UTILIZATION OF HYPERSPECTRAL DATA AND MACHINE LEARNING ALGRORITHMS FOR ESTIMATING CHLOROPHYLL CONTENTS IN WASABI LEAVES

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ABSTRACT: Chlorophyll content is effective indicator of photosynthesis and then it can be indicative of plant physiological activity. Nowadays, Hyperspectral remote sensing data are having been used for evaluating chlorophyll content. Especially, it has been shown that combining hyperspectral data and machine learning algorithms could be more effective to evaluate vegetation properties. The wasabi (*Eutrema japonicum*) plants were cultivated individually in Wagner plot (1/5000 a) containing 3L of tap water adjusted to a pH of 6.0 using HCl and NaOH. The 7 different treatments were applied including control, 0N, 2N, 0P, 2P, 0K, 2K, 0.5S, and 2S. A total of 100 wasabi leaves were sampled from plant tops among expanding leaves and reflectance was measured using Fieldspec4. The regression models were generated base on Random Forest (RF) machine learning algorithm using Original Reflectance (OR) and 5 preprocessing methods including First Derivative Reflectance (FDR), Continuum Removed (CR), Detrending (DT), Standard Normal Variate (SNV), and Multiplicative Scatter Correction (MSC) for estimating chlorophyll contents from reflectance. The objectives of this study were to examine the potential hyperspectral remote sensing approaches and to identify an optimized preprocessing technique for estimating chlorophyll content. The results indicate that all preprocessing technique were effective for improving estimation accuracies. Based on the ratio of performance of deviation (RPD), SNV was the best preprocessing technique with the RPD value of 2.48.

1. INTRODUCTION

Wasabi (*Eutreme japonivum*) belongs to Brassicaceae family and has white flowers with 4 petals. The grown-out plant can reach up to 30-50 cm in height. Wasabi has been cultivated in Japan for more than a thousand years, and nearly half of total wasabi rhizome consumed in Japan is produced in the Shizuoka prefecture (Hege, 2019). There has been a recent increase in global demand for Japanese cuisine, leading to an increased demand for wasabi production. Wasabi requires specific growing conditions including north-facing gorges and an abundance of cold and clean flowing water. It takes at least 10 months to cultivate wasabi, and recent environmental climate changes have adversely affected wasabi production. Wasabi seeds are small, about 2-3 mm long and 1 mm wide. In addition, optimal culture methods are poorly understood and its production depends on the experience of skilled farmers. Enhancing detection of nutritional and environmental stresses as well as diseases that result in lower yields may improve wasabi cultivation and facilitate its production by incipient farmers. Chlorophyll content can be indicative of plant physiological activity since it is an effective indicator of photosynthesis, since chlorophyll absorbs sunlight and uses the energy to synthesize carbohydrates from CO_2 and H_2O (Gitelson, 2006) and then chlorophyll within the leaf also has a close relationship with nitrogen, an essential plant nutrient (Bojovic, 2009). Thus, chlorophyll contents are powerful for detecting disease as well as nutritional and environmental stresses on plants (Datt, 1999, Sonobe, 2020a, 2020b). To investigations of chlorophyll content can be precisely using spectroscopic techniques such as ultraviolet and visible-light (UV-Vis) spectroscopy and high-performance liquid chromatography (HPLC) (Sonobe, 2021). However, these techniques are costly, labor-intensive and bulky equipment (Kalaji, 2017). On the other hand, leaf chlorophyll content and leaf dry weight are related to leaf reflectance over different spectral regions and several researches proposed methods for simultaneous determination of them (Feret, 2008, 2011). Also, this issue may be addressed using hyperspectral remote sensing an alternative tool for measuring chlorophyll content in the field (Amiruddin, 2020, Feret, 2008, Golhani, 2020, Vahtmae, 2020). To improving the accuracy of chlorophyll estimation, methods for removing noise and correcting the slope or base shift from the spectra are needed. Applying pre-processing techniques is way to identify vegetation characteristics from hyperspectral reflectance (Adenan, 2021). First derivative reflectance (FDR) analysis has been widely used to identify vegetation properties from reflectance data; derivative spectral indices outperform conventional broad-band spectral indices like the near-infrared / red reflectance ratio (Demetriadesshah, 1990). Continuum-removal (CR) transformation is a normalization technique that improves the statistical distinction between vegetation types in the visible spectrum at the cost of blurring the differences of the spectra near 400 nm and in the near-infrared part of the spectrum (Schmidt, 2003). Standard normal variates (SNV) and multiplicative scatter correction (MSC) are effective for reducing the noise or baseline shift in the raw reflectance data that is caused by light scattering (Liang, 2020). De-trending (DT) is also used to eliminate the

effect of additive interference of scattered light from particles (Barnes, 1989). Thus, this study evaluates the performance and suitability of five machine learning algorithms in conjunction with five pre-processing techniques for analyzing the original reflectance data obtained from wasabi leaves.

Regression techniques based on machine learning algorithms are becoming an appealing approach for quantifying vegetation properties such as chlorophyll content from remotely sensed data (Biau, 2016). The advantages of machine learning algorithms include their ability to solve large nonlinear problems autonomously using datasets from multiple variables, and to implement a great resilient framework not only for data-driven decision making, but also for the establishment of expert knowledge in the algorithm. Random forest (RF) algorithm has been successfully applied for both classification and regression to estimation chlorophyll contents in leaves. The main objectives of this study were to evaluate the potential of hyperspectral remote sensing data for chlorophyll contents estimation in wasabi leaves and to evaluate the best pre-processing method for reducing noise.

2. DATA AND METHODS

2.1 Measurements

The wasabi clonals plants were cultivated individually in Wagner pots (1/5000 a) containing 3 L of tap water adjusted to a pH of 6.0 using HCl and NaOH in Shizuoka University. After 1 week, slightly modified 0.1x solutions of Hoagland (Hoaglands.XXX), which is one of the most popular solution compositions for growing plants and contains macronutrients of 0.25 mM KNO_3, 0.25 mM Ca $(NO_3)24H_2O$, 0.375 mM $(NH_4)2SO_4$, 0.2 mM $MgSO_47H_2O$, 0.2 mM $NaH2PO_42H_2O$, 0.25 mM KCl, and 0.25 mM $CaCl_22H_2O$, and micronutrients of 5 µM EDTA–Fe (III), 2.5 µM H_3BO_3, 0.2 µM $MnSO_45H_2O$, 0.2 µM $ZnSO_47H_2O$, 0.05 µM $CuSO_45H_2O$, and 0.05 µM $Na_2MoO_42H_2O$ and 0.58 mM SO_4^{2-}(sulfate-ion), were supplied stepwise for 1 week each at 1/100 and 1/10 strengths to adapt the plants to the hydroponic system under standard nutrient solution conditions. The SO_4^{2-}, K^+, and Ca^{2+} concentration was adjusted using K_2SO_4 and $CaSO_42H_2O$.

Table 1. Growing conditions of Wasabi plants

Treatment	N	S	K	S
Control	1 (1.5 mM)	1 (0.2 mM)	1 (0.5 mM)	1(0.58 mM)
0 N	0	1	1	1
2 N	2	1	1	1
0 P	1	0	1	1
2 P	1	2	1	1
0 K	1	1	0	1
2 K	1	1	2	1
0 S	1	1	1	0
0.5 S	1	1	1	0.5
2 S	1	1	1	2

The FieldSpec4 was used to obtain hyperspectral reflectance in 1 nm steps across the entire wavelength domain from 400 to 2500 nm from a leaf clipping (Malvern Panalytical, Almelo, Netherlands). A splice correction function implemented in ViewSpec Pro (Analytical Spectral Devices Inc., USA) was used to reduce the inconsistency caused by the three detectors: visible and near–infrared (VNIR) portions of the electromagnetic spectrum, short wave infrared ($SWIR_1$) and short-wave infrared ($SWIR_2$). Nir–infrared (VNIR) portions of the electromagnetic spectrum, short wave infrared ($SWIR_1$) and short-wave infrared ($SWIR_2$) portions of the electromagnetic spectrum. To determine which wavelengths differed significantly in relation to slag fertilization ($p < 0.05$), a stepwise linear discriminant analysis was performed in a multiple regression model using a combination of forward and backward stepwise regressions (Draper, 1998). A dual–beam scanning ultraviolet–visible spectrophotometer (UV–1900, Shimadzu, Japan) was used to precisely measure chlorophyll content (Chla+b), then Porra's method (1989) was applied. Dimethyl-formamide was used to prepare extracts. The below equations (1 to 3) were used to calculate chlorophyll–a (Chl–a) and b (Chl–b) content (in µg ml^{-1}). Finally, units were converted to µg cm^{-2} using the area of leaf discs, since leaf optical properties are sensitive to chemistry in terms of quantity per surface area.

$$\text{Chl–a (µg ml}^{-1}) = 12.00 \times (A_{663.8} - A_{750}) - 3.11 \times (A_{646.8} - A_{750}) \quad (1)$$

$$\text{Chl–b (µg ml}^{-1}) = 20.78 \times (A_{646.8} - A_{750}) - 4.88 \times (A_{663.8} - A_{750}) \quad (2)$$

$$\text{Chla+b} = \text{Chl–a} + \text{Chl–b} \quad (3)$$

where A is the absorbance, and the subscripts are the wavelengths (in nm).

2.2 Data Analysis

The measurements were divided into three groups (a training dataset (50%), a validation dataset (25%) and a test data dataset (25%)) using a stratified sampling approach. To ensure the robust results, this approach was repeated 100 times before generating the regression models. At each iteration, the genetic algorithm (GA) based approach was applied to select effective wavelengths. RF regression generates multiple classification and regression trees (CART) based on randomly bootstrapped samples of training data (Breiman, 2001) by generalizing the binomial variance and by nodes that use a split variable from a group randomly selected variable. Because the previous study described the effectiveness of RF (Sonobe, 2018), it was also used in this study. RF differs from CART in growing non-deterministically to decorrelation the trees and lessen variance using two-stage randomization scheme related to a bootstrap sample and random variable selection (Nofrizal, 2021). The number of trees (ntree) and the number of variables used to split the nodes (mtry) are normally defined by the user. For tuning these hyperparameters, Bayesian optimization was applied using the Gaussian process. The processes were conducted using the statistical software R version 3.5.5.

2.3 Pre-processing techniques

2.3.1 First Derivative reflectance (FDR)

FDR is an effective technique for removing background effects and enhancing subtle spectral features as well as enhancing weak spectral features which are effective for evaluating target parameters (Meng, 2020). FDR has been applied to enhance specific points such as the green peak and the red edge inflection point (REIP) (Cho,2006).

2.3.2 Continuum removed (CR)

According to (Clark, 1984), CR consist for removing the continuous features of spectra and is often used to isolate specific absorption features present in the spectrum to minimize the noisy partially. The continuum is represented by a mathematical function used to separate and highlight specific absorption bands of reflectance spectrum (Mutanga, 2004). CR allowed the normalization of the spectra of the spectra and thereby facilitated the identification of significant absorption features that ranged across the vis-NIR spectrum. CR were performed in R programming language software.

2.3.3 Standard normal variated (SNV)

SNV correction is able to correct multiplicative scattering noises (such as the influence of sample size and scattering interference) caused by the surface structure of the sample. This scatter correction is a row-oriented transformation which standardizes the spectra using its mean and standard deviation manually. The process is started by calculating the mean and standard deviation for each i spectrum of m x 1 column vector xi. Then subtracts every data point of xij with the mean and divides it with the standard deviation, or mathematically simply as

$$x_{ij\ (SNV)} = x_{ij\ (SNV)} = (x_{ij} - \bar{x_i})/s(x_i) \quad (4)$$

However, because SNV parameter estimation does not use least square fitting, the method is extremely sensitive to any noisy points in the spectrum. As a result, SNV pre-treatment can have negative effects when a high number of noisy points are found in the spectrum (Barnes, 1984).

2.3.3 Standard normal variated (SNV)

Supposed to (Lu, 2020), MSC has similar benefit with SNV which used effectively to remove the baseline effect both translation and offset in the spectra (Lindgren, 1994). The correction is done by applying the multiplicative and additive spectral correction in the original spectra corresponds to the mean. Through this calculation, the corrected spectra would have relatively consistent baseline (Chen, 2021). For each *j* of *m* wavelengths, calculate the mean of all *n* spectra called it as *m x* 1 column vector of a standard spectrum **m**. Then, perform of simple linear regression $xij = \alpha i + bimj + eij$ on each i spectrum of m x 1 column vector $\mathbf{x}_i$ of *n* spectra in **X** (as dependent variable) relative to the *m x* 1 vector m (as independent variable). Given solution using ordinary last square (or simply as LS) method, the regression coefficient parameter bi and intercept αi are used to correct the baseline scatter by subtracting each spectrum $\mathbf{x}_i$ with α_i and divided by b_i or formulized as

$$x_{ij\ (MSC)} = (x_{ij} - \alpha_i)/\ b_i \quad (5)$$

2.3.4 Detrending (DT)

De-trending is a simple baseline correction method; the baseline is assumed to be a second-degree polynomial function of wavelength and is subtracted from the spectrum

2.4. Statistical criteria

The root-mean square errors (RMSE) and the ratio of performance to deviation (RPD) were calculated to evaluate the performance of these regression models. RMSE is defined by

$$\mathrm{RMSE} = \sqrt{\frac{1}{n}\sum_{i=0}^{n}(\hat{y}_i - y_i)^2} \quad (6)$$

where n is number of samples, y_i is the measured value, and $\hat{y}_i$ is the estimated value.

RPD was also calculated by

$$\mathrm{RPD} = \mathrm{SD}/\mathrm{RMSE} \quad (7)$$

where SD is the standard deviation of the real chlorophyll content as calculated from the test data measurements. The regression models are categorized into three groups: category A (RPD > 2.0), category B (1.4 ≥ RPD ≥ 2.0) and category C (RPD < 1.4). It has been claimed that category B can be improved by using different calibration strategies, but properties in category C may not be reliably predicted (Chang, 2001).

3. RESULTS

3.1 Chlorophyll content

The measured chlorophyll contents per leaf area (cm^2) ranged from 42.20 to 94.38 µg and the maximum value were obtained from the 0.5 S treatment while the minimum values were obtained from the 0N. Although, the average of chlorophyll content estimation is 29.20 µg and standard deviation of 7.43.

3.2 Spectral Reflectance and their correlation

Spectral reflectance in each fertilizer treatment for the wasabi plants shown in Figure 1. The N treatment made the reflectance higher over visible domain higher, while it made the reflectance values smaller over shortwave infrared domain. Although, according to figure 1, the treatment made the reflectance higher over visible domain higher, while it created the reflectance values smaller than shortwave infrared domain. Stepwise A linear discriminant analysis (p < 0.01) showed the reflectance values at 1451, 1952, 2088 and 2332 nm were advantages for identifying each treatment achieving the overall accuracy of 0.97.

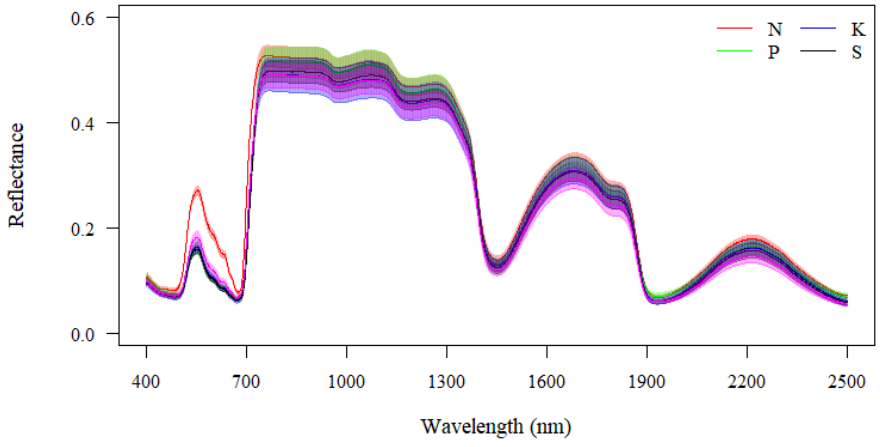

Figure 1. Spectral reflectance for each treatment

However, correlations of each spectral wavelength with a chlorophyll content determined spectral reflectance of in the near-infrared was positively correlated and the highest values was confirmed at 1232 nm (r = 0.27). And then, negative correlations were confirmed near green peak and REIP, and the two bottoms were identified at 553 nm (r = -0.55) and 713 nm (r = -0.67).

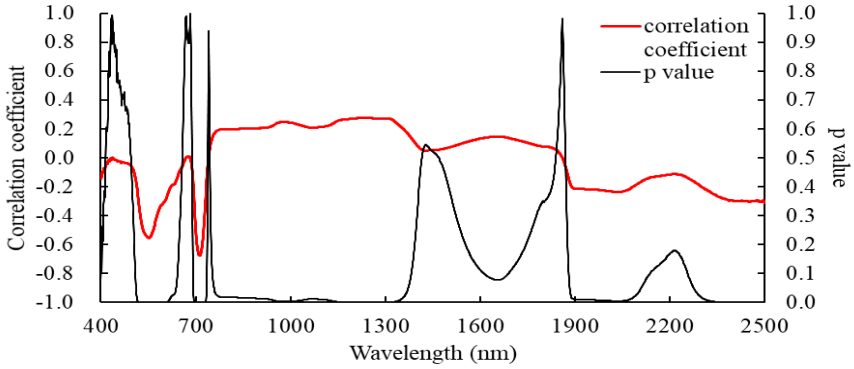

Figure 2. Correlation of each spectral and chlorophyll contents estimation

3.3 Accuracy validation

Table 2 shows the performance of five pre-processing method for chlorophyll content estimation and the Figure 2 shown the Taylor diagram of pre-processing method performance. Detrending was selected as the best pre-processing solution for estimating chlorophyll content 100 times.

Table 2. RMSEs and RPDs (cm^2) of 100-time estimation results

No	Pre-processing technique	RMSE	RPD
1	FDR	4.006	1.842
2	CR	3.949	1.869
3	SNV	3.703	1.993
4	MSC	3.617	2.040
5	Detrending	3.603	2.048

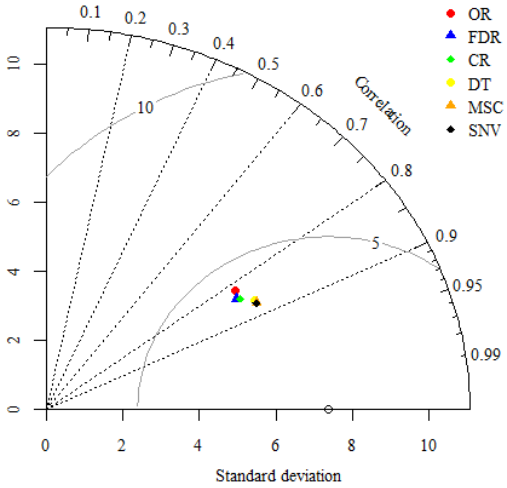

Figure 3. Tailor diagram showing the performance of each pre-processing methods

4. CONCLUSION

Although pre-processing techniques are potentially useful tool for improving estimation accuracies of chlorophyll content from hyperspectral data, the combinations of the pre-processing techniques and machine learning algorithms have been obscure. This study evaluated five pre-processing techniques with random forest (RF) regression for estimating chlorophyll contents from hyperspectral reflectance. The superior usefulness of Detrending (DT) was confirmed and the combination of DT and RF was most effective for estimating chlorophyll content.

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