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Nondestructive Measurement of Grape Leaf Chlorophyll Content Using Multi-spectral Imaging Technology and Calibration Models

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Abstract. Nondestructive measurement of grape leaf chlorophyll content is essential for precision vineyard management. Multi-spectral imaging technology was adopted for image acquisition of grape leave. For each leaf, a color (R-G-B) image and a near-infrared (NIR) image were taken. These images were then transformed into three vegetation indices, e.g. RVI, NDVI and GNDVI. Calibration models were established, by single-variable linear regression, multi-variable linear regression and BP-ANN. Three color space systems, e.g. R-G-B, CIE XYZ and HIS, were examined with the purpose of model optimization. A total of 112 leave were divided into a calibration set(62) and an independent validation set(50). A SPAD-502 chlorophyll meter was used for reference measurement. The single-variable linear regression result shows that the NDVI index is most significant for the measurement of leaf chlorophyll content with coefficient of determination (r^2) of 0.70 for calibration set and 0.69 for independent validation set. It is found that the model for R-index produces higher accuracy than those for G- and B-index, which confirms that chlorophyll content can be correlated with R-grayscale values. By comparison, the multi-variable linear regression models based on R-G-B-NIR achieves higher prediction accuracy with r^2 of 0.8174. To further improve the prediction accuracy, several BP-ANN models were developed. The best result was achieved for R-G-B-NIR with r^2 of 0.99 for independent validation set. It is concluded that multi-spectral imaging technology coupled with BP-ANN calibration model of R-G-B-NIR grayscales is promising for nondestructive measurement of grape leaf chlorophyll content. This method proposed in the study is worthy of being further examined for *in situ* determination of nutrition diagnose of grape plant.

Introduction

Chlorophyll plays an essential role in plant photosynthesis through light absorption and nutrition synthesis. It has been confirmed that chlorophyll content has strong correlation with nitrogen status in leave[1]. Traditionally, grape chlorophyll content is measured by the way of Aron[2] which is time-consuming and skill-needed. In addition, the Aron method must process or preprocess samples in the mean of leave destuction. Thus, it is necessary to develop cheaper and nondestructive methods for the measuremnt of leave chlorophyll content not only for laboratory experiment but also for in-field application.

Multi-spectral imaging technology is the one that captures information at several frequencies across visible and near-infrared spectral range. It becomes a powerful research tool in agriculture, biology, and object detection, and industrial product quality management [3-7]. A previous research created a relationship between chlorophyll content and red-edge chlorophyll index in grapes[8]. However, no published reports could be found in the aspect of grape leave chlorophyll determination by calibration models based on vegetation indices and color space systems.

This study aims to extract optical vegetation indices from multi-spectral images of grape leave via image processing techniques. Various calibration methods were adopted to calibrate these optical characteristics with leaf chlorophyll content. The prediction performance of the established calibration models are evaluated by an independent validation set.

Materials and Methods

Sample origins and instrument preparation. Grape leave were picked from a local vineyard in the suburb of Hangzhou city, China. A multi-spectral imaging system used in the study consists of a multi-spectral camera, wide-spectral light source and a data analyzer [7]. The multi-spectral camera can split incident optical signals into a color image (R-G-B combination) and a near infrared (NIR) image. These multi-spectral images were transmitted from the camera into a computer through RJ45-connected cables. These images were processed using the data analyzer, which were accomplished by a high performance computer with image processing software. The collection of sample images was done at ambient temperature (18-20°C).

Reference measurement of chlorophyll content of grape leave. After multi-spectral images were taken, the chlorophyll content in each grape leaf was measured by a SPAD-502 meter (Minolta Camera, Japan).

Images pretreatment. To reduce the noise, each leaf was photographed for 10 times. It had been proved that many high frequency noises could be eliminated by average. After that, balance correction of light source was applied to further reduce alignment error.

Color space systems and vegetation indices. Apart from original R-G-B color space, two extra color space systems, e.g. CIE XYZ and HIS, were examined with the purpose of model optimization.

Vegetation indices: Vegetation indices (VI) values are often used to indicate the growing status of green plants [9].

$$RVI=NIR/R,\ NDVI=(NIR-R)/(NIR+R),\ GNDVI=(NIR-G)/(NIR+G).$$

R-G-B system: The RGB system was established by decomposing color images into three individual monochromic images of R, G and B.

CIE XYZ system: CIE XYZ systems is based on direct measurements of the human eye, specifically the three cone cell receptors, and it is the basis and gold standard which is used to describe many other systems[10].The CIE XYZ system can be transformed from R-G-B system by the following equation.

$$\begin{bmatrix} X \\ Y \\ Z \end{bmatrix} = \begin{bmatrix} 0.4125 & 0.3575 & 0.1804 \\ 0.2127 & 0.7152 & 0.0721 \\ 0.0193 & 0.1192 & 0.9502 \end{bmatrix} \cdot \begin{bmatrix} R \\ G \\ B \end{bmatrix}$$

HIS system: HIS system is always used in describing the human visual system due to the fact that men's visual sensitiveness to brightness is far stronger than sensitiveness to colour tint. Image analysis and processing can be greatly simplified in HIS system[11]. Three independent components were introduced.

$$H = \frac{2 \cdot G - R - B}{4},\quad I = \frac{R + G + B}{3},\quad S = \frac{R - B}{2}$$

where H, I and S represent hue, light intensity and chromatic information, respectively.

Calibration methods. In this study, linear regression methods and BP-ANN were proposed to bridge the relationship between mean optical reflectance of multi-spectral images with chlorophyll content of leaf samples.

Linear Regression. Linear regression is a classical calibration method that combines one or more predictors (X-variables) with a corresponding single response(Y-vector)[12].

Back-Propagation Artificial Neural Network (BP-ANN). BP-ANN is one of popularly-used neural networks trained by error back-propagation algorithm. It consists of an input layer, a hidden layer and an output layer. BP-ANN can be used to approximate any a nonlinear function: f: $X \rightarrow Y$[13].

Results and Discussion

Original multi-spectral images. Before establishing calibration models, each color image was separated into three monochromic images. Figure 1 illustrates the original R-G-B image, the decomposed monochromic images and NIR image of a representative of grape leave.

R-G-B R G B NIR

Fig. 1 Original and decomposed images of a grape leaf

Sinple-variable linear regression models. Firstly, single variants of R, G, B, CIE-X, CIE-Y, CIE-Z, H, I, S, and RVI, NDVI, GNDVI were used to build calibration models (Table 1). By comparison, R, NDVI, CIEX and I perform best in the R-G-B, RVI-NDVI-GNDVI, CIE XYZ and HIS system respectively. It is worth noting that all these best single variables come from R channel of images. This confirms that during plant photosynthesis, leave absorb red light while reflect green light[14].

Table 1 Sinple linear regression models

No.	Calibration			Prediction	
	Linear function	r^2_{cal}	RMSE[a]	r^2_{pre}	RMSE
1	ch= 45.9245-0.5607*R	0.5615	3.3268	0.5469	3.3817
2	ch= 41.5598-0.3463*G	0.4304	3.7915	0.4102	3.8581
3	ch= 39.7960-0.3871*B	0.3335	4.1014	0.3098	4.1735
4	ch= 0.8176+7.7170*RVI	0.6805	2.2839	0.6676	2.8965
5	ch= -23.5943+89.2927*NDVI	0.6991	2.7557	0.6878	2.8071
6	ch= -1.1267+56.7687*GNDVI	0.5370	3.4185	0.5217	3.4747
7	ch= 42.9743-0.4592*CIE-X	0.4652	3.6740	0.4467	3.7372
8	ch= 42.2264-0.3850*CIE-Y	0.4481	3.7322	0.4288	3.7971
9	ch= 40.1812-0.3561*CIE-Z	0.3512	4.0467	0.3282	4.1178
10	ch= 36.2877-1.9888*H	0.3094	4.1751	0.2846	4.2494
11	ch= 42.3686-0.4254*I	0.4404	3.7579	0.4210	3.8230
12	ch= 31.3166-1.5359*S	0.1075	4.7463	0.0736	4.8357

[a] The unit is SPAD

Multi-variable linear regression models. Since the results of single-variable linear regression models are not as accurate as for pratical use, multi-variable linear regression models were built, respectively, based on the combinations of R-G-B-NIR, RVI-NDVI-GNDVI and CIE X-Y-Z-NIR. The results were shown in Table 2. Performance of these models based on R-G-B, CIE XYZ and HIS systems are identical. This is due to the fact that both the systems of CIE XYZ and HIS were linear-transformation of R-G-B system. It can be observed that R-G-B-NIR achieves higher accuracy with r^2=0.8365, RMSE=2.0312 for calibration set and r^2=0.8174, RMSE=2.1463 for independent validation set compared to the sinple-variable linear regression models in Table 1. This could be explained that chlorophyll content information in leave distributes in the whole spectral range from B, G, R to NIR rather than in an individual one.

Table 2 Multiple-variables linear regression models

No.	Calibration			Prediction	
	Linear function	r^2_{cal}	RMSE[a]	r^2_{pre}	RMSE
1	ch= -22.11+1.31*RVI-90*NDVI-13.29*GNDVI	0.7036	2.7351	0.6819	2.8335
2	ch= 36.99-1.36*R-0.57*G+1.19*B+0.18*NIR	0.8365	2.0312	0.8174	2.1463
3	ch= 36.99-3.81*X+0.79*Y+1.92*Z+0.18*NIR	0.8365	2.0312	0.8174	2.1463
4	ch= 36.99-0.64*H-0.74*I-2.56*S+0.18*NIR	0.8365	2.0312	0.8174	2.1463

[a] The unit is SPAD

BP-ANN models. To further improve the accuracy of predicting, four BP-ANN models were developed, respectively, based on R-G-B-NIR, RVI-NDVI-GNDVI, X-Y-Z-NIR and H-I-S-NIR. The number of neurons in hidden layer was tried from 10 to 14 in order to find optimal network topology. The learning rate was set as 0.05 and epochs was 8000. The BP-ANN was programmed by Matlab software. The results were listed in Table 3.

Table 3 Performance of BP-ANN models

Neuron number in hidden layer	R-G-B-NIR		RVI-NDVI-GNDVI		X-Y-Z-NIR		H-I-S-NIR	
	r^2	Error [a]	r^2	Error	r^2	Error	r^2	Error
10	0.9630	4.9291	0.9348	4.1938	0.6351	11.497	0.8041	8.7509
11	0.9813	2.2836	0.9251	6.0780	0.8106	7.2964	0.8161	7.2902
12	0.9868	1.9761	0.9519	3.0454	0.7327	8.0447	0.9073	6.9990
13	**0.9945**	**1.4418**	0.9303	5.0193	0.7782	6.8625	0.8621	8.6580
14	0.9884	2.9270	0.9369	5.7892	0.7874	6.4359	0.9002	7.4690

[a] The unit is SPAD

Apparently, the BP-ANN model based on R-G-B-NIR with 13 hidden layers performs best with r^2 of 0.9945 and Error of 1.4418. It is better than the result of MLR model based on R-G-B-NIR (Table 2). It might be due to the fact that BP-ANN is a non-linear statistical modeling tool which changes its structure based on external information during the learning phase. The correlation between measured and predicted values for the independent validation set using MLR model and BP-ANN model are compared in Fig.2.

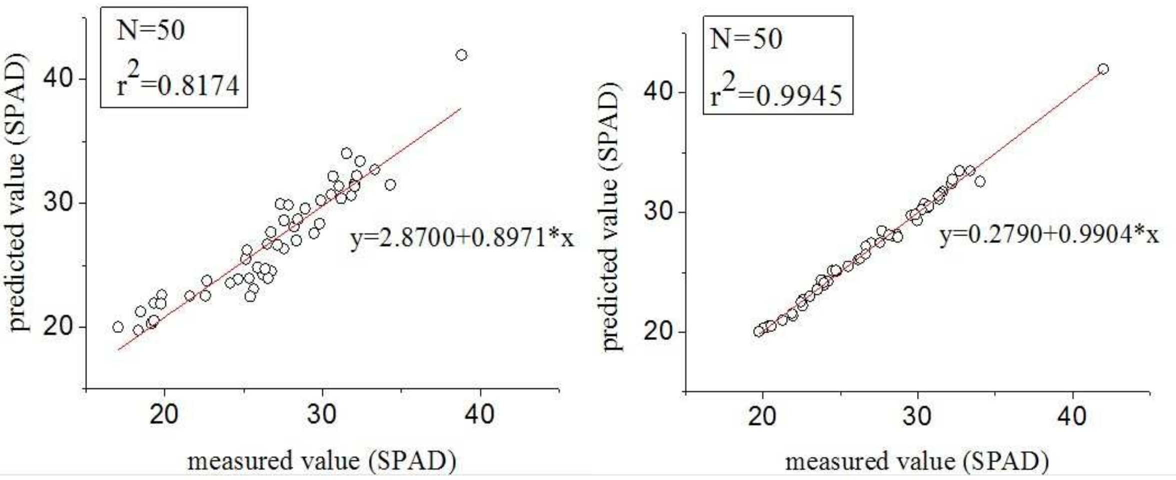

Fig. 2 Prediction Performance of MLR and BP-ANN models based on R-G-B-NIR

Conclusions

In this study, calibration models between grapes leave chlorophyll content and multi-spectral image characteristics were established by different calibration methods, e.g. single-variable linear regression, multi-variable linear regression and BP-ANN. The results show that R and R-related color indices can produce higher accuracy for predicting chlorophyll content of grape leave. Furthermore, the BP-ANN model based on R-G-B-NIR can achieve much higher prediction performance than other models under investigation. It is concluded that multi-spectral imaging technology, if coupled with appropriate calibration methods, is promising for nondestructive measurement of chlorophyll content of grape leave. This technology is worthy of being further examined for *in situ* nutrition diagnose of grape plant.

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