations located at Kuala Selangor, Peninsular Malaysia (N 3.30° E 101.32° and N 3.34° E 101.37°). Both sites had a mean temperature of 27.3°C and a dew point of 23°C. Quantitative assessment of OS disease severity was performed at a 4.2 ha plot within the study sites based on percentage of symptomatic fronds from total fronds in an oil palm tree. Quantitative assessment of K deficiency was based on leaf K content (percentage of dry matter, % DM). Spectral reflectance from symptomatic leaves affected by OS disease and K deficiency were obtained separately from two mature oil palm plots located approximately 10 km apart.

Frond number 21 was sampled systematically from thirty two oil palm trees that exhibited K deficiency symptom. Trees with K deficiency were identified based on visual symptom. Similarly, OS disease assessment was performed on frond number 21 among forty oil palm trees that exhibited bright orange lesions. An additional ten nonsymptomatic oil palm trees were sampled systematically based on the absence of OS disease or K deficiency symptoms. From each sampled frond, leaves were sub-sampled from three different sections, which served as observation replicates. Each section comprised ten leaf sub-samples. The sub-samples were subject to spectral reflectance acquisition, followed by leaf K analysis and CCCVd detection. OS disease severity assessment was conducted prior to leaf sampling from oil palm trees with OS disease symptoms.

OS disease severity assessment was conducted on forty oil palm trees based on visual OS disease symptom identification. The number of fronds with bright orange lesions and the total number of frond were recorded. The percentage of OS disease severity for each tree was calculated as follows:

$$\text{Disease severity } (\%) = \frac{\text{Number of fronds with orange spotting symptom}}{\text{Total number of fronds per palm}} \times 100$$

Spectral reflectance from the sampled oil palm leaves was obtained under controlled halogen lighting. A portable spectroradiometer (Model: ASD Fieldspec Pro®) was used to measure the spectral

reflectance from the adaxial part leaf number 21. The spectroradiometer, which was calibrated using a Teflon disk prior to each spectral measurement, was positioned 25 cm from the target with an effective field of view of 10 cm. The spectral wavelength ranged from 300 to 1050 nm, and was recorded at 9 nm intervals but interpolated to 1 nm interval using ViewSpec Pro Version 4.07. However, spectral reflectance at 300-399 nm and 1001-1050 nm were removed due to presence of noise in the data.

Leaf K content was determined using the dry ash method. 1 g of leaf sample was weighed into a porcelain crucible and placed in a muffle furnace. The furnace temperature was gradually increased up to 300°C. After an hour, the furnace temperature was raised to 500°C and maintained until the leaf sample turned into grayish white ash. The crucible containing grayish white ash was removed from furnace and left to cool and moistened with few drops of deionised water. Then, 2 ml of concentrated hydrochloric acid was added and heated on a hot plate. 10 ml of nitric acid (20 % v/v) was added into the ash and the crucible was placed in a water bath for 1 hr. The mixture was then transferred to a volumetric flask (100 ml). The solution was shaken and filtered with Whatman® Number 2 filter paper.

A total of forty leaf samples from OS-infected trees and ten from healthy trees were tested for CCCVd presence using the Dot-Blot method.

OS disease severity (%) and leaf K content (% DM) data were subject to descriptive statistical analysis. Separability of spectral reflectance between OS-infected leaves and K-deficient leaves was evaluated using Student's t-test. Both analyses were conducted using SPSS and MS Excel.

3. Results

CCCVd was detected in the all forty leaf samples from OS-infected oil palm trees. However, four out of ten leaf samples from nonsymptomatic trees showed CCCVd presence. As such, reflectance was acquired from six leaf samples which were free of CCCVd and deemed as healthy samples.

Descriptive statistics for leaf K and OS disease severity is given in Table 1. Leaf K values ranged from 0.45 to 0.98 % with mean of 0.73 % and coefficient of variance of 20 %. OS disease severity ranged from 6.25 to 73.08 % with mean of 40.45 % and coefficient of variance of 53 %. OS disease severity was clustered into four classes of ten observations due to the high coefficient of variance. Measured reflectances from OS-infected leaves were classed based on OS disease severity values which were 1-20 %, 21-40 %, 41-60 % and 60-80 %.

Table 1. Descriptive statistics of leaf K content and OS disease severity

	Leaf K content (% DM)	OS disease severity (%)
Number of samples (*n*)	32	40
Maximum	0.98	73.08
Minimum	0.45	6.25
Mean	0.73	40.45
Standard deviation	0.14	21.60
Coefficient of variance	20	53

Reflectance between OS-infected and K-deficient leaves was significantly different ($p < 0.05$) at 400-538 nm and 667-688 nm wavelengths (Figure 2). K-deficient leaves exhibited higher reflectance at this region than OS-infected leaves. Highest reflectance difference was found at 400 nm ($p < 0.01$) where reflectance of leaves exhibiting K deficiency is higher than OS disease. The reflectance of OS-infected and that of K-deficient leaves exhibited the green peak at 555 nm wavelength, with an average reflectance value of 0.15. Green peak is the wavelength exhibiting maximum reflectance in the green region of spectral reflectance from plant leaf. The reflectance of K-deficient leaves is higher than that of OS-infected leaves at 552 nm wavelength. A study conducted by Thomas and Gausman (1977) relating the spectral reflectance with leaf chlorophyll content in cotton showed that chlorophyll and carotenoid contents are best represented at 550 nm wavelength, which is close to the green peak value recorded from oil palm leaf. This indicates that K deficiency may have affected the leaves more than OS disease. At the visible red region, K-deficient leaves exhibited higher reflectance than that of OS-infected leaves at 667-688 nm wavelengths. Typically, chlorophyll *a* pigment triggers a significant absorbance value and is inversely proportional to reflectance values at the visible red region (Gitelson and Merzlyak, 1993). Therefore, OS-infected oil palm leaves may contain a higher count of chlorophyll *a* in comparison to K-deficient leaves. The infra-red region, however, did not show any significant difference between OS-infected and K-deficient leaf reflectance (Figure 2), possibly due to high standard deviation values corresponding to these wavelengths.

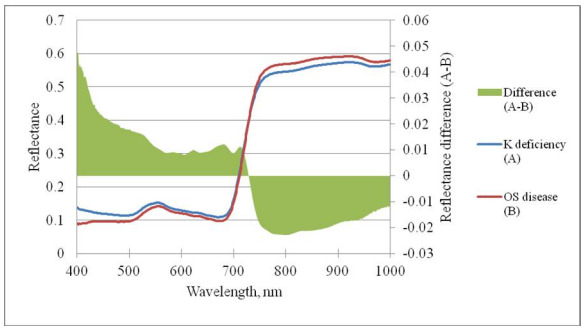


Figure 2. Comparison of spectral reflectance between OS-infected and K-deficient oil palm leaves

Reflectance of K-deficient leaves and that of healthy leaves were significantly different at 400-428 nm, 520-703 nm and 739-924 nm wavelength regions. In comparison to healthy leaves, K-deficient leaves showed a higher reflectance at the PAR region but lower reflectance at the NIR region. Typically, stressed leaves exhibit higher reflectance in the PAR region and lower reflectance at the NIR region (Björkman and Demmig-Adams, 1994). Our findings infer that K deficiency had inflicted stress on the affected leaves.

Reflectance of K-deficient leaves showed significant difference in comparison to reflectance of leaves across all four classes of OS disease severity (Table 2). At PAR region, reflectance comparison between leaves with K deficiency and 1-20 % of OS disease severity had the least significant range which was 400-408 nm wavelengths. A broad range of significant difference in leaf reflectance at this region was registered between K-deficient leaves and leaves with 21-40 %, 41-60 % and 61-80 % of OS diseases severity. At NIR region, reflectance from leaves with 1-20 %, 21-40 % and 41-60 % of OS disease severity was significant in comparison to leaf reflectance of K-deficient leaves. However, reflectance comparison of K-deficient and 61-80 % OS-infected leaves did not significant difference at this region.

Table 2. Wavelengths that significantly discriminates between K-deficient leaves and OS disease severity

OS disease severity (%)	*Discriminative wavelengths (nm)
1-20	400-408, 734-1000
21-40	400-538, 673-790, 699-711
41-60	400-725
61-80	400-450

(*Significant at $p < 0.05$; two-tailed)

Reflectance at the PAR region from leaves with 1-40 % OS disease severity was higher compared to that of K-deficient leaves. Therefore, PAR region can be used to discriminate between OS-infected and K-deficient oil palm trees. Reflectance from leaves with 1-20 % OS disease severity is significantly higher than that of all other classes at the infra-red region (Figure 3). The reflectance in the infra-red region is mainly dominated by leaf internal structure, leaf anatomy and characteristics of the epidermal surface (Jensen, 2002).

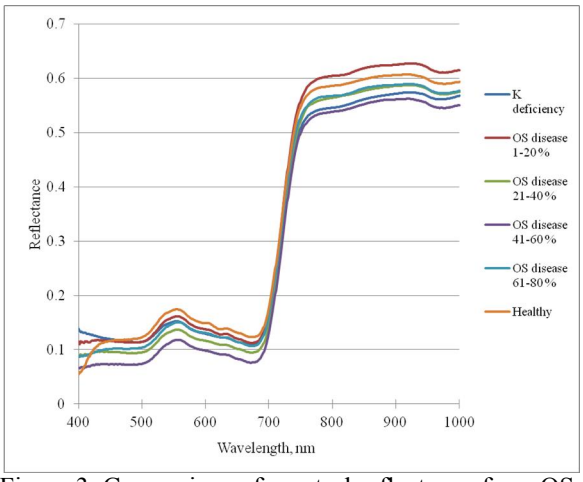


Figure 3. Comparison of spectral reflectance from OS-infected (four classes of disease severity), K-deficient and healthy leaves

4. Discussions

This work shows that K-deficient leaves could be discriminated from healthy leaves using spectral reflectance. K-deficient leaves also could be discriminated from the four classes of OS-infected leaves. PAR region is suitable to be used for the discrimination between symptomatic OS-infected and K-deficient leaves. As such, spectral discrimination of K deficiency and OS disease leaf symptoms could aid plantation management in disease identification to curb stress factor or to eliminate source point of infection. This study was focused on a mature oil palm stand. We suggest further work to investigate the separability of both these disorders using multispectral reflectance. Such an investigation should consider the growth stages of oil palm, i.e. 1-9 months after seeding (nursery stage) and 1-6 years after field planting (immature stage).

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