Prediction of SPAD Values Using Dominant Wavelength in Mung Bean Microgreens

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Abstract

The Soil and Plant Analysis Development (SPAD) value is a significant parameter indicating chlorophyll content, particularly in the green parts of plants. Conventional SPAD meters determine this value by measuring the transmission and absorption of red and infrared radiation at a single point (2×3 mm^2 sensor size). However, obtaining a comprehensive value for an entire leaf requires multiple measurements, increasing processing time. In this study, a non-destructive method for predicting SPAD values was developed using image processing techniques to determine dominant wavelength values from leaf photographs. A custom-designed photo box with controlled 6000 lux white LED lighting was used to capture images at a fixed distance of 15 cm. Images were processed using Color Picker (2024) software, where green components of the leaf were analyzed to extract dominant wavelength values. The results demonstrated that SPAD values could be accurately predicted using dominant wavelength data, with a 98.33% accuracy for the linear model (RMSE: 1.308) and 98.43% for the polynomial model (RMSE: 5.467). The findings indicate that a linear model provides a more precise correlation. This novel approach enhances the efficiency of SPAD measurement and offers a rapid, non-destructive alternative to conventional methods.

Keywords: Dominant wavelength, Microgreen, Mung bean, SPAD

Introduction

Soil Plant Analysis Development (SPAD) is a reliable indicator for representing chlorophyll content in plants. The SPAD-502m (Konica Minolta, Inc., Japan) is currently the most widely used portable chlorophyll meter (Donnelly, Yu, Rehberg, Meyer, Young, 2020). An introduction to the SPAD-502 is available on the official website (Konica, 2024). This device determines the relative chlorophyll content by measuring the light transmittance coefficient of leaves in the red and near-infrared regions. Additionally, the conversion equations between SPAD values and nitrogen-related features are often used as intermediate variables for conversion with other color indexes (do Amaral *et al.*, 2019; Ye, Abe, Zhang, Yoshimura, 2020). The SPAD-502 Plus measures absorbance at two different wavelengths: 660 nm (red) and 940 nm (near-infrared). Red light is strongly absorbed by chlorophyll, while near-infrared light serves as a reference wavelength to account for variations in leaf structure. The fundamental theoretical principles underlying these chlorophyll content meters were detailed by Markwell, Osterman and Mitchell (1995) and Richardson, Duigan and Berlyn (2002).

The sensors used in SPAD measurement devices are very small. For instance, the sensor size for the Konica Minolta SPAD-502Plus is 2×3 mm^2. Chlorophyll levels in plant leaves are not homogeneous, as can be seen in the mung bean microgreen leaf shown in Figure 1. Therefore, multiple repeated measurements are required to obtain an average value using SPAD measurement devices. This not only slows down the process but also makes it challenging to obtain a value representing the entire leaf. This study aims to achieve a representative SPAD value by using a parameter obtained from the entire leaf rather than a single point. For this purpose, it is intended to use the dominant wavelength value obtained from the visual image of the leaf.

Fig. 1. Mung bean microgreen leaf

The dominant wavelength was determined by processing the images in the CIE 1931 color space, where colors are represented using x and y chromaticity coordinates. This value can be used as a measure representing the entirety of color characteristics within an image area (Schanda, 2007). To find the dominant wavelength value, the coordinates in the CIE 1931 xyz color space need to be determined first (Post, 1997). Figure 2 illustrates how the dominant wavelength value is found using x and y coordinates on the CIE color space via the white point (CIE, 2004).

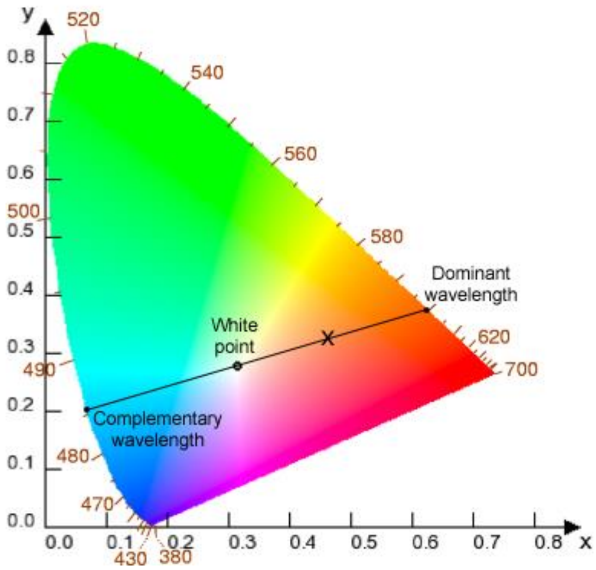

Fig. 2. Dominant wavelength

There are numerous studies in the literature that focus on developing devices to estimate

SPAD values based on color characteristics. In their study, Tan, Zhou, Zhao, He, and Qiu (2021) established a system that predicts SPAD values through RGB mapping. The system includes a container illuminated with white LEDs, within which photographs are taken for mapping purposes. By performing RGB mapping on the obtained images, the system makes predictions using a developed model. The R^2 values of the models range between 91% and 97%. Hassanijalilian *et al.* (2020) introduced a technique for assessing leaf SPAD values using a smartphone camera and a standard calibration board, achieving an R^2 of 0.86 and an RMSE of 3.2 SPAD units. However, their study did not include the distribution of the predicted SPAD values. Conversely, Zhang *et al.* (2019) developed a system called Leaf Scanner, which determined the chlorophyll content distribution across an entire leaf based on the transmittance signal from visible and near-infrared LEDs captured by an integrated camera. In both studies, the model's input was a vector of average color features from the entire leaf, with the reference SPAD value obtained by averaging measurements from multiple spots on the leaf. The prediction performance for the average SPAD value of an entire leaf was found to be satisfactory in these studies. In their study, León, Viña, Frezza, Chaves, and Chiesa (2007) attempted to predict the SPAD values of lettuce leaves using CIE Lab values obtained from a colorimeter. The R^2 values for these predictions ranged from 68% to 77%. Hu *et al.* (2010) investigated the relationships between SPAD values and CIE Lab*, as well as RGB values obtained through image processing. The study demonstrated a strong relationship between the L, R, and G parameters and SPAD values. Yadav, Ibaraki, and Gupta (2010) aimed to estimate chlorophyll content in micro-propagated potato plants using RGB image analysis. The researchers analyzed digital images of leaves, focusing on RGB color values, and compared these with SPAD measurements. Their method achieved a high correlation (R^2 = 0.95) between RGB values, particularly the green channel, and chlorophyll content, demonstrating the potential of digital image analysis as a non-destructive method for chlorophyll estimation. Diago *et al.* (2016) investigated the use of RGB image analysis for estimating grapevine yield and leaf area under field conditions. They applied supervised classification methodology on RGB images of grapevine canopies. The research found strong correlations between RGB analysis and SPAD measurements (R^2= 0.93) and in leaf area estimation (R^2= 0.95). These results demonstrated the effectiveness of RGB imaging in viticulture for rapid, non-destructive assessment of plant parameters.

A comprehensive review of the existing literature reveals a notable absence of research specifically utilizing dominant wavelength as a predictor for SPAD values. While various spectral approaches have been employed to estimate chlorophyll content, the direct application of dominant wavelength in SPAD value estimation appears to be an unexplored area in current scientific discourse. This gap in literature presents a potential avenue for novel research in the field of non-destructive chlorophyll content assessment.

Microgreens, which are considered superfoods, have recently gained significant attention in the context of functional nutrition. They are particularly favored in the diets of vegetarian and vegan individuals due to their bioactive compounds. Chlorophyll holds a prominent position among these bioactive compounds. Therefore, the measurement of chlorophyll is essential in the production of microgreens. In this context, the cotyledon leaves of mung bean microgreens, commonly used in microgreen production, were used in this study to predict SPAD values.

Materials and Methods

During the experiments, SPAD values of mung bean microgreen samples were measured using a Konica-Minolta 520 Plus model SPAD meter (Figure 3). The device operates by measuring the transmittance and absorbance of red (660 nm) and near-infrared (940 nm) light at a 2×3 mm^2 sensing area.

Given the small sensing area, measurements were conducted at nine different points on each leaf, and the average SPAD value was recorded.

To compare SPAD measurements with image processing results, the leaves were also photographed under standardized conditions. These images were processed using Color Picker (2024) software to extract RGB values, specifically analyzing the green channel intensity. The dominant wavelength values were subsequently calculated from the color space coordinates using the described transformation equations. The extracted dominant wavelength values were then statistically correlated with the SPAD measurements.

Fig. 3. SPAD measurement device

Determination of dominant wavelength

The dominant wavelength was determined by processing the images in the CIE 1931 color space, where colors are represented using x and y chromaticity coordinates. To extract the necessary color data, the Color Picker (2024) software was used to obtain RGB values from the leaf images, particularly focusing on the green channel. These RGB values were then used to compute dominant wavelength through chromaticity coordinate transformations.

The first step in the process involved normalizing the RGB color components from the standard 8-bit range (0-255) to a floating-point scale between 0 and 1 as follows:

$$R' = \frac{R}{255}, \quad G' = \frac{G}{255}, \quad B' = \frac{B}{255} \tag{1}$$

Since raw RGB values are not perceptually linear, a gamma correction was applied according to the sRGB standard:

$$R = \begin{cases} \frac{R'}{12.92}, & if\ R' \leq 0.04045 \\ \left(\frac{R'+0.055}{1.055}\right)^{2.4}, & otherwise \end{cases} \tag{2}$$

The same transformation was applied to G and B components before converting them into the CIE 1931 XYZ color space. The RGB-to-XYZ conversion was performed using the standard linear transformation matrix:

$$\begin{bmatrix} X \\ Y \\ Z \end{bmatrix} = \begin{bmatrix} 0.4124564 & 0.3575761 & 0.1804375 \\ 0.2126729 & 0.7151522 & 0.0721750 \\ 0.0193339 & 0.1191920 & 0.9503041 \end{bmatrix} \cdot \begin{bmatrix} R \\ G \\ B \end{bmatrix} \tag{3}$$

Once the XYZ tristimulus values were obtained, they were converted into chromaticity coordinates (x, y) using the following equation:

$$x = \frac{X}{X+Y+Z}, \quad y = \frac{Y}{X+Y+Z} \tag{4}$$

The calculated (x, y) chromaticity coordinates were then mapped onto the CIE 1931 chromaticity diagram. A straight line was drawn from the white reference point (D65 illuminant) through the averaged chromaticity coordinates of the leaf color. The intersection of this line with the spectral locus boundary of the CIE diagram determined the dominant wavelength value.

To standardize the lighting conditions for image processing, a photographing setup was prepared. A closed photo box with white interior walls was designed to ensure uniform light distribution. The interior was illuminated by six white LEDs, each with a power of 1.5 watts, maintaining a consistent illumination of 6000 lux across all measurements. The product to be photographed was fixed at a distance of 15 cm from the mobile phone camera, which has a resolution of 48 megapixels and captures images in the visible spectrum (400-700 nm). The camera was positioned perpendicularly to the leaf surface to minimize angle-related distortions and ensure consistency in dominant wavelength measurements.

The photographed product was placed on a non-reflective matte black background made of 2 mm thick polystyrene foam (Figure 4). This background was used to prevent unwanted reflections and ensure accurate color representation. Due to the intensity and spectral composition of the illumination, the black background may appear as dark blue in the captured image. This optical effect occurs because of the interaction between the camera sensor's color perception and the spectral reflectance of the black material under strong illumination. However, the actual background material is black, as verified under controlled lighting conditions.

Fig. 4. Captured image of the leaf placed on a matte black background for image processing.

The interior of the photo box was illuminated by six white LEDs, each with a power of 1.5 watts. The illumination intensity inside the photo box was fixed at 6000 lux. The photo box was made of plastic, with a diameter of 12 cm and a height of 15 cm (Figure 5). The outer surface of the photo box was coated with aluminum to prevent interference from external radiation. In the experimental setup, a controlled lighting environment was established to ensure consistency in light intensity across all measurements. This lighting condition remained constant throughout all image acquisitions to minimize variations in dominant wavelength readings due to external factors. Since SPAD values are based on the absorption of red (660 nm) and near-infrared (940 nm) light, it was essential to maintain a stable light environment that does not interfere with these absorption properties. The white LEDs used in the setup emit a broad spectrum of visible light (400-700 nm), ensuring that the captured images accurately represent the leaf's natural color without artificial spectral bias. The extracted dominant wavelength values were then statistically correlated with the SPAD measurements to determine their relationship.

Fig. 5. Photo box

As part of the study, a standardized method based on image processing was developed to determine dominant wavelength values. In this study, the dominant wavelength values were determined from the large surface areas of the leaves. The images were non-filtered photographs taken inside an artificially illuminated photo box developed specifically for this research. RGB values were extracted using the Color Picker image processing software (Color Picker, 2024), and the dominant wavelength was calculated using the described transformation methods.

In the Color Picker software, only the green components of the leaf images, excluding the black background, were processed to determine the dominant wavelength value representing the entire leaf surface.

Mung bean microgreens were grown under soilless hydroponic conditions using a fully artificial photosynthetic lighting method. The temperature inside the production chamber was maintained at 23-25 °C with a humidity range of 60-65%. Inside the chamber, lighting was applied for 16 hours at a Photosynthetic Photon Flux Density (PPFD) level of 100 μmol.m^{-2}s^{-1}, resulting in a Daily Light Integral (DLI) of 5.76 mol m^{-2} day. PPFD values were measured using a Corvus brand PAR meter (Figure 6). The lighting duration was controlled by a timer. No chemicals were used during the microgreen cultivation stage, and the lighting process was carried out using purple-colored growth LED lamps (Figure 7).

Fig. 6. PAR Measurement device by Corvus

Fig. 7. Images of mung bean microgreens under (a) purple-colored growth LED lamps, and (b) natural light

After the production of mung bean microgreens, leaves with different color characteristics were selected and prepared for measurement. A total of 100 samples were tested for SPAD measurement, and it was determined that 19 of them represented different color characteristics (Figure 8). SPAD measurements were taken at 9 different points on each of the 19 leaves using the Konica Minolta SPAD-502 Plus device. The average SPAD value of these nine measurements was calculated to represent the overall chlorophyll content of each leaf.

To obtain the corresponding dominant wavelength value, the entire leaf surface was analyzed in the captured image. The dominant wavelength was determined by averaging the values obtained from all pixels across the entire leaf area. This ensured that the extracted dominant wavelength represented the overall chlorophyll distribution rather than being limited to specific measurement points. The xy coordinates of the obtained images were determined and used in the image processing workflow.

Fig. 8. Mung bean microgreen leaves representing different color characteristics

Results and Discussion

In the experiments, mutual distribution graphs of the average SPAD values measured with 9 repetitions and the wavelength values obtained by image processing from 19 leaf samples determined in 19 different characteristics are shown in Figures 9 and 10.

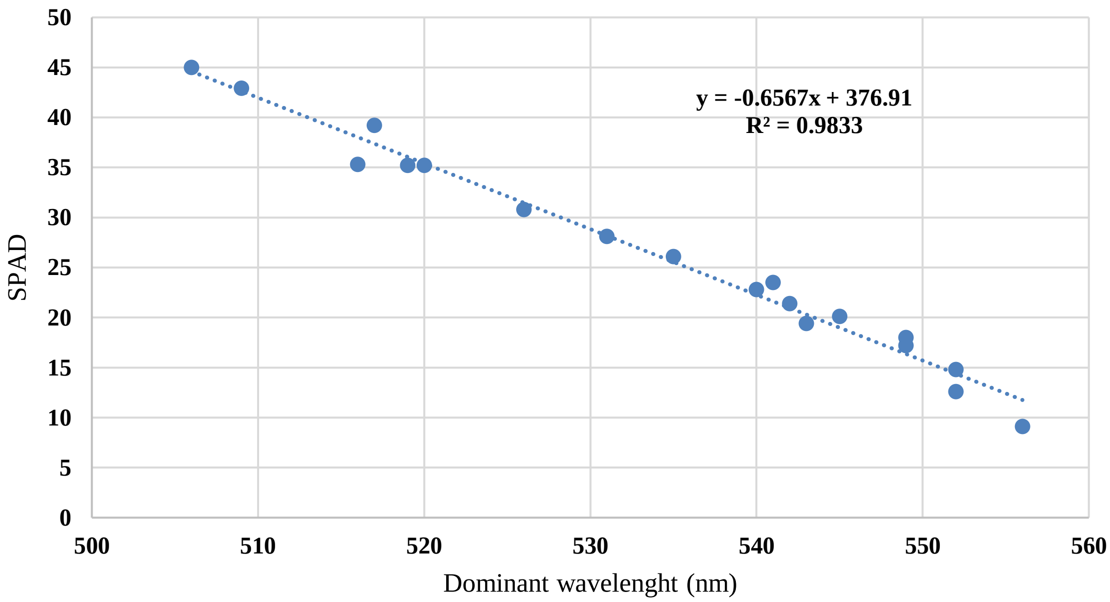

Fig. 9. Linear regression equation of the relationship between dominant wavelength and SPAD value

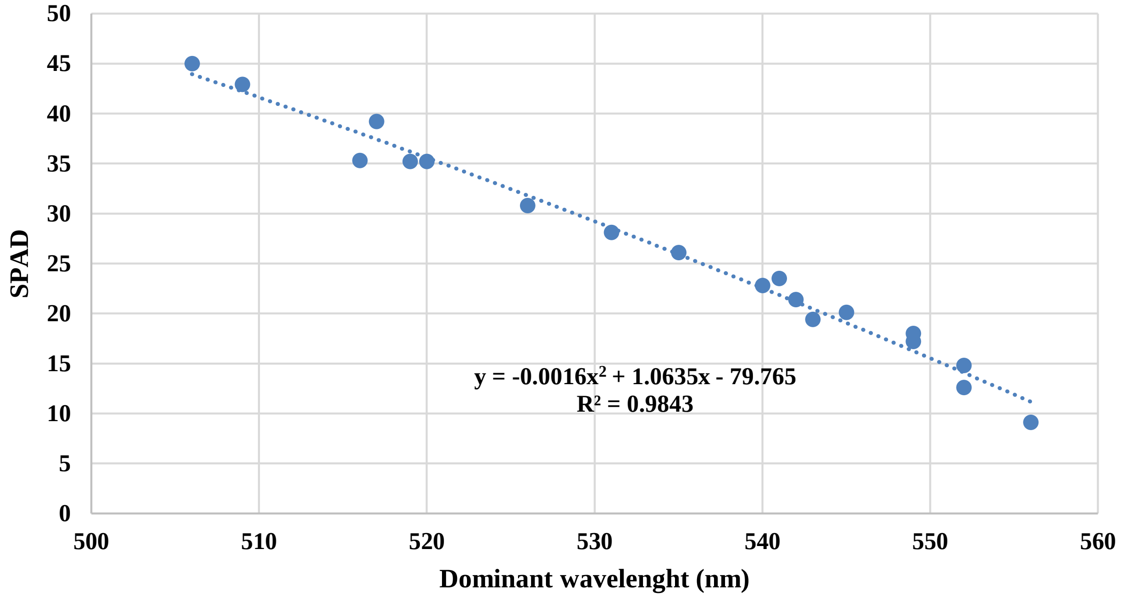

Fig. 10. Second-degree polynomial regression equation of the relationship between dominant wavelength and SPAD value

Based on the analysis results, strong relationships were observed between SPAD values and dominant wavelength values, which can be modeled with linear and second-degree polynomial equations. The R^2 value for the linear equation was found to be 98.33%, while for the second-degree polynomial equation it was 98.43%. These R^2 values indicate that both equations provide accurate predictions. However, the root mean square error (RMSE) values were different: the RMSE of the linear model was 1.308046, whereas the RMSE of the polynomial equation was 5.467945. Additionally, the values of the other error parameters, MBE (Main Bias Error) and MPE (Mean Percentage Error), have been calculated with a higher polynomial equation (Table 1). When the RMSE, MBE, and MPE values are evaluated together, it has been determined that the linear equation provides more accurate values.

Table 1- Statistical analysis results

	RMSE	MPE	MBE	R^2
Linear	1.308046	1.063011	0.020968	98.33
Polynomial	5.467945	25.504569	5.309631	98.43

Accordingly, it was concluded that the photo box developed in this study and the dominant wavelength values calculated using image processing can be used to predict SPAD values of mung bean microgreens.

The primary objective of this study was to determine whether dominant wavelength values extracted from image processing could serve as a reliable indicator of chlorophyll content, measured through SPAD values. The study primarily aimed to establish a correlation between these variables rather than directly predicting SPAD values from light intensity data.

However, as a secondary outcome, a linear regression model was applied to explore the potential for estimating SPAD values from dominant wavelength. The resulting equation demonstrated a strong correlation, suggesting that dominant wavelength could be used as a predictive parameter in future research. Nevertheless, further validation across different plant species and environmental conditions is required to develop a generalized predictive model.

It was found that converting the obtained dominant wavelength values to SPAD values with Equation 1 yielded more successful results.

$$SPAD = -0.6567\,DW + 376.91 \qquad (5)$$

DW - Dominant wavelength (nm)

The accuracy of SPAD estimations conducted within the scope of this study has surpassed all previously reported predictions utilizing spectral values in the literature. This superior performance strongly indicates that the approach of employing dominant wavelength for SPAD value estimation is a valid and efficacious methodology. The results demonstrate the potential of this novel technique to enhance the precision and reliability of non-destructive chlorophyll content assessment, thereby contributing significantly to the field of plant physiology and agricultural monitoring.

The developed system and procedure can be further validated by testing them on different types of leaves, which will allow for the formulation of equations applicable across various leaf types. Additionally, developing a single equation for different plant leaf types could potentially replace existing devices in SPAD measurement. This approach has the potential to broaden the system's applications.

Limitations and Considerations

The results obtained in this study have shown successful and applicable outcomes for mung bean microgreen material. The photo box used was designed to minimize the effects of environmental conditions (such as irradiance, light spectrum, etc.). However, in the future, repeating these experiments with different types of leaves will provide data on the broader applicability of the developed method. Depending on the quality of the obtained data, new equations and coefficients could be developed for different leaves, thereby expanding the application of the method.

Conclusion

This study developed a novel non-destructive method for predicting SPAD values in mung bean microgreens using dominant wavelength analysis. Traditional SPAD meters face limitations due to their point-specific measurements (sensor area: 2×3 mm²) and the need for repeated sampling to represent whole-leaf chlorophyll content, which reduces efficiency. Our image-processing approach addressed this by extracting dominant wavelength values from leaf images captured under standardized conditions (6000 lux LED lighting, 15 cm fixed distance) using Color Picker (2024) software.

Results revealed a statistically robust correlation (>98% R^2) between SPAD values and dominant wavelength. The linear regression model (SPAD = −0.6567 × DW + 376.91) outperformed the polynomial model (RMSE: 5.467) with higher accuracy (98.33%) and lower error (RMSE: 1.308), establishing it as the optimal predictive tool. Compared to conventional methods, this technique offers:

Whole-leaf representation in a single measurement,

Significant time reduction in data acquisition,

Non-destructive preservation of leaf integrity.

A current limitation is validation solely on mung bean microgreens. Future work should test the model across diverse plant species and leaf morphologies to enhance generalizability. Integration into mobile applications for real-time field monitoring is also recommended. This approach holds transformative potential for enhancing SPAD measurement efficiency in agricultural monitoring and precision farming.

Authors Contribution

R. Külcü: Conceptualization, Methodology, Data acquisition, Data pre and post processing, Statistical analysis, Visualization, Review and editing services

A. Süslü: Supervision, Technical advice, Validation

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پیش‌بینی مقادیر شاخص SPAD با استفاده از طول موج غالب در ریزسبزی ماش

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چکیده

مقدار شاخص کلروفیل (تحلیل خاک و توسعه گیاه SPAD) پارامتر مهمی است که نشان‌دهنده محتـوای کلروفیـل، بـه‌ویژه در قسـمت‌های سـبز گیاهان است. دستگاه‌های مرسوم اندازه‌گیری SPAD، این مقدار را با اندازه‌گیری انتقال و جذب تابش قرمز و مادون قرمز در یک نقطـه واحـد (انـدازه حسگر ۲×۳ میلی‌متر مربع) تعیین می‌کنند. با این حال، به‌دست آوردن یک مقدار جامع برای کل برگ نیـاز بـه انـدازه‌گیری‌های متعـدد دارد کـه زمـان پردازش را افزایش می‌دهد. در این مطالعه، یک روش غیرمخرب برای پیش‌بینی مقادیر SPAD با استفاده از تکنیک‌های پردازش تصویر بـرای تعیـین مقادیر طول موج غالب از عکس‌های برگ توسعه داده شد. از یک جعبه تصویربرداری با نورپردازی LED سفید و شدت روشـنایی ۶۰۰۰ لـوکس بـرای گرفتن تصاویر از فاصله ثابت ۱۵ سانتی‌متر استفاده شد. تصاویر با استفاده از نرم‌افزار Color Picker (2024) پردازش شدند، کـه در آن اجـزای سـبز برگ برای استخراج مقادیر طول موج غالب تحلیل شدند. نتایج نشان داد که مقادیر SPAD را می‌توان با استفاده از داده‌های طول موج غالب، با دقـت ۹۸/۳۳ ٪ برای مدل خطی (RMSE ۱/۳۰۸) و دقت ۹۸/۴۳٪ برای مدل چندجمله‌ای (RMSE ۵/۴۶۷) پیش‌بینی کرد. بررسی دقیق‌تر نشـان داد کـه مدل خطی همبستگی دقیق‌تری ارائه می‌دهد. این رویکرد جدید، کارایی اندازه‌گیری SPAD را افزایش می‌دهد و جایگزینی سـریع و غیرمخـرب بـرای روش‌های مرسوم می‌باشد.

واژه‌های کلیدی: شاخص SPAD، طول موج غالب، ماش، میکروگرین

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