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Foliar Spectral Signature Analysis of Three Pepper (*Piper nigrum* L.) Cultivars in Malaysia

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ABSTRACT

Pepper (*Piper nigrum* L.) is an important cash crop for smallholders in the state of Sarawak, Malaysia. At present, there are more than eight pepper cultivars being planted in the state since the 1950s. In this study, the unique spectral signatures of three high yielding pepper cultivars in Malaysia were examined using a ground-based spectral sensor to detect varietal differences. Concurrent foliar spectral signatures, chlorophyll concentration, and Normalized Difference Vegetation Index (NDVI) of cultivar Kuching, Semongok Aman and Semongok Emas were recorded under normal field conditions. Foliar chlorophyll concentration and NDVI results showed that all the matured pepper vines under this assessment were in healthy condition. From a rough observation, except for a slight increase in the visible (VIS) region of the spectrum (500 to 699 nm) for cultivar Semongok Aman and Semongok Emas, spectral reflectance of all three pepper cultivars were similar in the near-infrared region (700 to 1000 nm). The results for First Derivative Curves analysis indicated that the 684.5 to 756.5 nm region showed the highest separation followed by region with the second lowest separation (504.5 to 540.5 nm). The highest value was obtained in the Red Edge Position (REP) for cultivar Kuching, Semongok Aman and Semongok Emas which were 709, 706 and 710, respectively. The highest value obtained in the VIS region for cultivar Kuching, Semongok Aman and Semongok Emas were 523, 524 and 527, respectively. These fundamental findings suggested that it is possible to discriminate pepper cultivars through field spectroscopy and first derivative analysis. In addition, this information may be used for further studies related to precision agriculture particularly in integrated nutrient and disease management.

Keywords: First derivative, *Piper nigrum*, red edge position, spectral signature, variety discrimination.

INTRODUCTION

There is a growing interest in precision agriculture and the recent advancement in hyperspectral technologies is driving accurate vegetation studies. Precision agriculture tools for application are particularly essential in making the right decisions in day-to-day farm management so interventions can be implemented to maximise profits. It is also used in the pest and disease management approach in which the crop health status can be made known accurately to prevent significant crop loss (Bazzichetto et al., 2018). Foliar spectral signature analysis can play an important role in the early detection and quantification of vegetation cover (Martin et al., 2018) and is now recognised as an important tool for agriculturalists and land managers in understanding and managing many crop issues (Bazzichetto et al., 2018). For instance, the spectral diversity of plant cultivars represents the variations existing in spectral patterns that can be detected by spectroradiometers (Wang and Gamon, 2019). Studies have shown that each plant cultivar has a unique spectral reflectance due to dissimilar physical and biochemical characteristics that ultimately help to facilitate varieties-level identification (Matongera et al., 2017). Apart from varietal identification, foliar spectral signature analysis can also assist in issues related to crop disease and nutrient management.

Black pepper (*Piper nigrum* L.), which will simply be referred to as pepper in the rest of this paper, is a significant economic crop to smallholders in Sarawak, Malaysia. In the year 2021, Malaysia has produced and exported approximately 7,000 metric tonnes of pepper valued at RM153.75 million thus highlighting its importance as an important cash crop (Malaysian Pepper Board, 2023). This study focused on the three main pepper cultivars in Malaysia namely Kuching, Semongok Aman and Semongok Emas, which are recommended by the Malaysian Pepper Board because of their high yielding traits as well as their ability to produce different quality of black and white peppercorns (Chen et al., 2018). This analysis is important as it has strong implications for monitoring pepper health based on varietal differences by using precision agriculture techniques. Therefore, the goal of this study was to examine the unique spectral signature of important pepper cultivars in Malaysia and to observe whether these cultivars can be differentiated using a ground-based spectral sensor. Subsequently, this information will be implemented for the upscaling of hyperspectral measurements to airborne sensors and could provide a reliable estimation of the overall farm condition through mapping inside the targeted areas and could also help in diagnosing disease and nutritional problems associated with pepper of different varieties.

MATERIALS AND METHODS

Study Site and Plant Materials

The experiment was conducted at a pepper farm located in Padawan, Sarawak, Malaysia (1°19'42" N, 110°14'39" E). The soil series at the study site was Bemang Series. Bemang series is an alluvial soil characterised by fine loamy/silty, siliceous/mixed, acidic, isohyperthermic, *Typic Dystruptes*, and alluvium from sedimentary rocks (Paramanathan, 2000). The soil profile generally has a friable consistence throughout and is well to moderately well-drained. The climate is tropical, moderately hot with average annual rainfall around 3500 to 4000 mm. The temperature in Padawan ranges from 20 to 36 °C with an average temperature around 23 °C in the early hours of the morning and rises to around 32 °C in the mid-afternoon (Sarawak Tourism Board, 2021). Three pepper cultivars (Figure 1) namely *P. nigrum* var. Kuching (Kuching), Semongok Aman (S. Aman) and Semongok Emas (S. Emas) under natural field conditions were randomly selected and assessed. The unique leaf characteristics of these varieties are shown in Table 1. The mature leaves of 8 replicates from each cultivar were used as samples. This gave a total of 24 leaves assessed in this study. The duration of the study was from February to July 2022.

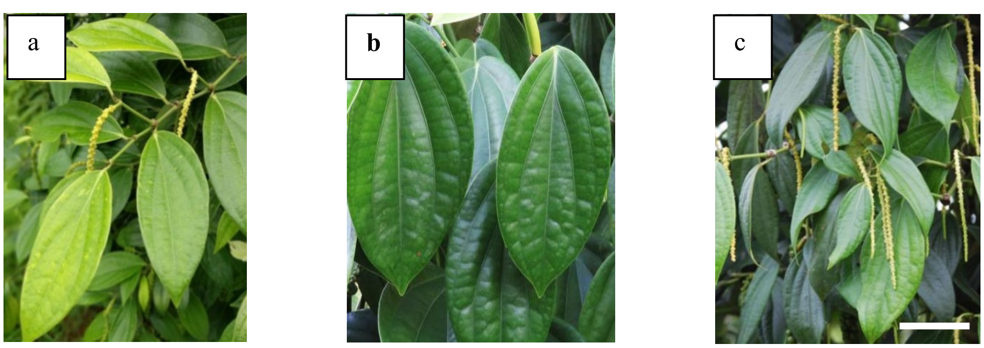

Figure 1. Matured leaves from three pepper varieties namely Kuching (a), Semongok Aman (b), and Semongok Emas (c). Scale bar = 6 cm.

Table 1. Leaf characteristics of three main pepper varieties in Malaysia (Chen et al., 2018)

Variety	Shape	Upper surface relief	Length:width ratio
Kuching	Ovate-lanceolate	Smooth	2.02
Semongok Aman	Ovate	Strongly raised between the main veins	1.70
Semongok Emas	Ovate-lanceolate	Mildly raised between the main veins	2.35

Pepper Chlorophyll Concentration and NDVI Measurement

As an indicator of the plant health status, measurements of the foliar chlorophyll concentrations and Normalized Difference Vegetative Index (NDVI) were obtained. The NDVI was recorded using an NDVI meter (CM1000, Spectrum, FieldScout, USA). Foliar chlorophyll content of pepper leaves was determined using a chlorophyll meter (MC-100, Apogee, USA). Readings were recorded when mature fully expanded leaves with the same orientation and the same layer in the crown (middle bottom) were still attached to the tree.

Pepper Foliar Spectral Reflectance Sampling

Following the method by Prospere et al. (2014), spectral reflectance measurements at wavelengths from 325 to 1075 nm were done using a spectroradiometer (PS-300, Apogee, Utah, U.S.A.) attached to an external single-leaf clip and contact probe device (ASD, Longmont, Colorado, USA). Reflectance data were measured on mature fully expanded leaves with the same orientation and the same layer in the crown (middle bottom) that were still attached to the tree.

Statistical Analysis

Values from foliar chlorophyll concentration and NDVI as well as specific wavelength values within the spectral reflectance regions were analysed using one-way analysis of variance (ANOVA) with statistical analysis software (SPSS version 15, Chicago, Illinois, USA). The Tukey's Honest Significance Difference (HSD) Test, at $\alpha = 0.05$ level of significance was done to compare the means and to determine differences among treatments.

Spectral Data Processing and Analysis

First, the spectral data were transformed into Excel format in individual files (Figure 2). Collected spectral data were averaged to obtain a consistent spectral feature based on the cultivars. The Unscrambler X chemometric program (Version 10.4, CAMO Software, Oslo, Norway) was used to produce spectral curves. Before spectral curves were produced, data were processed using the Multiplicative Scatter Correction and the second derivative Savitzky-Golay smoothing method. This is to suppress background noise, accentuate individual reflectance features and resolve the over-lapping spectral features. To detect the important wavelength regions, the mean first derivative curves for each pepper cultivar spectral data were analysed using Microsoft Excel (Office 2016, Redmond, Washington, USA) as shown in Figure 2.

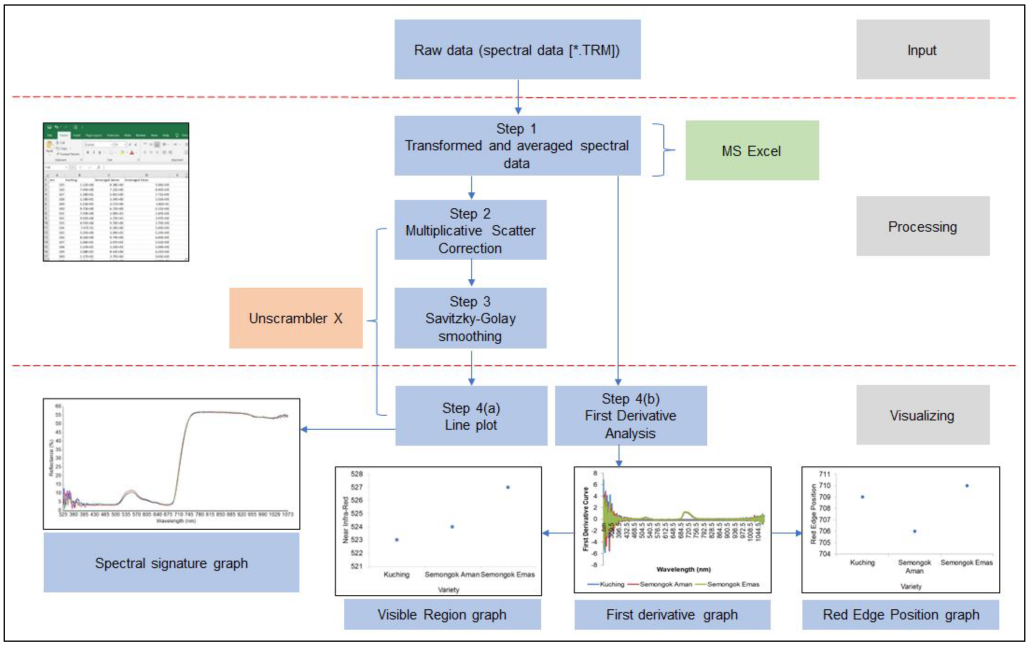

Figure 2. Spectral data processing and visualisation

Principal Component Analysis

A more in-depth analysis was made to study whether there were distinct differences among pepper cultivars at both the visible (VIS) and Red Edge Position (REP) regions. Principal Component Analysis (PCA) was performed on pepper foliar spectral reflectance curves at the VIS and RED Edge regions using The Unscrambler X programme. The varimax orthogonal rotation and cross validation methods were used to determine the number of components.

RESULTS AND DISCUSSION

Selected Physiological Characteristics of Pepper

The foliar chlorophyll concentration and NDVI of healthy mature pepper from different varieties are shown in Table 2. The results shown were consistent and typical of a healthy and mature plant including pepper. These results gave additional insights into understanding and confirmation of the plant's health status. Prospere et al. (2014) mentioned that obtaining knowledge of the plant foliar chlorophyll concentration and NDVI were particularly important in studies related to species or varietal discrimination especially when using the spectroradiometer instrument.

The spectral signature profiles for the three main pepper cultivars after necessary pre-processing are visualised graphically in Figure 3. The mean spectral reflectance of these cultivars was depicted from the 325 to 1075 nm wavelength region. It was found that all three cultivars gave almost similar spectral signatures. A slight increase in *P. nigrum* var. Semongok Aman reflectance was detected in the VIS region starting from 510 to 626 nm. Meanwhile, the reflectance for *P. nigrum* var. Semongok Emas demonstrated a slight increase from the 627 to 692 nm region. The slight increase of reflectance in these two cultivars might be associated with the morphological characteristics of the upper surface leaves relief as presented in Table 1. A study by Klančnik and Gaberščik (2015) documented that leaf thickness was among the most important morphological traits which explained the spectrum variability at the VIS regions for each plant cultivar. Results in Figure 3 showed that the spectral reflectance for the three pepper cultivars was similar in the near-infrared region, which is from 700 to 1000 nm. Therefore, a more in-depth spectral analysis is needed to identify the optimal wavelengths as the general rough observation of spectral reflectance signatures was not able to distinguish the differences among varieties.

Table 2. The foliar chlorophyll concentration and Normalised Difference Vegetation Index of healthy mature pepper varieties

Variety	Foliar chlorophyll concentration (µmol/m^2 of chlorophyll)	Normalized Difference Vegetation Index (NDVI)
Kuching	630.68 ± 17.45^a	0.92 ± 0.05^a
Semongok Aman	627.82 ± 19.56^a	0.91 ± 0.03^a
Semongok Emas	639.15 ± 34.05^a	0.89 ± 0.09^a

Note: Mean ± S.D., n = 24. Means with the same letter within columns are not statistically different using Tukey's at P > 0.05 probability level

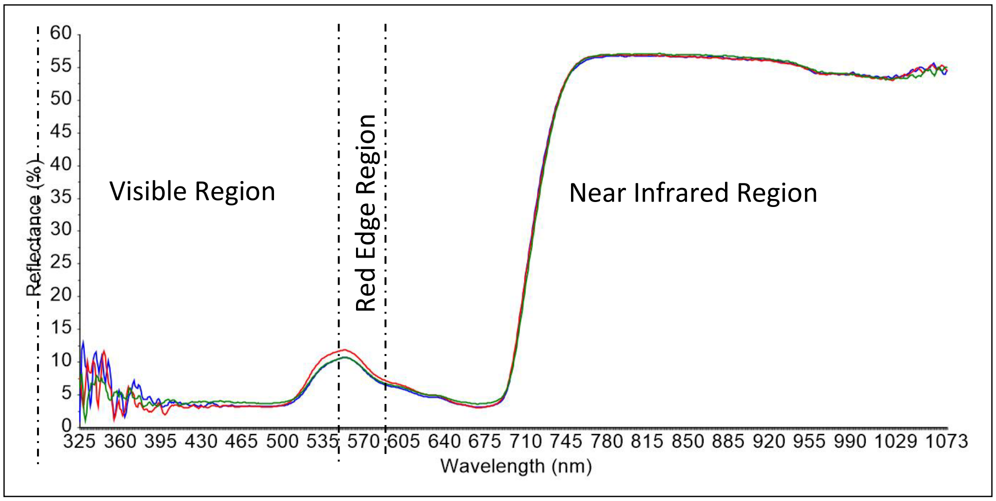

Figure 3. Mean spectral reflectance of *P. nigrum* leaves under field condition at wavelengths ranging from 325 to 1075 nm. The varieties were Kuching (■), Semongok Aman (■) and Semongok Emas (■).

Results from the first derivative analysis curves showed two increasing peak areas between 325 to 1075 nm wavelengths (Figure 4). These peaks were detected at the VIS and REP regions. The maximum value of the first derivative for wavelengths in the VIS region was between 505 to 545 nm whereas in the REP region, the highest peak was between 680 to 750 nm. Extraction of the VIS and REP is one of the simpler curve fitting techniques and is sensitive to chlorophyll concentration changes as well as leaf mass, surface thickness and structure which may be an important tool to differentiate pepper varieties (Sims and Gamon, 2002). Calamita et al. (2021) reported that both the VIS and NIR spectra are associated with internal leaf structures and their reflective scattering is principally due to the air in leaf cell walls and to the differences in leaf cellular constituents.

The bands derived from the maximum peaks of the derivative curve for each pepper cultivar are depicted in Table 3. This result suggested that the regions between 523 to 527 and 709 to 723 nm are important spectral wavelengths that can be further analysed to discriminate among pepper cultivars. Awad et al. (2019) stated that the range from 700 to 750 nm (Red Edge) was always significant for species discrimination. Prospere et al. (2014) mentioned that the Red Edge is the transition between the strong absorbance in the red region by chlorophyll pigment and the relatively high reflectivity in the NIR region attributed to the scattering effect caused by the internal leaf structure.

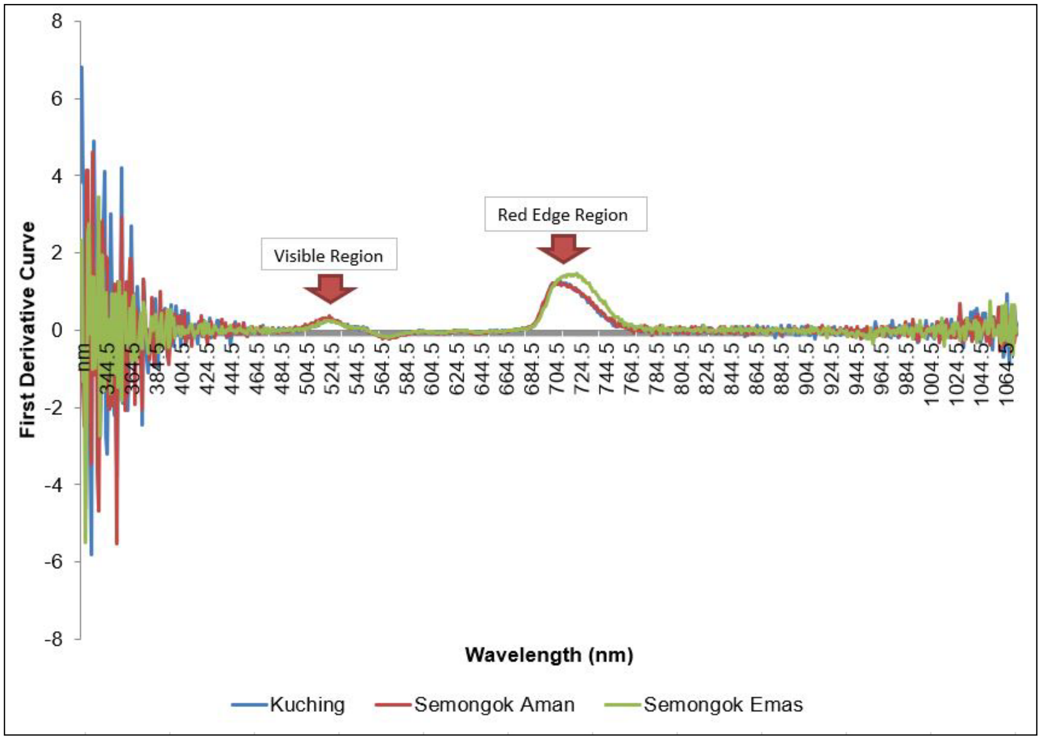

Figure 4. First derivative curves of pepper cultivars under field condition

Table 3. Peak bands for the mean pepper variety derivative curves at different region

Variety	Region	
	VIS (nm)	REP (nm)
Kuching	523	709
Semongok Aman	524	706
Semongok Emas	527	723

Results from the pepper variety comparison using PCA at the VIS region (523 to 527 nm) is shown in Figure 5. It was observed that at the 523, 524 and 527 nm bands, there were three distinct clusters representing the three main pepper varieties. The Kuching variety cluster was found to be in between the Semongok Emas and Semongok Aman clusters. This result showed that at the VIS regions of 523 to 527 nm, the Semongok Emas and Semongok Aman varieties can be clearly differentiated whereas the Kuching variety can be similar to either one of the aforementioned varieties.

Further analysis was done with the Tukey's Honest Significance Difference (HSD) Test, at $\alpha = 0.05$ level of significance to determine differences among pepper foliar reflectance bands of the VIS region (523, 524, and 527 nm). The peak reflectance bands result in Table 4 showed that the Semongok Aman has the highest values with 814 at 523 nm, 8.57 at 524 nm and 9.58 at 527 nm. These were followed by Kuching with 6.85 at 523 nm, 7.17 at 524 nm and 8.06 at 527 nm. Meanwhile the lowest values were for Semongok Emas with 6.67 at 523 nm, 6.90 at 524 nm and 7.55 at 527 nm. Statistical comparison result for the three pepper varieties showed that Semongok Aman was significantly different from both Kuching and Semongok Emas in the 523, 524 and 527 nm bands. There were no significant differences detected when comparing the Kuching and Semongok Emas varieties in the 523, 524 and 527 nm bands.

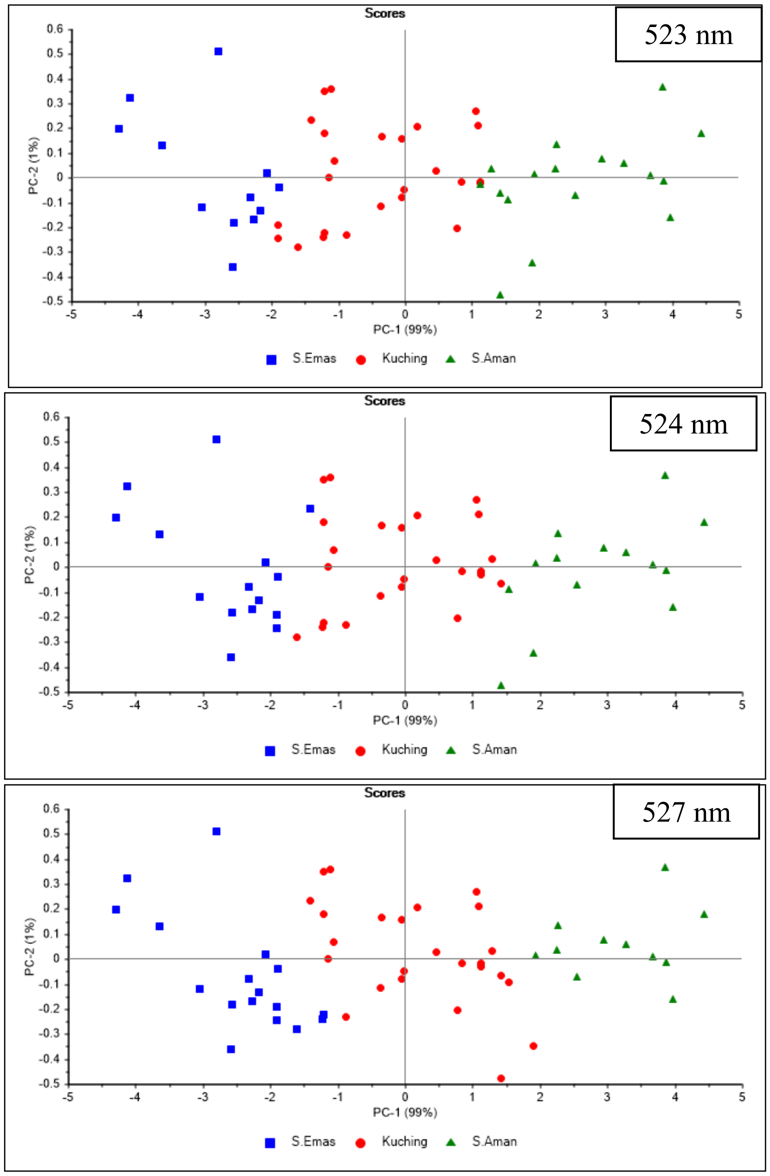

Figure 5. Variety comparison using the Principal Component Analysis (PCA) at the VIS region of 523, 524 and 527 nm

Table 4. VIS region peak reflectance bands of different pepper varieties

Variety	Peak reflectance bands (nm)		
	523	524	527
Kuching	6.85 ± 0.99^{b}	7.17 ± 1.04^{b}	8.06 ± 1.29^{b}
Semongok Aman	8.14 ± 0.70^{a}	8.57 ± 0.79^{a}	9.58 ± 0.91^{a}
Semongok Emas	6.67 ± 0.68^{b}	6.90 ± 0.73^{b}	7.55 ± 0.97^{b}

Note: Pepper vines under field conditions were randomly selected and assessed in this research (mean + S.D., n = 56). Figures with same letter superscript within columns are not statistically different using Tukey's at P > 0.05 probability level. The samples were Kuching (mature *Piper nigrum* var. Kuching), Semongok Aman (mature *Piper nigrum* var. Semongok Aman) and Semongok Emas (mature *Piper nigrum* var. Semongok Emas).

The PCA result for the three main pepper varieties at the REP regions of 706, 709, and 723 nm is depicted in Figure 6. It was observed that at the 706, 709 and 723 nm bands, there were three distinct clusters representing the three main pepper varieties. The Semongok Aman variety cluster was found to be in between the Semongok Emas and Kuching clusters. This result showed that at the Red Edge regions of 706 to 723 nm, the Semongok Emas and Kuching varieties can be clearly differentiated whereas the Semongok Aman variety can be similar to either one of the aforementioned varieties.

Further analysis was done with the Tukey's Honest Significance Difference (HSD) Test, at $\alpha = 0.05$ level of significance to determine differences among pepper foliar reflectance bands of the Red Edge region (706, 709 and 723 nm). The peak reflectance bands result in Table 5 showed that for band 706, the Semongok Aman has the highest value (20.30), followed by Kuching (20.07) and Semongok Emas (16.11). For bands 709 and 723, the Kuching variety demonstrated the highest values of 25.96 and 43.30, respectivley, followed by Semongok Aman with 25.80 and 42.66, respectively, and Semongok Emas with 20.81 and 36.91, respectively. Statistical comparison result for the three pepper varieties showed that Semongok Emas is significantly different from both Kuching and Semongok Aman in the 706, 709 and 723 nm bands. There were no significant differences detected when comparing the Kuching and Semongok Aman varieties in the 706, 709 and 723 nm bands.

The reflectance bands in the VIS and NIR particularly the Red regions are commonly identified as key wavelengths in several studies. Due to the unique spectral signature and reflectance of plant cultivars in the same species, varietal discrimination can be made through spectral reflectance, which captures data in hundreds of narrow bands, and has shown promising results after performing PCA (Roslin et al., 2021). Roslin et al. (2021) added that all plant spectral signatures were very similar, and many overlapped species and varietal differences show up at specific wavelengths. For example, in the visible spectrum (450 nm to 700 nm) and the infrared region (700 nm to 990 nm), the spectra of different species and cultivars are separated by their different wavelengths. In addition, similar observation by Abdulridha et al. (2016) mentioned that the leaf pigments, cell structure and water content are different for each plant species cultivars. Based on the differences in the plant cell structures which give unique spectral reflectance, plant varieties can be differentiated.

Table 5. Red Edge region peak reflectance bands of different pepper varieties

Variety	Peak reflectance bands (nm)		
	706	709	723
Kuching	20.07 ± 2.90^a	25.96 ± 3.29^a	43.30 ± 3.10^a
Semongok Aman	20.30 ± 2.63^a	25.80 ± 2.88^a	42.66 ± 2.90^a
Semongok Emas	16.11 ± 4.06^b	20.81 ± 5.16^b	36.91 ± 6.36^b

Note: Pepper vines under field conditions were randomly selected and assessed in this research (mean ± S.D., n = 56). Figures with same letter superscript within columns are not statistically different using Tukey's at P > 0.05 probability level. Treatments are Kuching – mature *Piper nigrum* var. Kuching, Semongok Aman – mature *Piper nigrum* var. Semongok Aman, and Semongok Emas – mature *Piper nigrum* var. Semongok Emas

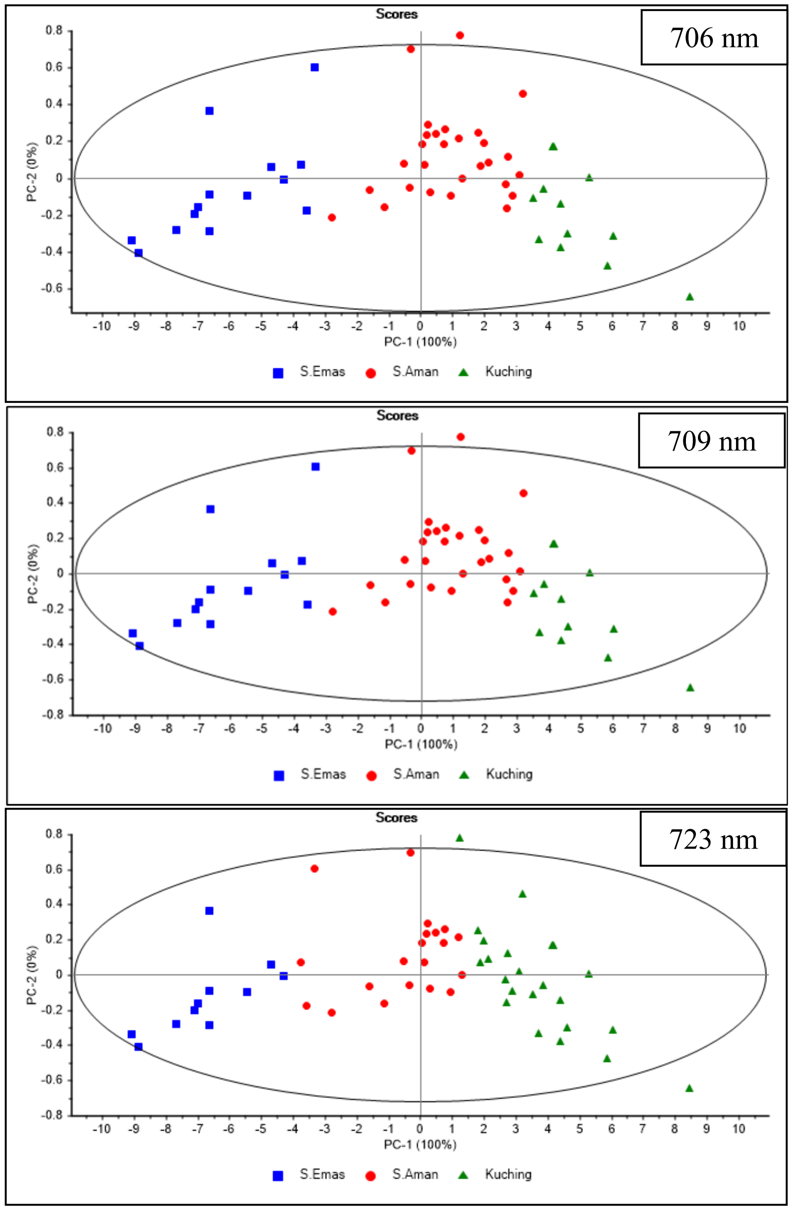

Figure 6. Variety comparison using the Principal Component Analysis (PCA) at the Red Edge region of 706, 709 and 723 nm

CONCLUSIONS

Our study showed that the first derivative curve analysis detected two regions for pepper variety discrimination at the VIS region (523 to 527 nm) and the Red Edge region (706 o 723 nm). The bands in the VIS region where maximum reflectance values were recorded for Kuching, Semongok Aman and Semongok Emas varieties were 523, 524 and 527 nm, respectively, and the bands in the Red Edge region where maximum reflectance values were recorded for Kuching, Semongok Aman and Semongok Emas varieties were 706, 709 and 723 nm, respectively. In the VIS region (523, 524, 527 nm), Semongok Aman recorded the highest reflectance values, followed by Kuching and Semongok Emas, and in the Red Edge region (706 nm), Semongok Aman recorded the highest reflectance values, followed by Kuching and Semongok Emas. On the other hand, in the Red Edge regions (709, 723 nm), Kuching recorded the highest reflectance values, followed by Semongok Aman and Semongok Emas. In the VIS region (523 to 527 nm), the Semongok Emas and Semongok Aman varieties can be clearly differentiated whereas the Kuching variety is similar to Semongok Emas, while in the Red Edge region (706 to 723 nm), the Semongok Emas and Kuching varieties can be clearly differentiated whereas the Kuching variety is similar to Semongok Aman. Our findings suggested that it is possible to identify and discriminate pepper cultivars through field spectroscopy and first derivative analysis for future monitoring in integrated plant nutrient and disease management. The fundamental result from this study will assist in identifying diseases and nutritional problems associated with pepper based on varieties as well as recognising pepper varieties at a larger scale using air-borne hyperspectral sensors. Further study may include a more in-depth multivariate data analysis to differentiate disease and nutrient related problems that occur among the three main pepper cultivars.

AUTHORS CONTRIBUTION

KMTS, ATHG, KPE, CYS and WCM performed and completed the experiments. KMTS, ATHG, KPE, ZJAM and WCM acquired and/or interpreted the data and KMTS, AOH and AS performed the statistical analysis. KMTS drafted the manuscript. Each author participated in the critical revision and final approval of the manuscript.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this paper.

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