Estimating SPAD, nitrogen concentration, chlorophyll a, b, and total chlorophyll in rice leaves using calibrated smartphone digital image

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TITLE

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ABSTRACT

The determination of nitrogen and chlorophyll content is often conducted by laboratory analysis. Devices with rapid analysis, mobility, and non-destructive testing are in demand. Smartphones have the potential to serve as tools for the evaluation of nitrogen and chlorophyll levels, since images contain essential information and color elements. A dataset of color parameter data from digital images of leaves that strongly correlate with nitrogen and chlorophyll content data can be used to create an equation for nitrogen and chlorophyll content assessments. A detailed examination was performed on 86 rice leaf samples from the maximum tillering and mature stage. Rice leaf photos were taken with a smartphone in natural outdoor lighting. Color calibration with Spydercheckr is needed to adjust for lighting conditions. Uncalibrated and calibrated image data are analyzed to determine RGB values, which are converted into CIE-Lab color space. The L*a*b color space had a significant correlation with SPAD parameters, nitrogen concentration, chlorophyll a, b, and total chlorophyll. This connection was higher after imagery calibration. The study found that smartphone images can predict SPAD values with 87.9% to 92.3% precision, depending on color space. Using smartphone digital picture L* and a* color spaces, N content can be estimated with 84.7% and 81.9% accuracy. Average accuracy for chlorophyll a, b, and total chlorophyll content was 65% to 76%. This study shows that smartphone images can estimate rice leaf SPAD and nitrogen content. However, chlorophyll a, b, and total chlorophyll concentration estimation needs improvement to enhance accuracy.

KEYWORDS

calibrated image; CIE-lab; color image processing; leaf color; nitrogen estimation

INTRODUCTION

The development and productivity of plants are predominantly influenced by the physiological mechanisms of photosynthesis and respiration, which facilitate the synthesis of carbohydrates and the generation of energy for plant metabolism. Both metabolic processes can function optimally when provided with the necessary nutrition. Photosynthesis, a biological process affected by chlorophyll in plants, necessitates the presence of nitrogen as a vital component. Hence, the assessment of plant development and production necessitates the consideration of chlorophyll value and nitrogen content in the leaves, as indicated by previous studies (Evans and Clarke, 2019;

Kobayashi and Matsuda, 2016; Stirbet et al., 2020). Prior research has demonstrated the significance of chlorophyll values in the assessment of nitrogen status in rice leaves, as evidenced by strong correlation values (Zhang et al., 2017; Liu et al., 2017; Gholizadeh et al., 2017; Turner and Jund, 1994).

Rice is a prominent staple grain globally, with Indonesia being a notable producer of this commodity. At present, Indonesia's food requirements continue to depend on the rice industry, thereby highlighting the significance of government initiatives aimed at enhancing rice yield (Silalahi et al., 2019; Sulistyorini and Sunaryanto, 2020). In order to enhance rice yield, it is important to administer the appropriate dosage of fertilizer, hence enabling the implementation of precision agriculture techniques. The leaf color chart (LCC) is a practical tool designed to assist farmers by providing suggestions on fertilizer application based on color indicators (Subedi et al., 2018; 2019). Nevertheless, the utilization of LCC is impeded mostly due to farmers' restricted accessibility to it. Furthermore, the qualitative evaluation of color is constrained by the use of only 4 or 6 shades of green, so resulting in an incomplete representation of the entire spectrum of green hues. Moreover, the predominant green hue exhibited by leaves mostly corresponds to the presence of chlorophyll. Consequently, relying just on apparent color characteristics may not be suitable for accurately assessing nitrogen deficit. Furthermore, in order to obtain accurate fertilizer recommendations based on chlorophyll and nitrogen levels, it is necessary to do laboratory analysis, a process that is both costly and time-consuming, and not readily available to farmers.

There is a pressing demand for a swift analytical approach that exhibits enhanced accuracy, precision, portability, and universal applicability among wetland rice cultivators. This method should possess the capability to effectively assess the nitrogen and chlorophyll levels in the leaves, thereby serving as a crucial determinant for determining the appropriate dosage of nitrogen fertilizer to be administered by farmers. The utilization of hyperspectral, multispectral, and infrared cameras, along with other diverse devices, is subject to financial limitations and occasional lack of portability (Caballero et al., 2020; Wang et al., 2021; Wu et al., 2022). The utilization of widely accessible smartphone cameras has the potential to evaluate the levels of nitrogen and chlorophyll in plants, as the digital images produced by these cameras can offer valuable insights into the color characteristics of leaves. One limitation associated with utilizing digital cameras for color measurement is the susceptibility of the captured color to environmental lighting conditions. Consequently, it becomes imperative to calibrate the color output generated by smartphone cameras.

Variations in color features might arise while capturing digital photographs of an item at different points in time. To address this issue, the calibration of color pixels becomes imperative. The color features obtained can be regarded as standardized colors that can be employed for the calibration of color features in smartphone cameras (Souza et al., 2018). Additionally, the utilization of

SpyderCHECKR's datacolor standard color palette, which serves as a means to calibrate colors and is seamlessly integrated with the SpyderCHECKR software48, is employed. Certain studies employ a methodology akin to the utilization of the SpyderCHECKR color palette. This involves the placement of multiple calibration objects with distinct colors, enabling the calibration of leaf color features based on the color characteristics generated at various time points. The Spydercheckr color palette is widely utilized in the domain of photography as a calibration tool to achieve precise color reproduction. The study conducted by Sunoj et al. (2018) demonstrates that color calibration matrix equations can be constructed using a standard palette of colors. The utilization of digital leaf photos enables the evaluation of plant health by analyzing the colorimetric properties of leaves. The correlation between leaf color and leaf chlorophyll concentration is the most significant, thus indicating that leaf chlorophyll content can serve as a reliable indicator of the overall health status of plants. Thus, the purpose of this study was to determine the potential of utilizing both calibrated and uncalibrated camera images to estimate chlorophyll and nitrogen content in rice leaves.

MATERIALS AND METHODS

SPAD, Nitrogen and Chlorophyll content measurement

This study focuses on the relationship between chlorophyll content and leaf N content with image data readings through both calibrated and uncalibrated smartphone cameras. Chlorophyl and leaf N content were measured on fully expanded upper leaves at maximum tillering and harvest stages. A total of 86 plant leaf samples were taken from rice plants of different ages, at maximum tillering period and mature period, thus the values obtained were wide distributed. The quantification of chlorophyll content in leaves using chlorophyll meter and laboratory methods. Leaf chlorophyll content was determined using a SPAD-502 chlorophyll (Konica Minolta Sensing Inc., Tokyo, Japan). This measuring technique involved assessing the transmittance of light at two specific wavelengths at 650 nm (red) and 940 nm (infrared). Chlorophyll content analysis was also calculated using the Arnon method in laboratory. The optical absorption at wavelengths 663 nm and 645 nm was measured using a spectrophotometer device. The instrument was calibrated using a control sample consisting of 80% acetone. The determination of chlorophyll a, chlorophyll b, and total chlorophyll for each sample was conducted utilizing the following formulas:

$$Chl\ a = \frac{(12.7\ x\ D_{663}) - (2.69\ x\ D_{645})\ x\ V}{1000\ x\ W}$$

$$Chl\ b = \frac{(22.9\ x\ D_{645}) - (4.68\ x\ D_{663})\ x\ V}{1000\ x\ W}$$

$$Chl\ total = \frac{(20.2\ x\ D_{645})\ +\ (8.02\ x\ D_{663})\ x\ V}{1000\ x\ W}$$

The variables "V," "W," and "D" represent the volume of acetone 80% (20 ml or 25 ml), the fresh weight, and the optical absorption at wavelengths 663 nm and 645 nm, respectively.

Image acquisition and processing

Both nitrogen content analysis, chlorophyll using a spectrophotometer, and chlorophyll using SPAD 502 are considered as ground truth values that will be correlated with image data from both calibrated and uncalibrated cameras. Subsequent to the removal of the leaves, digital image of the external environment was conducted employing a smartphone camera equipped with the subsequent specifications and settings (Table 1).

Calibration using the spydercheckr48 color palette is performed on photos generated from the camera in order to reproduce colors better as in the original, thus can be used to analyze chlorophyll or nitrogen content better than without calibration. Calibration is done with software Adobe Lightroom Classic version 11.4.1 (Adobe, San Jose, CA, USA) and Spydercheckr version 1.6 (Data color, Lawrenceville, NJ, USA). Uncalibrated and calibrated image were then RGB-valued using ImageJ, an open-source image processing software (Schneider et al., 2012), and then converted to L*a*b. The L*a*b color space is considered superior for analyzing the color range from yellow to green in leaf color due to its enhanced perceptual uniformity compared to other color spaces, such as RGB or CMYK. The utilization of the Lab* color space for the analysis of the yellow to green spectrum in leaf coloration facilitates the comprehension of the impact of chlorophyll content on the visual appearance of leaves. Correlation analysis and figures were generated using Origin Pro 2021 version 9.8.0.200 (OriginLab Corporation OriginLab Corporation, Northampton, MA, USA).

Additionally, the acquired model was employed to transform the non-calibrated camera images into SPAD values, nitrogen content, chlorophyll a, chlorophyll b, and total chlorophyll. The values derived from the model were subsequently compared with the empirical values obtained from SPAD measurements and laboratory analysis, encompassing nitrogen content, chlorophyll a, chlorophyll b, and total chlorophyll. The accuracy computation involved the utilization of the discrepancy between the modelled and actual data.

RESULTS AND DISCUSSION

Relationship of SPAD and Nitrogen content with leaf chlorophyll content

The SPAD meter is a tool that enables rapid and non-invasive quantification of chlorophyll concentrations in plant foliage. This measurement provides a direct assessment of photosynthetic efficacy and the overall health of the plant. The correlation between chlorophyll and SPAD meters originates in the capacity of SPAD meters to offer a quantitative assessment of chlorophyll levels in plant foliage. SPAD meters provide the capability to assess plant health and productivity by quantifying leaf greenness. The utilization of this linkage has the potential to facilitate the monitoring of plant conditions, identification of nutrient deficiencies or plant stress, and provision of crucial information for the optimization of plant growth. There was a significant relationship between the SPAD value and chlorophyll content, encompassing chlorophyll a, b, and total. As the SPAD value increases, there was a corresponding increase in the chlorophyll content. The regression coefficients for chlorophyll a, b, and total were 0.6733, 0.5130, and 0.6422, respectively (Figure 3). The primary pigment responsible for absorbing sunlight during the process of photosynthesis in plants is chlorophyll. Consequently, greater SPAD values are indicative of more efficient rates of photosynthesis.

CIE-lab color space relationship with SPAD value

The color spaces L*, a*, and b* had notable correlation coefficients with the SPAD value (Figure 4). The calibrated image exhibited a stronger correlation value in the L* color space. The calibrated L* color space exhibited a broader range than the uncalibrated model. In the context of the a* color space, it can be observed that the uncalibrated image had a slight advantage compared to the calibrated image, despite both images demonstrating strong correlation values. The color space b* exhibited a significant rise, with the correlation value rising from -0.487 in the uncalibrated image to -0.797 after calibration. The color of the digital image demonstrated a strong correlation with the SPAD value, which becomes more pronounced when the digital image is calibrated. All of these color space values show a high correlation value that exceeds the use of RGB. Research by Ibrahim et al. (2021) on lettuce leaves showed RGB values only correlated with 0.794, 0.346, 0.387, respectively.

CIE-lab color space relationship with nitrogen content

Similarly, nitrogen content showed a significant correlation in all color spaces (Figure 5). The increase in correlation was found in the L* color space after calibration, from –0.668 to –0.809. When operating within the a* color space, the distinction between the calibrated and uncalibrated photographs was negligible. The b* color space demonstrates the most notable and prominent improvement. The uncalibrated digital image exhibits a correlation coefficient of -0.214, whereas the calibrated digital image demonstrates a correlation coefficient that was twice as large, measuring -0.548. The results indicate that the calibration process significantly improved the accuracy of the values in the digital image. The process of image calibration, when executed with accurate and well-defined color settings, is expected to produce an image that is identical to the original color of the sample. This is intended to enhance the accuracy of color information, hence facilitating the detection of nitrogen content.

CIE-lab color space relationship with chlorophyll a

Chlorophyll a, being the primary pigment involved in the process of photosynthesis, plays an essential role in the absorption of solar energy. It possesses a distinctive capability to capture light throughout the red and blue wavelength ranges, typically spanning from approximately 430 to 662 nm (Bartolome et al., 2020; Zepka et al., 2019). The calibrated image exhibited a modest rise in the L* color space (R=-0.836, P<0.01) in contrast to the uncalibrated image (R=-0.648, P<0.01), despite both images displaying a strong correlation. This effect was also observed in the a* color space. In contrast, the b* color space exhibited a substantial enhancement in the calibrated image captured by a smartphone. Before the calibration, there was no substantial relationship between the color space b* and chlorophyll a, as indicated by no significant correlation (R=-0.099, P>0.05). Nevertheless, the process of calibration utilizing spydercheckr 48 demonstrated a strong correlation between the color space b* and the camera that performed calibration. The color space b* is a metric used to quantify the location of colors along the blue-to-yellow axis. Within the framework of b*, the horizontal axis of the Lab color space represents the blue-to-yellow axis, progressing from left to right. Positive values of b* are indicative of a greater yellow coloration, whilst negative values of b* are indicative of a greater blue coloration.

CIE-lab color space relationship with chlorophyll b

Chlorophyll b is a photosynthetic pigment that serves the purpose of absorbing light energy within the wavelength range of 453-642 nm, which is beyond the capacity of chlorophyll a. Chlorophyll b expands the range of light absorption in plants by effectively capturing light at distinct blue and red wavelengths. This broadening of the light spectrum enhances photosynthetic efficiency and confers an adaptive advantage in response to variations in external light conditions. Furthermore, the involvement of chlorophyll b in the energy transfer to chlorophyll a during the light-dependent phase of photosynthesis is important (Bartolome et al., 2020; Zepka et al., 2019). Similar to the correlation observed between the CIE-Lab color space and chlorophyll a, the association between the CIE-Lab color space and chlorophyll b exhibited a noticeable increase in the b* color space. Prior to calibration, the data exhibited an insignificant correlation, as shown by a R value of 0.032. However, following the calibration process, a significant correlation was observed, with a R value of -0.328 (see Figure 7).

CIE-lab color space relationship with total chlorophyll

The aforementioned trend continued in relation to the aggregate measurement of chlorophyll, which includes both chlorophyll a and chlorophyll b. The correlation value in the L* color space exhibited a significant increase from R=-0.593 (P<0.01) to R=-0.801 (P<0.01) at the calibration process. The color space a* exhibited a minor decline, with a uncalibrated value of -0.735 (P<0.01), then decreases to -0.577 (P<0.01) when the image performs calibration. In the same way, the color space b* demonstrated significant effects of digital image calibration, with a correlation coefficient of R=-0.047 (P>0.05) observed before calibration, which improves to -0.437 (P<0.01) applying calibration.

Estimated SPAD, Nitrogen, and chlorophyll content using smartphone digital image

The utilization of calibrated images indicating increased correlation across various variables (SPAD, nitrogen, chlorophyll a, chlorophyll b, and total chlorophyll) offers the possibility of facilitating the analysis of parameter values. The process of calibrating an image is often complex due to the necessity of software assistance, which can be time-consuming and impractical for farmers. Hence, it is imperative to establish a correlation between the uncalibrated image and the analysis of crop parameters. This can be achieved by correlating the CIE-lab values of the image data before and after calibration, resulting in the derivation of an equation. The three color space values L*, a*, and b* showed significant correlation with values of 0.864, 0.918, and 0.842, respectively. Color calibration is important because it is related to lighting conditions, where different light conditions allow the image color to be different, which in turn results in different accuracy (Astika and Khayati, 2019; Sunoj et al., 2018).

The trial results from 11 digital image samples showed that the accuracy of SPAD with the L*, a*, and b* color spaces was more than 85% (Table 2). This indicated that the CIE-lab color space has the potential to assist observers in analyzing through smartphone cameras. Employing a color space alternative to RGB enables the attainment of an enhanced color range due to its expansive gamut of colors, perhaps leading to improved outcomes (Sunoj et al., 2018; Shrivastava & Pradhan, 2021). The CIE-Lab color space demonstrated an adequate degree of accuracy, averaging around 80% in most cases. However, it is worth noting that the b* color space exhibited a slightly lower accuracy rate of 79%. The utilisation of CIE-Lab color space proves beneficial in the examination of nitrogen levels in rice leaves, particularly subsequent to the calibration of the image with a color standard. In contrast, the accuracy values of chlorophyll a and chlorophyll b were rather low, ranging from 62.3% to 74.6% in color space a* and from 66.4% to 73.5% in color space b*. The exacerbation of this issue was evident in the lowest accuracy values recorded in these two color spaces, which demonstrate only 19.0% and 26.2% respectively. Hence, it was imperative to implement enhancements in order to achieve a minimum accuracy threshold of 85%. Both chlorophyll a and b have an impact on the overall chlorophyll measurement. The accuracy of total chlorophyll is relatively low because of the low accuracy values of chlorophyll a and b. The L* color space exhibits an accuracy value of 75.7%, whilst the a* and b* color spaces demonstrate accuracies of 70.8% and 66.1% respectively.

CONCLUSION

There was a significant correlation between plant parameters (SPAD, N content, chlorophyll a, b, and total chlorophyll) and images obtained from smartphones based on the CIE-Lab color space. The correlation value increased when calibration was done first using a color palette. With the high correlation value, the use of smartphone camera images has great potential in estimating these plant parameters. The accuracy of SPAD and nitrogen content values was found to be high, ranging from 79.0% to 92.3%, depending on the L*a*b color space used. In meanwhile, it is observed that the accuracy values for chlorophyll a and b remain relatively low, ranging from 65.3% to 74.6%. Similarly, the accuracy of the total chlorophyll content was reported to be between 66.1% and 75.7%.

ACKNOWLEDGMENTS

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TABLES AND FIGURES

MATERIALS AND METHODS

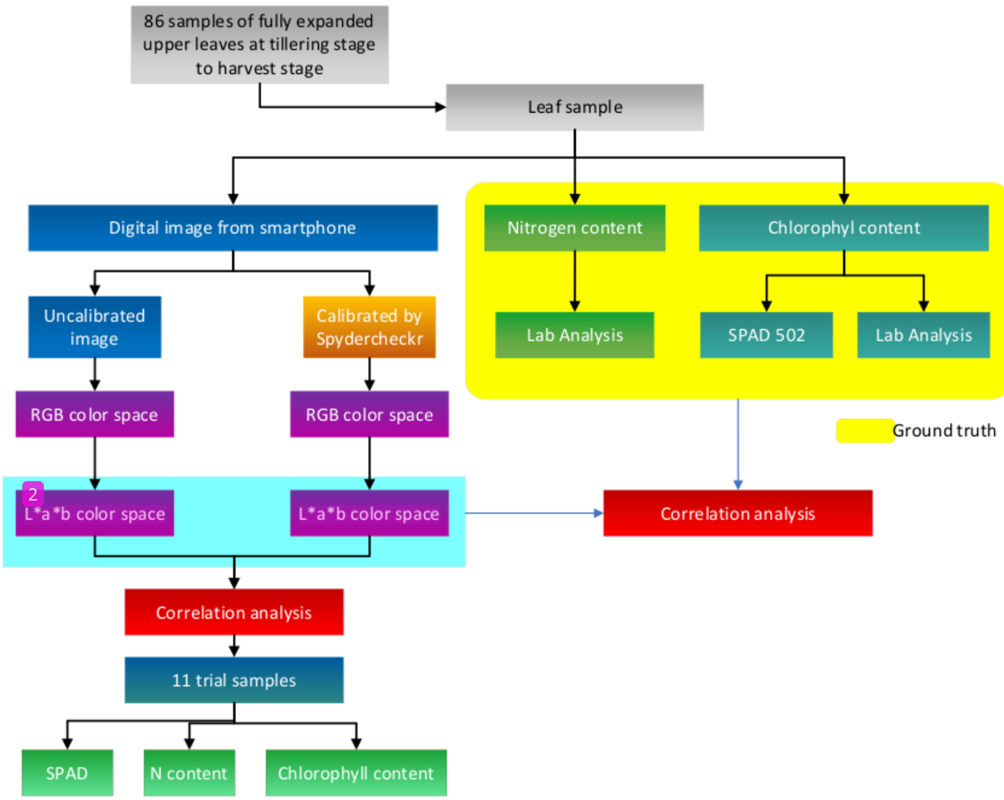

Figure 1. Procedure leaf sampling and analysis

Table 1. Smartphone camera spesifications and settings for obtaining digital image data

Spesifications			Settings		
Brand	:	Vivo	ISO	:	100
Model	:	V23e	Shutter speed	:	1/400
Resolution	:	64MP	White balance	:	Sunshine
Aperture	:	f/1.8	Autofocus	:	On
Focal length	:	26 mm	Camera height	:	47.6 cm
Feature	:	HDR	Zoom	:	3.0 x

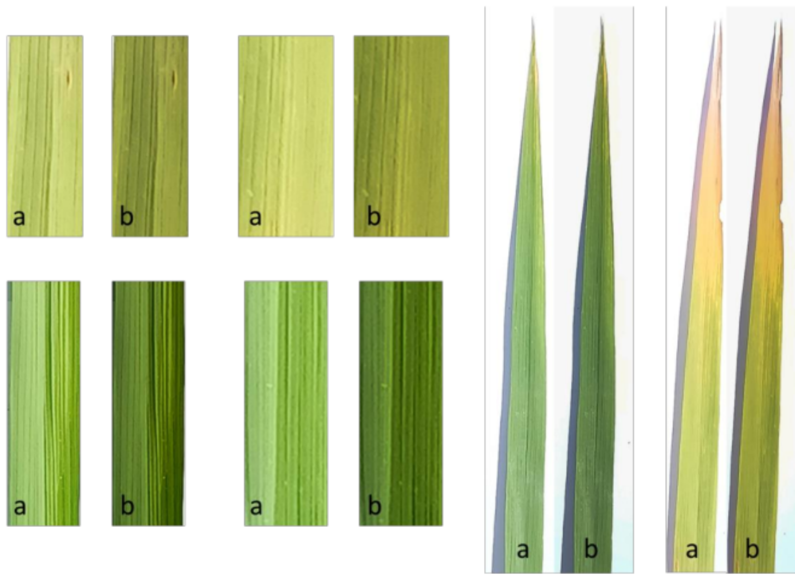

Figure 2. Images of leaf color digital image changes (a) before calibration and (b) after calibration using spydercheckr 48

RESULTS AND DISCUSSION

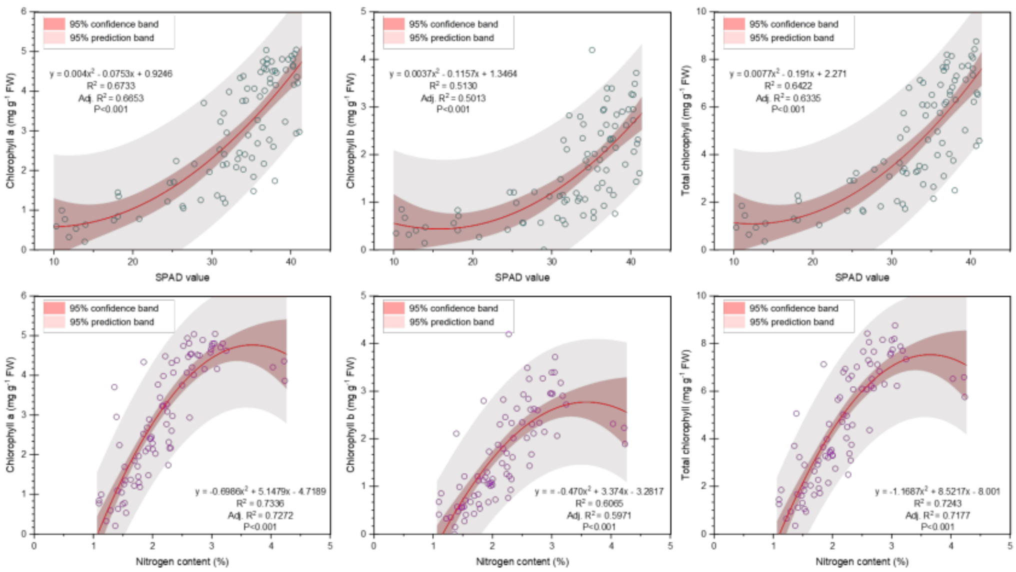

Figure 3. Relationship between SPAD value or nitrogen content and chlorophyll a, b, and total content

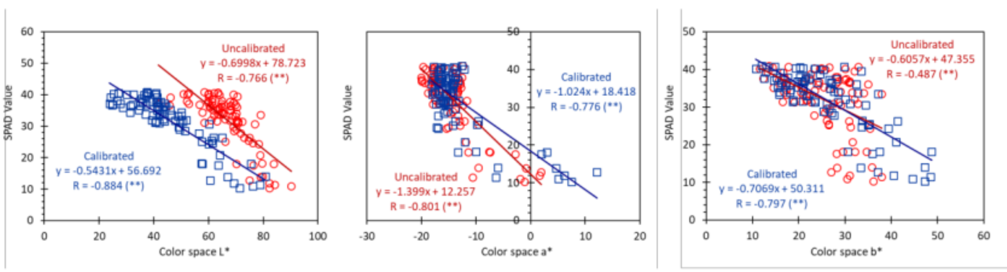

Figure 4. Comparison of correlation between SPAD and L*a*b color space digital images with and without calibration

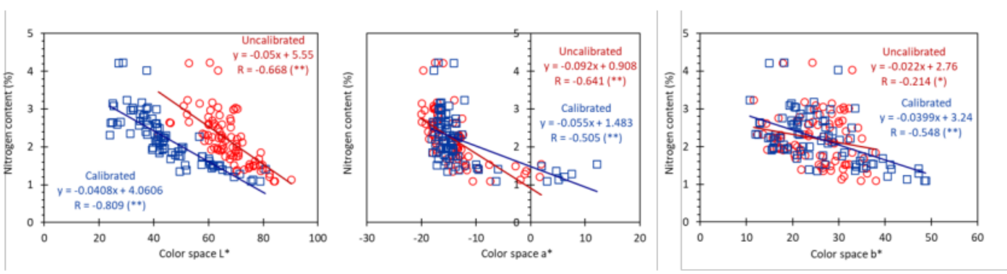

Figure 5. Comparison of correlation between nitrogen content and L*a*b color space digital images with and without calibration

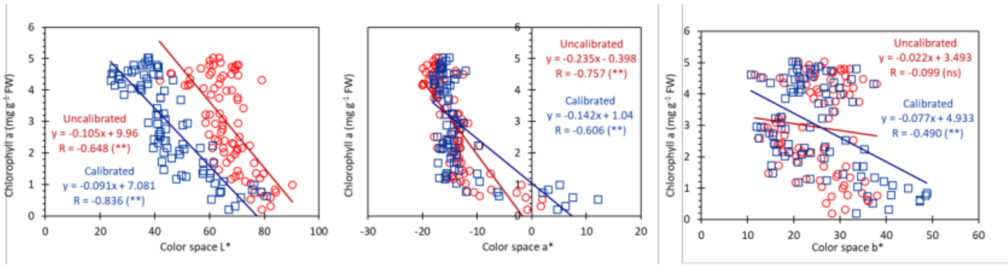

Figure 6. Comparison of correlation between chlorophyll a and L*a*b color space digital images with and without calibration

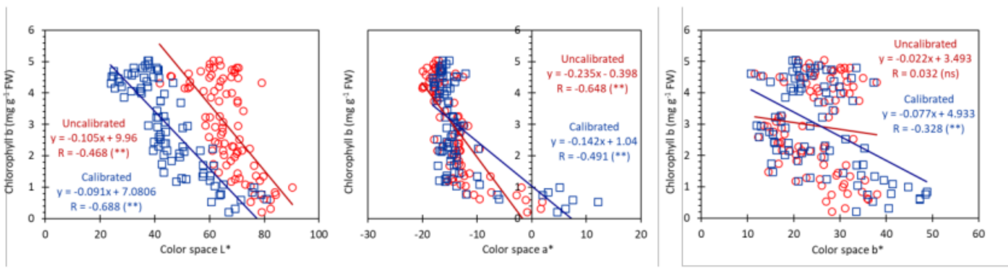

Figure 7. Comparison of correlation between chlorophyll b and L*a*b color space digital images with and without calibration

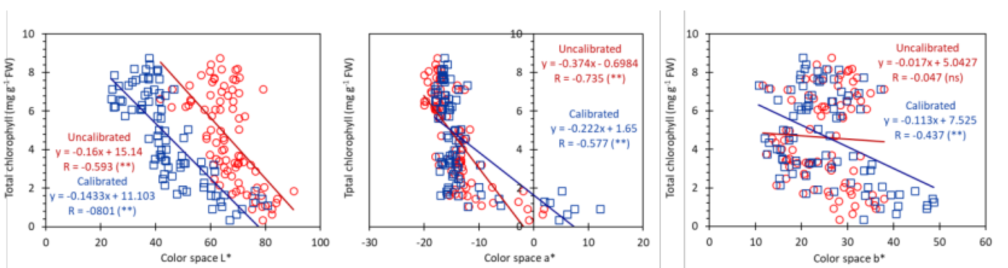

Figure 8. Comparison of correlation between total chlorophyll and L*a*b color space digital images with and without calibration

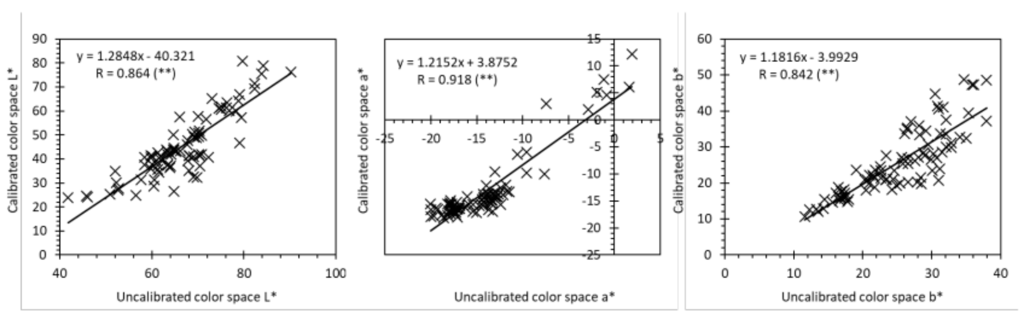

Figure 9. Correlation of color space L*a*b values between calibrated and uncalibrated images

Table 2. Accuracy analysis of color from trial samples of smartphone digital images

Color space L*					
	SPAD	N content	Chlorophyll a	Chlorophyll b	Total chlorophyll
Average	92.3	84.7	74.6	73.5	75.7
Std. Dev.	7.0	9.1	14.0	16.0	14.8
RMSE	12.6	0.19	1.16	0.57	3.24
Min	76.3	68.9	48.5	51.0	53.4
Max	98.8	96.4	96.1	91.9	97.4
Color space a*					
	SPAD	N content	Chlorophyll a	Chlorophyll b	Total chlorophyll
Average	87.9	81.9	70.6	69.9	70.8
Std. Dev.	8.9	13.4	23.0	18.8	18.2
RMSE	17.6	0.21	0.95	0.44	2.57
Min	66.5	52.1	19.0	26.2	34.5
Max	96.72	99.65	94.52	98.98	92.76
Color space b*					
	SPAD	N content	Chlorophyll a	Chlorophyll b	Total chlorophyll
Average	87.9	79.0	65.3	66.4	66.1
Std. Dev.	8.9	10.0	18.5	12.7	13.5
RMSE	23.2	0.29	1.69	0.70	4.46
Min	74.4	62.5	30.5	54.1	44.1
Max	98.5	98.3	91.7	94.6	89.7

Estimating SPAD, nitrogen concentration, chlorophyll a, b, and total chlorophyll in rice leaves using calibrated smartphone digital image

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