Jurnal Riset Biologi dan Aplikasinya, Volume 7, Issue 1, March 2025

Jurnal Riset Biologi dan Aplikasinya
https://journal.unesa.ac.id/index.php/risetbiologi

# Assessing Foliar Chlorophyll Content with SPAD-502 Chlorophyll Meter: A Comparison with Spectrophotometer Method in Various Plants

**Siti Nurbaiti[1], Asri Fajar Milasari[1], Wiko Arif Wibowo[1], Elvian Indah Nilamsari[2], Diah Rachmawati[1*]**
[1]Department of Tropical Biology, Faculty of Biology, Universitas Gadjah Mada
Jalan Teknika Selatan, Sekip Utara, Yogyakarta, Indonesia, 55281
[2]Division of Biological Science, Graduate School of Science and Technology, Nara Institute of Science and Technology
Ikoma 630-0101, Japan
* Corresponding author, e-mail: drachmawati@ugm.ac.id

## Article History

Received : 1 February2025
Revised : 18 February 2025
Accepted : 26 March 2025
Published : 31 March 2025

## Keywords

Chlorophyll analysis, Non-destructive measurement, Spectrophotometry; Leaf pigment

## ABSTRACT

Measuring chlorophyll content in plants is one of the main points that has never been ignored in various plant biology and agronomy research studies. Chlorophyll content is a parameter of growth and development, diagnosis of nutritional status, and response to environmental conditions. This study aimed to compare and determine the relationship between SPAD-502 chlorophyll meter readings and chlorophyll content obtained from acetone extraction followed by spectrophotometry measurement. Various leaves with different colors and thicknesses were used to determine the reliability of SPAD readings across diverse morphological traits. The results showed higher SPAD readings in leaves with a greener color. SPAD value was found to have a good linear relationship (r 0.8 and $R^2$ 0.64) and a positive correlation with total chlorophyll content, though variations due to leaf thickness suggest the need for correction factors. Furthermore, SPAD has potential as a rapid, non-destructive tool for monitoring plant health and nutrient status in agriculture, plant breeding, and horticulture. This tool can contribute to optimizing crop yield and managing fertilization practice, especially where the maintaining leaf integrity is essential for both commercial and aesthetic value. Species-specific calibration models are recommended to enhance measurement accuracy.

**How to cite**: Nurbaiti, S., Milasari AF., Wibowo WA., Nilamsari EI & Rachmawati D. (2025). Assessing Foliar Chlorophyll Content with SPAD-502 Chlorophyll Meter: A Comparison with Spectrophotometer Method in Various Plants. *Jurnal Riset Biologi dan Aplikasinya*, 7(1): 50-56. DOI: 10.26740/jrba. V7n1.p.50-56

## INTRODUCTION

Plants, as the first trophic level in energy flow of ecosystems, play an important role in photosynthesis, which is required for growth, development, and reproduction. Environmental conditions greatly influence photosynthesis and its efficiency, with high temperatures and water availability being key limiting factors that can restrict and reduce plant productivity (Chanaphai et al., 2023). One of the most critical components of photosynthesis is chlorophyll, a green pigment that plays a major role in capturing sunlight energy.

Measuring chlorophyll levels in plants is essential for understanding plant health and productivity. Accurate information regarding plant chlorophyll levels can help identify plant nutritional conditions, diagnose plant stress, monitor plant health, and optimize cultivation practices to increase crop yields and product quality (Oliveros et al., 2021; Wang et al., 2023). Because leaf greenness is often correlated with nitrogen content, given that approximately 70% of a leaf's nitrogen is contained in chloroplasts, chlorophyll content serves as a useful indicator of a plant's nutritional status (Ghosh et al., 2023; Khoddamzadeh & Souza Costa, 2023; Wicharuck et al., 2024). Moreover, chlorophyll levels have an impact on the development and quality of fruit and seeds containing embryos, which are crucial for plant propagation and food security (Pallavolu et al., 2023). Consequently, breeding programs and genetic engineering efforts often aim to enhanced nutrient-use efficiency, making chlorophyll assessment vital in plant science.

Traditionally, chlorophyll quantification relies on chemical extraction followed by spectrophotometric measurements (Tan et al., 2021). However, this method is destructive, time-consuming, and the use of chemicals potentially affects the environment (Wicharuck et al., 2024). The use of non-destructive plant chlorophyll-measuring tools, such as SPAD (Soil Plant Analysis Development), has emerged as an alternative and is now the most widely used tool that allows direct and rapid measurement of plant chlorophyll levels and provides reliable results (H. H. Lin et al., 2020; Zhang et al., 2022). Recent studies have validated the accuracy of SPAD readings by comparing them to spectrophotometric measurements, hyperspectral imaging, and reflectance spectrum analysis (Hu et al., 2024; Oliveros et al., 2021; Wang et al., 2023).

Despite its advantages, the relationship between SPAD readings and spectrophotometric chlorophyll content remains variable across different plant species and leaf characteristics. Variations in leaf thickness, morphology, and species-specific characteristics can influence the accuracy of SPAD reading. In the thick, waxy leaves of Arabica coffee, SPAD has been utilized for monitoring nitrogen status (Wicharuck et al., 2024). Meanwhile, in other thick-leaved species like orchids, SPAD has been used to estimate chlorophyll content or to measure chloroplast movement induced by blue light, but without correlation analysis with the extracted chlorophyll content (Banjare et al., 2023; Cho et al., 2024; Ko et al., 2020; Lin et al., 2019). Therefore, in this study, we aimed to determine the relationship between SPAD-502 chlorophyll meter readings and chlorophyll content measured via UV-vis spectrophotometry. Here, we extend the use of the SPAD across multiple ornamental plant species with different leaf characters and develop predicted equations to provide a reference for the rapid, non-destructive estimation and determination of chlorophyll in batch samples.

## MATERIALS AND METHODS

This study was conducted from August to October 2024 at the Laboratory of Plant Physiology, Faculty of Biology, Universitas Gadjah Mada, Yogyakarta, Indonesia. The material used in this research consisted of six species of ornamental plants planted and grown in the areas around the Faculty of Biology (7°46′10″S 110°22′44″). The research methods included leaf sampling, SPAD measurement, chlorophyll extraction and measurement, and data analysis. Sampling was carried out in the field using a purposive sampling method, considering the different colors and the thickness of leaves.

In SPAD measurements (Konica Minolta Sensing, Inc., Tokyo, Japan), five SPAD readings were taken from each leaf. The average reading was presented as the SPAD value. Immediately following SPAD measurement, the leaves were extracted for chlorophyll measurement using a spectrophotometer. A total of 100 mg of leaves was ground with liquid nitrogen in a mortar and pestle, and then 10 ml of 80% (v/v) chilled acetone was added. The samples were kept in the dark for one hour at 4° C, followed by filtering with filter paper. The absorbance was measured using a spectrophotometer (simpliNano, Biochrome) at 645 nm and 663 nm, and chlorophyll content was determined by the method of Arnon (1949) as shown in the equation below and expressed on fresh weight (mg $g^{-1}$). Leave analysis was also carried out by making anatomical preparations from fresh samples. Digital images of anatomical sections were captured using a microscope and OptiLab Advanced Plus (Miconos) then followed by measurement of the leaf tissue thickness using image analysis software of image raster.

$$\text{Chl a (mg g}^{-1}) = [(12.7 * A663) - (2.69 * A645)] \times V/1000 \times W$$

$$\text{Chl b (mg g}^{-1}) = [(22.9 * A645) - (4.68 * A663)] \times V/1000 \times W$$

$$\text{Chl Total (mg g}^{-1}) = [(20.2 * A645) + (8.02 * A663)] \times V/1000 \times W$$

Note:

A = Optical density at the respective wavelength (nm)
V = Final volume of chlorophyll extract in 80% acetone
W = Fresh weight of the tissue extracted

Statistical analysis was carried out to evaluate the differences between groups using Analysis of Variance (ANOVA) and continued with the Duncan test ($p<0.05$) for significance. The analysis was performed using IBM SPSS Statistics 21 (IBM Corp., Armonk, NY, USA). Regression analysis was conducted to determine the relationship between chlorophyll levels measured by spectrophotometer and SPAD values. The significance of the correlations between measurements was determined by Pearson's correlation. The simple regression equation (Y = a + bx) was used with the 95% limits of confidence. The SPAD value was the independent variable, and the chlorophyll content was the dependent variable (Ates & Kaya, 2021; Li et al., 2020; Ruiz-Espinoza et al., 2010).

## RESULTS AND DISCUSSION

The use of SPAD to measure chlorophyll content was adapted and compared to the conventional method of chlorophyll extraction followed by spectrophotometric measurement. Several leaf plants were used in this study (Figure 1). Among the leaves of orchids, *Dendrobium hybrid* (a) is greener than *Dendrobium* sp. (b) and *Dendrobium fimbriatum* (c). The leaves of *Dendrobium hybrid* and *Dendrobium* sp. are thicker than *Dendrobium fimbriatum.* In addition, the leaf surface of *Dendrobium* sp. exhibits a purple color. In *Bougainvillea spectabilis*, leaves in (d) are greener and older than in (e), which are younger leaves. In *Euodia ridleyi*, the leaves in (g) are dominant in green, while (h) are dominant in yellow. The cross-sections of these leaves are presented in Figure 2.

Table 1 shows the result of both measurements of chlorophyll content. In SPAD measurement, the results indicated that leaves with a greener color had a higher value of SPAD unit. *Dendrobium hybrid* had higher SPAD values compared to *Dendrobium* sp. and *Dendrobium fimbriatum.* A similar pattern was depicted from the SPAD values between *Bougainvillea spectabilis,* where leave (1d) was greener than leaf (1e). Moreover, a clear comparison showed in the leaves of *Euodia ridleyi*, where leaves the leaves predominantly green in color (1g) had significantly higher SPAD values than the yellow leaves (1h).

According to Table 1, the total chlorophyll content measured by spectrophotometer ranged from 0.32 to 3.52 mg $g^{-1}$. In a comparison among the orchids, *Dendrobium* sp. had the lowest chlorophyll content and showed no difference from the yellow leaves of *Euodia ridleyi* (1h). *Dendrobium hybrid* had higher chlorophyll content than *Dendrobium fimbriatum.* However, both orchids showed insignificantly different from the green leaves of *Euodia ridleyi* (1g). *Bougainvillea spectabilis* (1d) had the highest content of chlorophyll in both methods of measurement and showed no significant difference with *Dracaena fragnans.* This pattern of chlorophyll content is also observed in seasonal color changes, where chlorophyll content tends to be higher in deeper green leaves in June than in April (Li et al., 2020).

Correlation relationships between SPAD and spectrophotometer measurement were analyzed, and the results are shown in Table 2. The data showed significant ($p < 0.05$) linear correlations between SPAD values and chlorophyll content. Based on the equation, the regression coefficients (0.033; 0.011; and 0.044 in Chl *a*, *b*, and total, respectively) were positive, meaning that the direction of influence of SPAD on spectrophotometer value was positive. The correlation coefficients (r), which represent the degree of association between SPAD values and chlorophyll content, were found in 0.77 – 0.80 with a high level of significance ($p < 0.01$). In this study, a significant coefficient determination ($R^2$) was obtained in 0.63 (Chl *a*), 0.60 (Chl *b*), and 0.64 (Chl total), meaning that SPAD values affecting spectrophotometer results were around 60%. Among Chl a, Chl b, and total chlorophyll, the highest values for both (r) and ($R^2$) were observed for total chlorophyll. Studies quantifying the relationship between chlorophyll concentration in leaves and SPAD readings usually describe the relationships as linear (Kumar & Sharma, 2019; Ruiz-Espinoza et al., 2010).

Research by Oliveros et al. (2021) found a linear model of the relationship between chlorophyll content and SPAD chlorophyll meter in three sugarcane cultivars under two different fertilization rates. A linear correlation was also found between SPAD and nitrogen concentration in grape leaves, which reflects the chlorophyll content, and it was stated that an SPAD chlorophyll meter could be appropriate for estimating nitrogen contents in grapevine leaves (Ates & Kaya, 2021). Measurement by Li et al. (2020) that used two methods of chlorophyll extraction showed a linear fit between SPAD and the actual chlorophyll content, with the relationship being stronger when chlorophyll content was expressed in area than in weight. The positive relationships observed in this study align with majority of reported studies that use linear regression to quantify the relationship between SPAD readings and chlorophyll content.

Regardless of the linear and positive relationship found in this study, it was noted that the results in orchid leaves showed different patterns. In orchids, high SPAD values were not proportionally followed by high chlorophyll content obtained by spectrophotometer measurements. *Dendrobium* sp., which has thicker leaves and a greater SPAD value, showed a lower total

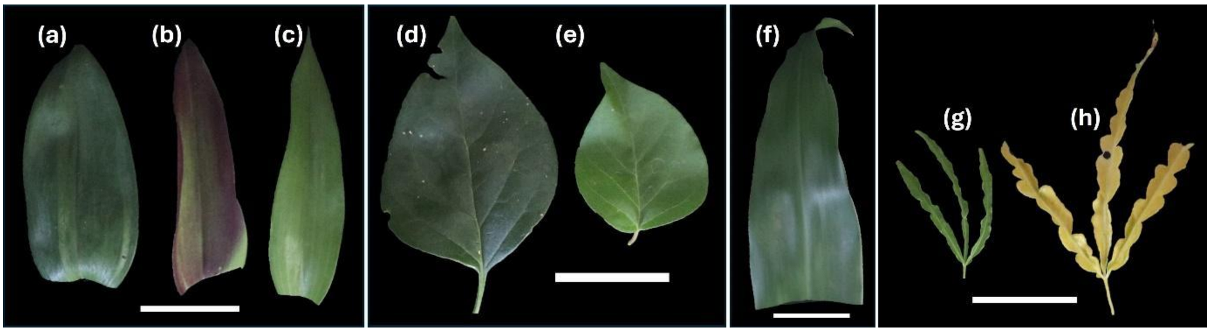


**Figure 1.** Various leaves samples with different colors. *Dendrobium hybrid* (a); *Dendrobium* sp. (b); *Dendrobium fimbriatum* (c); *Bougainvillea spectabilis* (d, e); *Dracaena fragrans* (f); *Euodia ridleyi* (g, h). Bar = 5 cm

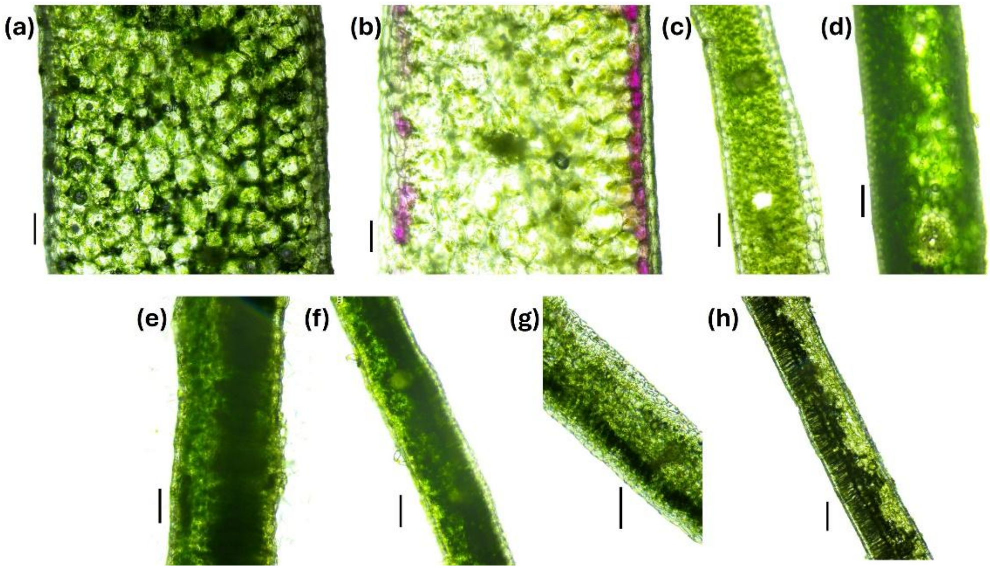


**Figure 2.** Cross section of various leaves used for chlorophyll measurement. *Dendrobium hybrid* (a); *Dendrobium* sp. (b); *Dendrobium fimbriatum* (c); *Bougainvillea spectabilis* (d, e); *Dracaena fragrans* (f); *Euodia ridleyi* (g, h). Bar = 1 mm.

**Table 1.** Chlorophyll content measured by SPAD reading meter and spectrophotometer

| Code | Sample | SPAD* | Chlorophyll (mg g$^{-1}$) | | |
|---|---|---|---|---|---|
| | | | Chl a* | Chl b* | Total* |
| 1a | *Dendrobium hybrid* | 58.25 ± 5.24$^{e}$ | 0.75 ± 0.07$^{b}$ | 0.35 ± 0.01$^{b}$ | 1.10 ± 0.08$^{b}$ |
| 1b | *Dendrobium* sp. | 34.32 ± 7.93$^{c}$ | 0.18 ± 0.02$^{a}$ | 0.11 ± 0.04$^{a}$ | 0.29 ± 0.05$^{a}$ |
| 1c | *Dendrobium fimbriatum* | 31.36 ± 1.68$^{c}$ | 0.65 ± 0.08$^{b}$ | 0.19 ± 0.08$^{ab}$ | 0.84 ± 0.12$^{b}$ |
| 1d | *Bougainvillea spectabilis* | 71.35 ± 2.48$^{f}$ | 2.56 ± 0.24$^{d}$ | 0.96 ± 0.06$^{d}$ | 3.52 ± 0.20$^{d}$ |
| 1e | *Bougainvillea spectabilis* | 48.90 ± 1.53$^{d}$ | 1.78 ± 0.25$^{c}$ | 0.67 ± 0.27$^{c}$ | 2.45 ± 0.50$^{c}$ |
| 1f | *Dracaena fragrans* | 64.95 ± 6.34$^{ef}$ | 2.58 ± 0.39$^{d}$ | 0.62 ± 0.08$^{c}$ | 3.20 ± 0.39$^{d}$ |
| 1g | *Euodia ridleyi* | 18.37 ± 0.90$^{b}$ | 0.73 ± 0.05$^{b}$ | 0.20 ± 0.05$^{ab}$ | 0.93 ± 0.09$^{b}$ |
| 1h | *Euodia ridleyi* | 4.43 ± 0.32$^{a}$ | 0.21 ± 0.05$^{a}$ | 0.11 ± 0.04$^{a}$ | 0.32 ± 0.08$^{a}$ |

Note: * Means within columns followed by same lowercase letters showed no differ significantly at $p < 0.05$ using Duncan test

**Table 2**. Relationship between chlorophyll content measured by spectrophotometer (Y) and SPAD reading (X) in various plants

| Components | Simple regression | Correlation coefficients (r) | Coefficients of determination ($R^2$) |
|---|---|---|---|
| Chl *a* | Y = 0.033x -0.209 | 0.79* | 0.63 |
| Chl *b* | Y = 0.011x -0.042 | 0.77* | 0.60 |
| Total Chl | Y = 0.044x -0.249 | 0.80* | 0.64 |

*Correlation is significant at 0.01 level of probability

chlorophyll than *Dendrobium fimbriatum.* These results are likely affected by leaf thickness. The precision of the measurement can be influenced by various factors, including the growth stage, leaf thickness, leaf position, measurement points on the leaf, moisture content, cultivar, year, and genetic variations (Chang & Robison, 2003; Yuan et al., 2016). Thicker leaves in orchids tend to have a larger volume or greater mass from the same leaf area compared to thinner leaves, which may result in lower chlorophyll content values obtained by spectrophotometry. Specific Leaf Weight (SLW), the ratio of leaf area to leaf dry weight and an indicator of leaf thickness, was reported as an important factor affecting chlorophyll content. Adding the SLW factor in multiple regressions resulted in an increase in correlation and determination coefficients compared with those values shown in the simple regression equation of SPAD and chlorophyll content (Ruiz-Espinoza et al., 2010; Yuan et al., 2016). Additionally , research conducted by Jinwen et al. (2009) on rice leaves with different thicknesses found that chlorophyll content was significantly correlated with leaf thickness based on leaf area compared to fresh weight. Thicker leaf, which is induced by high light intensity, has higher chlorophyll a/b ratios and chlorophyll content per unit area, but lower chlorophyll content per unit weight or volume.

Chang & Robison (2003) noted that in converting SPAD values to chlorophyll concentrations, different formulas need to be formulated for different species. Their research comparing the SPAD value with the percentage of nitrogen as a result of nitrogen fertilizer treatment on various plants showed that the slope of the linear regression curve was significantly different between species. It was also found that adding a water content component to the linear regression equation could increase the coefficient of determination between N concentration and SPAD. To increase the accuracy, the SPAD measurement must be calibrated for each cultivar and species (Oliveros et al., 2021; Zulkarnaini et al., 2019). Moreover, several other studies report on nonlinear relationships between chlorophyll concentration and SPAD values (Sim et al., 2015; Wakiyama, 2016).

Non-destructive methods based on the absorbance and/or reflectance of light by the intact leave, typically produce a chlorophyll index value that expresses relative chlorophyll content rather than absolute chlorophyll content per unit area or concentration per gram of leaf tissue (Ruiz-Espinoza et al., 2010). Despite the need for further investigation, the results show that SPAD could indicate relative chlorophyll content, especially in applications where precise values are not essential or for ornamental plants that require aesthetic maintenance. This method would be particularly useful for researchers or producers in identifying the senescence, a symptom of foliar nutrient deficiencies and decreased chlorophyll content.

## CONCLUSION

The results of SPAD showed that leaves with a greener color had higher scores than leaves with a less green color. SPAD value showed a positive and linear relationship with the actual chlorophyll content from the manually extracted method of a spectrophotometer, but leaf thickness has to be a correction factor. Calibration for each cultivar and species is also needed to increase its accuracy, as leaf chlorophyll content is affected by environmental factors, leaf traits, and crop nutritional conditions. Furthermore, as a valuable non-destructive tool for chlorophyll estimation and nutrient assessment in batch samples, SPAD is beneficial in monitoring horticulture, ornamental plants, and urban landscaping, where maintaining leaf integrity is crucial for aesthetic and commercial value. It provides a simple and rapid for assessing plant health in these settings while preserving foliage quality.

## ACKNOWLEDGMENTS

This research was supported by the Academic Excellent Program from the Directorate of Research Universitas Gadjah Mada number 6530/UN1.P1/PT.01.03/2024 to SN.

## REFERENCES

Arnon, D. I. (1949). Copper Enzymes in Isolated Chloroplasts Polyphenoloxidase in *Beta vulgaris*. *Plant Physiology*, 24(1), 1–15. https://doi.org/10.1104/pp.24.1.1

Ates, F., & Kaya, O. (2021). The Relationship Between Iron and Nitrogen Concentrations Based on Kjeldahl Method and SPAD-502 Readings in Grapevine (*Vitis vinifera* L. cv. 'Sultana Seedless'). *Erwerbs-Obstbau*, 63, 53–59. https://doi.org/10.1007/s10341-021-00580-8

Banjare, B., Tiwari, S. P., & Ghritlahare, M. K. (2023). Effect of growth media and plant growth regulators on growth attributes of orchid (*Dendrobium* L.). *The Pharma Innovation Journal*, 12(6), 4667–4670.

Chanaphai, P., Jongrungklang, N., Puangbut, D., & Songsri, P. (2023). Response of Photosynthetic and Root Traits of Sugarcane Genotypes Under Drought and Recovery Conditions. *Sugar Tech*, 25(5), 1102–1114. https://doi.org/10.1007/s12355-023-01288-7

Chang, S. X., & Robison, D. J. (2003). Nondestructive and rapid estimation of hardwood foliar nitrogen status using the SPAD-502 chlorophyll meter. *Forest Ecology and Management*, 181(13), 331–338. https://doi.org/10.1016/S0378-1127(03)00004-5

Cho, A., Chung, S. W., & Kim, Y. J. (2024). Calcium ammonium nitrate applications for improved leaf growth and photosynthetic responses in the CAM orchid Phalaenopsis under elevated CO2 in a greenhouse. *Scientia Horticulturae*, 324, 112601. https://doi.org/10.1016/j.scienta.2023.112601

Ghosh, M., Roychowdhury, A., Dutta, S. K., Hazra, K. K., Singh, G., Kohli, A., Kumar, S., Acharya, S., Mandal, J., Singh, Y. K., Pathak, S. K., & Gupta, S. K. (2023). SPAD Chlorophyll Meter-Based Real-Time Nitrogen Management in Manure-Amended Lowland Rice. *Journal of Soil Science and Plant Nutrition*, 23(4), 5993–6005. https://doi.org/10.1007/s42729-023-01457-3

Hu, X., Cao, Y., Zhou, P., Wu, Y., & Korohou, T. W. (2024). Rapid and Nondestructive Evaluation of Rice SPAD Value under Disease Stress Using Hyperspectral Imaging Sensors. *Journal of Sensors*, 2024. 1–13. https://doi.org/10.1155/2024/3063206

Jinwen, L., Jingping, Y., Pinpin, F., Junlan, S., Dongsheng, L., Changshui, G., & Wenyue, C. (2009). Field Crops Research Responses of rice leaf thickness, SPAD readings and chlorophyll a/b ratios to different nitrogen supply rates in paddy field. *Field Crops Research*, 114(13), 426–432. https://doi.org/10.1016/j.fcr.2009.09.009

Khoddamzadeh, A. A., & Souza Costa, B. N. (2023). Best Nitrogen Management Practices Using Sensor-Based Smart Agriculture in Nursery Production of Cacao. *Horticulturae*, 9(4). https://doi.org/10.3390/horticulturae9040454

Ko, S. S., Jhong, C. M., & Shih, M. C. (2020). Blue light acclimation reduces the photoinhibition of *Phalaenopsis aphrodite* (Moth orchid). *International Journal of Molecular Sciences*, 21(17), 1–17. https://doi.org/10.3390/ijms21176167

Kumar, P., & Sharma, R. K. (2019). Development of SPAD value-based linear models for non-destructive estimation of photosynthetic pigments in wheat (*Triticum aestivum* L.). *Indian Journal of Genetics and Plant Breeding*, 79(1), 96–99. https://doi.org/10.31742/IJGPB.79.1.13

Li, J., Zhou, X., Zhou, J., Shang, R., Wang, Y., & Jing, P. (2020). Comparative Study on Several Determination Methods of Chlorophyll Content in Plants. *IOP Conference Series: Materials Science and Engineering*, 730(1), 23-37. https://doi.org/10.1088/1757-899X/730/1/012066

Lin, H. H., Lin, K. H., Huang, M. Y., & Su, Y. R. (2020). Use of non-destructive measurements to identify cucurbit species (*Cucurbita maxima* and *Cucurbita moschata*) tolerant to waterlogged conditions. *Plants*, 9(9), 1–15. https://doi.org/10.3390/plants9091226

Lin, Y. J., Chen, Y. C., Tseng, K. C., Chang, W. C., & Ko, S. S. (2019). Phototropins Mediate Chloroplast Movement in *Phalaenopsis aphrodite* (Moth Orchid). *Plant and Cell Physiology*, 60(10), 2243–2254. https://doi.org/10.1093/pcp/pcz116

Oliveros, N., Tinini, R., Costa, D. dos S., Ramos, R., Wetterich, C., & Teruel, B. (2021). Predictive models of chlorophyll content in sugarcane seedlings using spectral images. *Engenharia Agricola*, 41(4), 475–484. https://doi.org/10.1590/1809-4430-Eng.Agric.v41n4p475-484/2021

Pallavolu, L. A., Pasala, R., Kulasekaran, R., Pandey, B. B., Virupaksham, U., & Perika, S. (2023). Analysing the SPAD dynamics of water-stressed vs. well-watered sesame (*Sesamum indicum* L.) accessions and establishing their relationship with seed yield. *PeerJ*, 11. https://doi.org/10.7717/peerj.14711

Ruiz-Espinoza, F. H., Murillo-Amador, B., García-Hernández, J. L., Fenech-Larios, L., Rueda-Puente, E. O., Troyo-Diéguez, E., Kaya, C., & Beltrán-Morales, A. (2010). Field Evaluation of the Relationship between Chlorophyll Content in Basil Leaves and A Portable Chlorophyll Meter (SPAD-502) Readings. *Journal of Plant Nutrition*, 33(3), 423–438.

https://doi.org/10.1080/01904160903470463

Sim, C. C., Zaharah, A. R., Tan, M. S., & Goh, K. J. (2015). Rapid determination of leaf chlorophyll concentration, photosynthetic activity and NK concentration of *Elaies guineensis* via correlated SPAD-502 chlorophyll index. *Asian Journal of Agricultural Research*, 9(3), 132–138. https://doi.org/10.3923/ajar.2015.132.138

Tan, L., Zhou, L., Zhao, N., He, Y., & Qiu, Z. (2021). Development of a low-cost portable device for pixel-wise leaf SPAD estimation and blade-level SPAD distribution visualization using color sensing. *Computers and Electronics in Agriculture*, 190, 106487. https://doi.org/10.1016/j.compag.2021.106487

Wakiyama, Y. (2016). The relationship between SPAD values and leaf blade chlorophyll content throughout the rice development cycle. *Japan Agricultural Research Quarterly*, 50(4), 329–334. https://doi.org/10.6090/jarq.50.329

Wang, N., Yang, G., Han, X., Jia, G., Li, Q., Liu, F., Liu, X., Chen, H., Guo, X., & Zhang, T. (2023). Study of the spectral characters–chlorophyll inversion model of *Sabina vulgaris* in the Mu Us Sandy Land. *Frontiers in Earth Science*, 10, 1–15. https://doi.org/10.3389/feart.2022.1032585

Wicharuck, S., Suang, S., Chaichana, C., Chromkaew, Y., Mawan, N., Soilueang, P., & Khongdee, N. (2024). The implementation of the SPAD-502 Chlorophyll meter for the quantification of nitrogen content in Arabica coffee leaves. *MethodsX*, 12, 1–5. https://doi.org/10.1016/j.mex.2024.102566

Yuan, Z., Cao, Q., Zhang, K., Ata-Ul-Karim, S. T., Tan, Y., Zhu, Y., Cao, W., & Liu, X. (2016). Optimal leaf positions for SPAD meter measurement in rice. *Frontiers in Plant Science*, 7, 1–10. https://doi.org/10.3389/fpls.2016.00719

Zhang, R., Yang, P., Liu, S., Wang, C., & Liu, J. (2022). Evaluation of the Methods for Estimating Leaf Chlorophyll Content with SPAD Chlorophyll Meters. *Remote Sensing*, 14, 1–17. https://doi.org/10.3390/rs14205144

Zulkarnaini, Z. M., Sakimin, S. Z., Mohamed, M. T. M., & Jaafar, H. Z. E. (2019). Relationship between chlorophyll content and soil plant analytical development values in two cultivars of fig (*Ficus carica* L.) as brassinolide effect at an open field. *IOP Conference Series: Earth and Environmental Science*, 250(1). https://doi.org/10.1088/1755-1315/250/1/012025.