

Correlation between SPAD and chlorophylls *a*, *b* and total in leaves from *Vaccinium corymbosum* L. cv. Biloxi, Legacy and Victoria in the high tropics

Correlación del SPAD y las clorofilas *a*, *b* y total en hojas de *Vaccinium corymbosum* L. cv. Biloxi, Legacy y Victoria en trópico alto



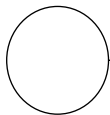
ELBERTH HERNANDO PINZÓN-SANDOVAL^{1, 2}
PEDRO JOSÉ ALMANZA-MERCHÁN¹
GERMAN EDUARDO CELY-REYES¹
PABLO ANTONIO SERRANO-CELY¹
GUILLERMO ALEXANDER AYALA-MARTÍNEZ¹

Blueberry cv. Biloxi.

Photo: E.H. Pinzón-Sandoval

ABSTRACT

Blueberries in the Ericaceae family are important, cultivable species for commercial and industrial purposes. Critical parameters for evaluating the nutritional and physiological status of plants include estimates of photosynthetic pigments such as chlorophylls; however, this is a destructive, expensive and time-consuming method that is not easily accessible for agricultural producers. For this reason, in recent years, several technological solutions have been made available, such as the SPAD-502 chlorophyll index meter, which has demonstrated efficiency in terms of rapid estimates of total chlorophyll content in the field. Therefore, the objective of this research was to evaluate the contents of chlorophylls *a*, *b* and total obtained with spectrophotometry and to determine the correlation with the non-destructive method (SPAD) in blueberry plant (*Vaccinium corymbosum* L.) cultivares Biloxi, Legacy and Victoria, in the vegetative phase under conditions of the municipality of Paipa-Boyaca (Colombia). The data obtained with the SPAD-502 ranged from 57.4 to 62.8 SPAD in the 'Victoria', 61.2 to 68.3 in the 'Biloxi', and 68.2 to 73.1 in the 'Legacy'. The content of chlorophylls *a*, *b* and total presented a positive and significant correlation with the SPAD index, with a correlation coefficient (*r*) greater than 0.91 and adjustment to simple linear regression models with a coefficient of determination (*R*²) greater than 0.90 in the three cultivars. This confirms the usefulness and importance of using SPAD for non-destructive estimation of chlorophyll content under field conditions.



Additional key words: blueberry; cultivars; photosynthetic pigments; physiological status.

¹ Universidad Pedagógica y Tecnológica de Colombia, Facultad de Ciencias Agropecuarias, Grupo de Investigación en Desarrollo y Producción Agraria Sostenible, Tunja (Colombia). ORCID Pinzón-Sandoval, E.H.: 0000-0001-9229-3450; ORCID Almanza-Merchán, P.J.: 0000-0002-9207-0617; ORCID Cely-Reyes, G.E.: 0000-0001-6312-3575; ORCID Serrano-Cely, P.A.: 0000-0002-1270-3024; ORCID Ayala-Martínez, G.A.: 0000-0001-8887-8800

² Corresponding author. elberth.pinzon@uptc.edu.co

RESUMEN

Los blueberry dentro de la familia Ericaceae, son especies cultivables importantes con fines comerciales e industriales. Dentro de los parámetros críticos para evaluar el estatus nutricional y fisiológico de la planta, es la estimación de pigmentos fotosintéticos como las clorofilas; sin embargo, este es un método destructivo, costo y demorado, al cual no pueden acceder fácilmente los productores agrícolas. Por esto en los últimos años, se han puesto a disposición varias soluciones tecnológicas, tales como el medidor de índice de clorofila SPAD-502 el cual ha demostrado su eficiencia en cuanto a la estimación rápida de los contenidos totales de clorofila en campo. Por lo anterior, el objetivo de la investigación fue evaluar los contenidos de clorofilas *a*, *b* y total obtenidas mediante espectrofotometría y su correlación frente al método no destructivo (SPAD) en plantas de blueberry (*Vaccinium corymbosum* L.) de los cultivares Biloxi, Legacy y Victoria, en fase vegetativa bajo condiciones del municipio de Paipa-Boyacá (Colombia). Los datos obtenidos con el SPAD-502 oscilaron entre 57,4 a 62,8 SPAD en la 'Victoria', 61,2 a 68,3 en la 'Biloxi' y 68,2 a 73,1 SPAD en la 'Legacy', el contenido de clorofilas *a*, *b* y total, presentaron una correlación positiva y significativa frente al índice SPAD con coeficiente de correlación (*r*) mayor a 0,91 y el ajuste a modelos de regresión lineal simple con coeficiente de determinación (R^2) superior a 0,90 en las tres cultivares evaluadas. Esto confirma la utilidad e importancia de usar el SPAD para la estimación no destructiva del contenido de clorofilas bajo condiciones de campo.

Palabras clave adicionales: arándanos; cultivares; pigmentos fotosintéticos; estatus fisiológico.

Received: 30-07-2022 Accepted: 08-09-2022 Published: 03-10-2022

INTRODUCTION

The Ericaceae family includes the *Vaccinium* genus, which has approximately 450 species worldwide, distributed mainly in the northern hemisphere (Lim, 2012). The blueberry is one of these species (*Vaccinium corymbosum* L.), is native to the United States, and is the most important species cultivated for commercial and industrial purposes because of its size and variety of antioxidants in the fruits (Miao *et al.*, 2022). These properties have generated great interest, especially in the nutritional pharmaceutical industry where it is known as a "super fruit" that is used in the treatment of various diseases (You *et al.*, 2011; Stevenson and Scalzo, 2012). In Colombia, the departments of Cundinamarca and Boyaca are the larger producers, where all production is used to satisfy local consumption (Fernández-Vargas *et al.*, 2020). Therefore, blueberry cultivation represents an opportunity for continuous production that could meet domestic demand and help meet the needs of countries in the northern hemisphere in the winter months (Cortés-Rojas *et al.*, 2016).

However, blueberry production can be affected by the shallow and highly branched root system, which has little or no root hairs, with consequent restrictions

on the ability of plants to absorb water and nutrients (Kim *et al.*, 2011). This can affect the synthesis of photosynthetic pigments such as chlorophylls (Yu *et al.*, 2015).

The mineral nutrition of crops is of great concerns for producers. Diagnostic tools such as soil or tissue analysis are currently used, which are expensive and time-consuming, limiting rapid decision-making (Güiza-Castillo *et al.*, 2020). Destructive methodologies are used to quantify photosynthetic pigments that, while being important in the evaluation of the physiological status of plants, are expensive and not easily accessible for producers (Amarante *et al.*, 2009).

The chlorophyll content in leaves is one of the more important variables when evaluating the physiological status of plants (Amarante *et al.*, 2009). The leaf chlorophyll concentration is generally determined by extracting leaf samples, with subsequent spectrophotometric measurements (Porra *et al.*, 1989; Parry *et al.*, 2014). Such determinations are destructive, costly and time consuming and, therefore, may not be practical. There are also methods that use measurement

equipment, which estimate the chlorophyll concentration index quickly, non-destructively, and directly in the field (Güiza-Castillo *et al.*, 2020). These methods exploit the optical properties of leaves and are based on the reflectance and/or absorbance of radiation by chlorophyll (Uddling *et al.*, 2007).

In recent years, several technological solutions have been made available for real-time detection of the concentration of some photosynthetic pigments, such as chlorophylls and their relationship with nutrients such as nitrogen, improving plant management and nutritional values in crops (Edalat *et al.*, 2019). Direct chlorophyll measurement equipment is generally easy to use, does not require any specific user training, and takes measurements quickly with little data processing (Gianquinto *et al.*, 2006). However, given the relatively small sampling area, strict sampling protocols and calibration curves between readings and the chlorophyll concentration in tissue samples must be developed for practical uses (Netto *et al.*, 2005; Padilla *et al.*, 2019).

Measuring the chlorophyll index Soil Plant Analysis Development (SPAD) (Ghosh *et al.*, 2020) has been increasing in agriculture because it provides a fast, simple and convenient method to non-destructively measure the chlorophyll content of crops and leaves (Li *et al.*, 2019; Zhang *et al.*, 2020, Aldana *et al.*, 2014). In addition to their ease, small size, and relatively low cost, chlorophyll meters are well-suited for the field because they provide rapid assessments of chlorophyll concentrations in different crops (Padilla *et al.*, 2018). These benefits have created much interest in the rapid analysis of the total chlorophyll index (Mehrabi and Sepaskhah, 2022).

The SPAD has been widely used to directly determine the chlorophyll index, which has been correlated with elements such as nitrogen and magnesium in the leaves of different plant species, such as grapes (Castañeda *et al.*, 2018; Ates and Kaya, 2021), guava (Güiza-Castillo *et al.*, 2020), soybean (Yokoyama *et al.*, 2018), and wheat (Mehrabi and Sepaskhah, 2022). Therefore, the present study aimed to estimate the concentrations of chlorophylls *a*, *b* and total in blueberry leaves (*Vaccinium corymbosum* L.) from the Biloxi, Legacy and Victoria *c* using the spectrophotometric method to observe the correlation with the non-destructive SPAD method cultivars under the conditions of the municipality of Paipa (Boyaca).

MATERIALS AND METHODS

This study was carried out on the Tinguavita Experimental Farm of the Universidad Pedagógica y Tecnológica de Colombia (UPTC) in the municipality of Paipa (Boyaca, Colombia), with the coordinates 5°45' N and 73°45' W, at 2,525 m a.s.l., with an average temperature of 15.5°C and average monthly rainfall of 80.5 mm according to the weather station located on the farm during this research, which was carried out during the first semester of 2022.

Biloxi cultivar plants were used, which have medium-sized fruits, a light blue color, high firmness values, and excellent flavor. Legacy is a vigorous and productive cultivar, and its berries are medium-sized and very firm, with good flavor and little scaring (San Martín, 2013). Victoria is a vigorous, late-ripening cultivar with large, easy-to-harvest fruits. The plants were sourced from Fallcreek - Farm & Nursery, Inc. For each cultivar, 30 plants were selected 6 months after transplantation with an approximate height of 45 cm. The plants were planted under crystal-colored anti-hail mesh with a shading percentage of 3% according to the manufacturer's technical information, in 60 L bags with rice husks, pine shavings and black soil substrate. The plants were placed 0.45 m apart, with 1.4 m between rows. To guarantee a weekly water supply of 0.8 L/plant, a drip irrigation system was employed with a crown arrangement using 4 drippers of 1 L h⁻¹. Nutrition was managed following the recommendation of Hirzel (2013), adding in kg 60 of N, 40 of P, 70 of K, 14 of Ca, 8 of Mg, and 8 of S. The micronutrient source guaranteed in kg 0.2 of B and 0.3 of Zn, distributed in a cycle of 52 weeks.

To quantify the total chlorophyll index (SPAD), 30 plants were selected, 10 leaves were taken from the branches in the middle-third that were fully-expanded, and measurements were taken at 10 a.m. to avoid photoinhibition and leaves with low photosynthetic activity according to Deok Han *et al.* (2022a) and Deok Han *et al.* (2022b). The measurements were taken with a SPAD-502® (Konica Minolta Camera Co., Osaka, Japan), reporting the average for each plant.

The contents of chlorophylls *a* (chl *a*) and *b* (chl *b*) was determined with spectrophotometry using a Thermo Scientific™ Genesys™ 10 UV spectrophotometer, following the methodology described by Solarte *et al.* (2010): taking leaf circles with a radius of 0.5 cm from the middle part of the leaves (between

the central rib and the margin), which were macerated to 0.5 g, and adding 14 mL of 80% acetone (v/v), previously cooled at $-10\text{ }^{\circ}\text{C}$ for 5 min, followed by vortex for 2 min and centrifugation for 10 min at 4,000 rpm. The supernatant was removed to a 25 mL volumetric flask, which was covered with paper aluminum, with previously-cooled 80% acetone (v/v). The entire process was carried out with low illumination, taking absorbance readings at wavelengths of 663 nm for chl *a* and 647 nm for chl *b*. The analytical blank was 80% acetone (v/v). The contents of chl *a*, *b* and *a+b* were calculated according to Lichtenthaler (1987) for 80% acetone (v/v) (Equations 1, 2 and 3), expressed in mg g^{-1} fresh weight (FW).

$$\text{Chl } a = 12,25A_{663} - 2,79A_{647} \quad (1)$$

$$\text{Chl } b = 21,50A_{647} - 5,10A_{663} \quad (2)$$

$$\text{Chl total}(a+b) = 7,15A_{663} + 18,71A_{647} \quad (3)$$

Statistical analysis

The data obtained for chl *a*, *b* and *a+b* were correlated with the total chlorophyll index (SPAD) using the Pearson correlation (*r*), evaluated with the Shapiro-Wilk test ($P < 0.05$). Subsequently, simple linear regression was performed with the lm function. The correlation analysis was done with the statistical package Corplot from R Core Team (2022).

RESULTS AND DISCUSSION

According to the results of the Pearson correlation analysis, a linear association was observed between the SPAD method and the contents of chl *a*, *b* and *a+b* in the leaves, with positive and significant correlation coefficients in the cultivars and values that ranged between 0.93 and 0.99 (Fig. 1). These results agree with those reported by Güiza-Castillo *et al.* (2020), who studied guava plants at different phenological stages and observed that the SPAD chlorophyll index presented a positive correlation with the contents of chl *a*, *b* and *a+b*, quantified with spectrophotometry, reporting correlation values greater than 0.91. These results also agree with Lee *et al.* (2019), who indicated that, when evaluating Arisoo variety apple plants, an *r* correlation of 0.95 was presented between SPAD readings and chl *a+b* content values.

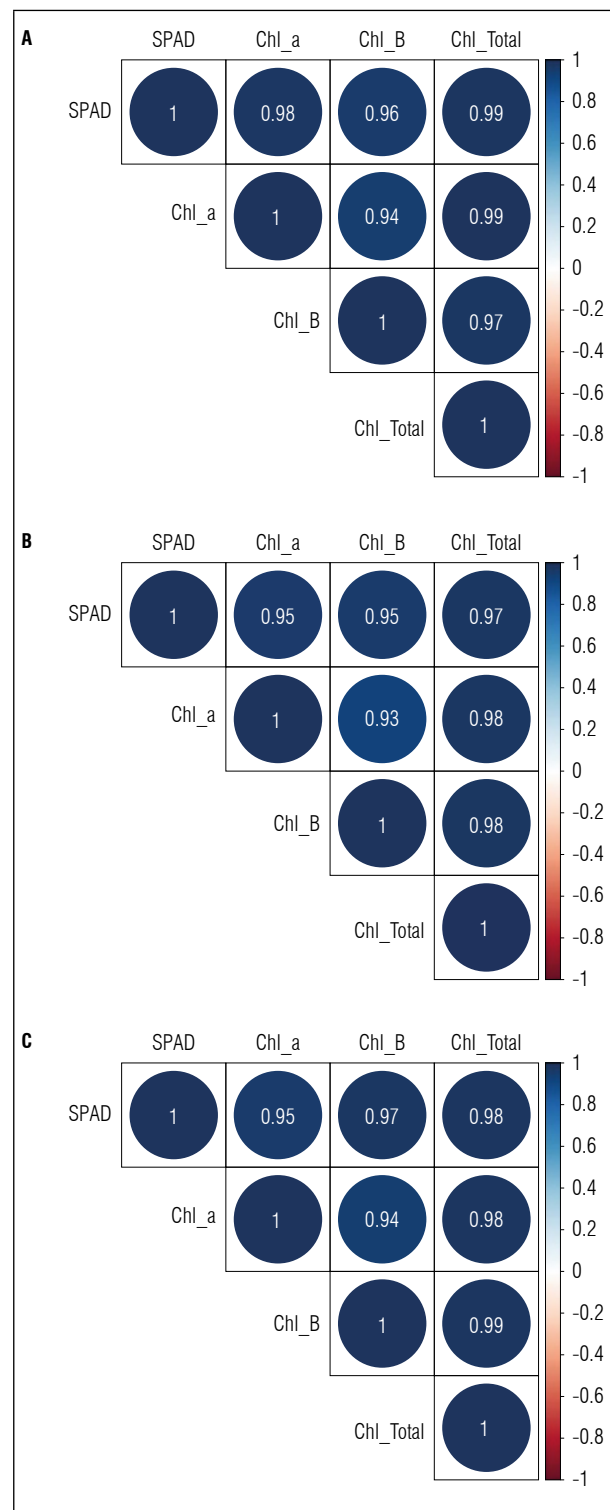


Figure 1. Pearson correlation diagram for the SPAD chlorophyll index and contents of chlorophylls-chl *a*, *b* and *a+b* in the blueberry cvs. Legacy (A), Victoria (B) and Biloxi (C).

The Legacy cultivar presented a total chlorophyll index that ranged between 68.2 and 73.1 SPAD units. The chl *a* concentration presented values that ranged between 0.77 and 1.36 mg g⁻¹ FW, the chl *b* had values that ranged between 0.31 and 0.66 mg g⁻¹ FW, and the chl *a + b* had a minimum value of 1.08 and a maximum value of 1.98 mg g⁻¹ FW (Fig. 2).

The Victoria cultivar had a total chlorophyll index between 57.4 and 62.8 SPAD units. The concentration of chl *a* had values that ranged between 1.11 and 1.56 mg g⁻¹ FW, chl *b* had values that ranged between 0.40 and 0.76 mg g⁻¹ FW, and the chl *a + b* had a minimum value of 1.57 and a maximum value of 2.32 mg g⁻¹ FW (Fig. 3).

The Biloxi cultivar had a total chlorophyll index that ranged between 61.2 and 68.3 SPAD units. The chl *a* concentration values ranged between 1.20 and 1.67 mg g⁻¹ FW, chl *b* had values that ranged between 0.42 and 1.18 mg g⁻¹ FW, and the chl *a + b* had a minimum value of 1.62 and a maximum value of 2.85 mg g⁻¹ FW (Fig. 4).

The results indicated that the quantification of the chlorophyll content, either directly or indirectly, varies between cultivars of the same species. This was also observed by Deok Han *et al.* (2022b), who, when evaluating three blueberry cultivars, found variations in the SPAD value, depending on the cultivar, the position of the leaf, and when the measurements were taken. The same authors indicated that SPAD is a useful tool to characterize different blueberry cultivars that can determine whether they are suitable for their environment.

The leaves with light green tones were associated with low SPAD values, and dark green tones were associated with high SPAD values. Deok Han *et al.* (2022a) indicated that the SPAD value of leaves is related to maturity since leaves in the apical position are relatively immature, and leaves in the basal position are comparatively old, causing the total chlorophyll content to vary. Likewise, Castañeda *et al.* (2018) observed that, in *Vitis vinifera* plants, as leaves acquired darker green tones, the SPAD value increased. Therefore, the plant and the leaves must be taken into account when evaluated with these types of meters to obtain greater reliability in the results. In the present study, the leaves were taken from the middle-third, with no evidence of high variation in the data (Fig. 2, 3, 4). Similar results were described by Castillo and Ligarreto (2016), who, when evaluating the SPAD

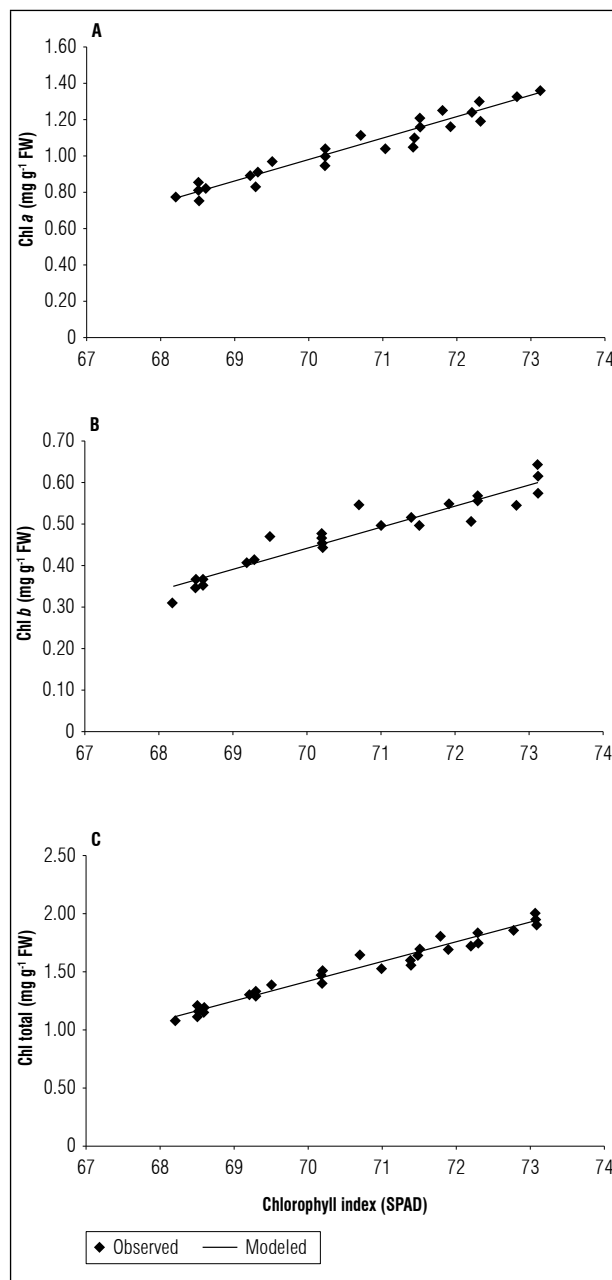


Figure 2. Relationship between the SPAD chlorophyll index and the contents of chlorophylls-chl *a* (A), *b* (B) and *a + b* (C) in blueberry cv. Legacy.

method in maize plants, concluded that the best area for taking samples is the middle-third of plants.

The results indicated that the SPAD method presented a high correlation with the content of photosynthetic pigments: chl *a*, *b* and *a + b*. In the evaluated cultivars, it was observed that the linear regression

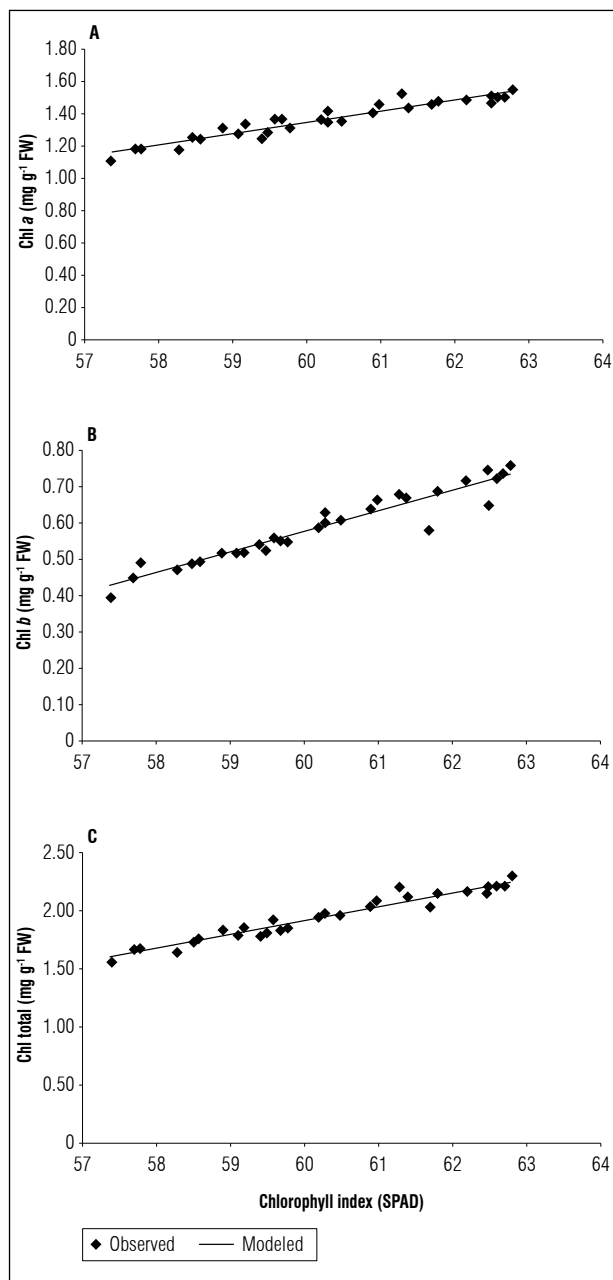


Figure 3. Relationship between the SPAD chlorophyll index and the contents of chlorophylls-chl *a*, *b* and *a+b* in blueberry cv. Victory.

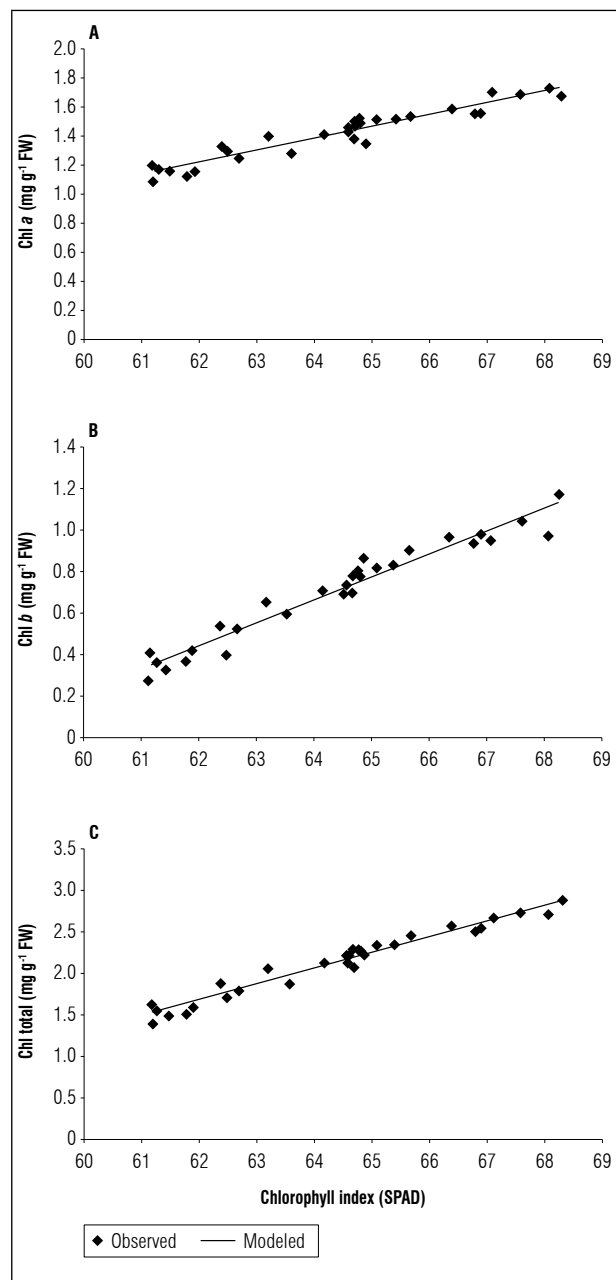


Figure 4. Relationship between the SPAD chlorophyll index and the contents of chlorophyll-hl *a* (A), *b* (B) and *a+b* (C) in blueberry cv. Biloxi.

represented the behavior of the contents of chl *a*, *b* and *a+b* in terms of the total chlorophyll index (SPAD), where the R^2 value was greater than or equal to 0.90 ($n=30$; $P \leq 0.001$) in the three cultivars. This indicated that there was a positive relationship between the destructive method and the SPAD method (Tab. 1).

The results agreed with the behavior of other species with respect to the mathematical adjustment of their relationships, such as in soybean, where linear regression best described the relationship between SPAD readings and concentrations of photosynthetic pigments (Fritschi and Ray, 2007). However, the results differed from those reported by Castañeda



Table 1. Linear regression parameters between SPAD units and chlorophyll content in three blueberry cultivars.

Cultivar	Variable	Intercept	P-value	slope	P-value	R ²	Equation lineal model
Biloxi	Chl <i>a</i>	-3.8011	<0.001	0.081	<0.001	0.91	Chl <i>a</i> = 0.08103*SPAD - 3.8011
	Chl <i>b</i>	-6.3354	<0.001	0.1095	<0.001	0.94	Chl <i>b</i> = 0.1095*SPAD - 6.3354
	Chl <i>a+b</i>	-10.116	<0.001	0.1901	<0.001	0.95	Chl <i>a+b</i> = 0.19018*SPAD - 10.1160
Legacy	Chl <i>a</i>	-7.2883	<0.001	0.118	<0.001	0.95	Chl <i>a</i> = 0.1180*SPAD - 7.2883
	Chl <i>b</i>	-3.1641	<0.001	0.0515	<0.001	0.91	Chl <i>b</i> = 0.0515*SPAD - 3.1641
	Chl <i>a+b</i>	-10.4368	<0.001	0.1694	<0.001	0.97	Chl <i>a+b</i> = 0.1694*SPAD - 10.4368
Victoria	Chl <i>a</i>	-2.919	<0.001	0.0712	<0.001	0.9	Chl <i>a</i> = 0.0712*SPAD - 2.9190
	Chl <i>b</i>	-2.8372	<0.001	0.0569	<0.001	0.9	Chl <i>b</i> = 0.0569*SPAD - 2.8372
	Chl <i>a+b</i>	-5.446	<0.001	0.1229	<0.001	0.94	Chl <i>a+b</i> = 0.1229*SPAD - 5.4460

et al. (2018), who indicated that, in grape leaves, polynomial equations generated the best correlation between the SPAD method and the destructive method. Donnelly *et al.* (2020) observed that the quadratic model showed the best fit when correlating the SPAD method and the chlorophyll content extracted from the leaves of different tree species.

This method must be validated for different species and their cultivars under field or greenhouse conditions because, as observed here, there are morphological variations, such as increased or decreased leaf thickness, physiological variations, such as a greater or lower content of photosynthetic pigments, environmental variations, such as longer or shorter exposure to light, and genotype variations (Uddling *et al.*, 2007; Castañeda *et al.*, 2018; Güiza-Castillo *et al.*, 2020).

CONCLUSIONS

This study evidenced a significant correlation between the total chlorophyll index measurements (SPAD) and the concentrations of chlorophylls *a*, *b* and total in leaves from the middle-third of blueberry cultivars Legacy, Victoria and Biloxi in the vegetative stage in the high tropics. Linear regression equations allow extractable chlorophyll contents to be inferred from the total chlorophyll index (SPAD).

This research confirmed the usefulness and importance of using the SPAD method for the non-destructive determination of chlorophyll contents in

different blueberry cultivars under field conditions because it is an easy, fast and efficient method.

Acknowledgments

The authors thank the Government of Boyacá, Convocatoria 865 de 2019, for funding this research through the project “Patrimonio Autónomo Fondo Nacional de Financiamiento para la Ciencia, la Tecnología y la Innovación Francisco José de Caldas”, contract No. 80740-690-2020, and the Asociación de productores de arándanos de Boyacá-ASOBLU for its contributions and support.

REFERENCES

Aldana, F., P.N. García, and G. Fischer. 2014. Effect of water-logging stress on the growth, development and symptomatology of cape gooseberry (*Physalis peruviana* L.) plants. *Rev. Acad. Colomb. Cienc.* 38(149), 393-400.

Amarante, C.V.T., O.Z. Zanardi, A. Miqueloto, C.A. Steffens, J. Erhart, and J.A. de Almeida. 2009. Quantificação da área e do teor de clorofilas em folhas de plantas jovens de videira “Cabernet sauvignon” mediante métodos não destrutivos. *Rev. Bras. Frutic.* 31(3), 680-686. Doi: <https://doi.org/10.1590/S0100-29452009000300009>

Ates, F. and O. Kaya. 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. Doi: <https://doi.org/10.1007/s10341-021-00580-8>

Castañeda, C.S., P.J. Almanza-Merchán, E.H. Pinzón, G.E. Cely, and P.A. Serrano. 2018. Estimación de la concentración de clorofila mediante métodos no destructivos

- en vid (*Vitis vinifera* L.) cv. Riesling Becker. Rev. Colomb. Cienc. Hortic. 12(2), 329-337. Doi: <https://doi.org/10.17584/rceh.2018v12i2.7566>
- Castillo, Á.R. and G.A. Ligarreto. 2016. Relación entre nitrógeno foliar y el contenido de clorofila, en maíz asociado con pastos en el Piedemonte Llanero colombiano. Corpoica Cienc. Tecnol. Agropecu. 11(2), 122. Doi: https://doi.org/10.21930/rcta.vol11_num2_art:202
- Cortés-Rojas, M.E., P.A. Mesa-Torres, C.M. Grijalba-Rativa, and M.M. Pérez-Trujillo. 2016. Rendimiento y calidad de frutos de los cultivares de arándano Biloxi y Sharpblue en Guasca, Colombia. Agron. Colomb. 34(1), 33-41. Doi: <https://doi.org/10.15446/agron.colomb.v34n1.54897>
- Deok Han, G., S. Heo, J.M. Chio, and Y.S. Chung. 2022a. SPAD: potential phenotyping method for characterization of blueberry. Mol. Biol. Rep. 49(6), 5505-5510. Doi: <https://doi.org/10.1007/s11033-022-07430-0>
- Deok Han, G., D. Ho Jung, S. Heo, and Y. Suk Chung. 2022b. SPAD value difference between blueberry cultivar 'STAR' by planted ground and pot. Phyton 91(11), 2583-2590. Doi: <https://doi.org/10.32604/phyton.2022.022866>
- Donnelly, A., R. Yu, C. Rehberg, G. Meyer, and E.B. Young. 2020. Leaf chlorophyll estimates of temperate deciduous shrubs during autumn senescence using a SPAD-502 meter and calibration with extracted chlorophyll. Ann. For. Sci. 77(2), 30. Doi: <https://doi.org/10.1007/s13595-020-00940-6>
- Edalat, M., R. Naderi, and T.P. Egan. 2019. Corn nitrogen management using NDVI and SPAD sensor-based data under conventional vs. reduced tillage systems. J. Plant Nutr. 42(18), 2310-2322. Doi: <https://doi.org/10.1080/01904167.2019.1648686>
- Fernández-Vargas, Y., G.A. Puentes M., and N.C. Sanabria N. 2020. Planificación del sistema de producción - Recolección del agraz (*Vaccinium meridionale* Swartz) en el municipio de Ráquira, Boyacá - Colombia. Rev. Espacios 41(41), 8.
- Fritschi, F.B. and J.D. Ray. 2007. Soybean leaf nitrogen, chlorophyll content, and chlorophyll a/b ratio. Photosynthetica 45(1), 92-98. Doi: <https://doi.org/10.1007/s11099-007-0014-4>
- Ghosh, M., D.K. Swain, M.K. Jha, and V.K. Tewari. 2020. Chlorophyll meter-based nitrogen management in a rice-wheat cropping system in Eastern India. Int. J. Plant Prod. 14(2), 355-371. Doi: <https://doi.org/10.1007/s42106-020-00089-2>
- Gianquinto, G., P. Sambo, and D. Borsato. 2006. Determination of SPAD threshold values for the optimisation of nitrogen supply in processing tomato. Acta Hortic. 700, 159-166. Doi: <https://doi.org/10.17660/ActaHortic.2006.700.26>
- Guiza-Castillo, L.-L., E.-H. Pinzón-Sandoval, P.-A. Serrano-Reyes, G.-E. Cely-Reyes, and P.-C. Serrano-Agudelo. 2020. Estimation and correlation of chlorophyll and nitrogen contents in *Psidium guajava* L. with destructive and non-destructive methods. Rev. Colomb. Cienc. Hortic. 14(1), 462-470. <https://doi.org/10.17584/rceh.2020v14i1.11341>
- Hirzel, J. 2013. Fertilización en arándano. pp. 31-42. In: Undurraga, P. and S. Vargas (eds.), Manual de arándano. INIA, Chillán, Chile.
- Kim, S.J., D.J. Yu, T.C. Kim, and H.J. Lee. 2011. Growth and photosynthetic characteristics of blueberry (*Vaccinium corymbosum* cv. Bluecrop) under various shade levels. Sci. Hort. 129(3), 486-492. Doi: <https://doi.org/10.1016/j.scienta.2011.04.022>
- Lee, Y., H.J. Kweon, M.-Y. Park, and D. Lee. 2019. Field assessment of macronutrients and nitrogen in apple leaves using a chlorophyll meter. HortTechnology 29(3), 300-307. Doi: <https://doi.org/10.21273/HORTTECH04217-18>
- Li, R., J. Chen, Y. Qin, and M. Fan. 2019. Possibility of using a SPAD chlorophyll meter to establish a normalized threshold index of nitrogen status in different potato cultivars. J. Plant Nutr. 42(8), 834-841. Doi: <https://doi.org/10.1080/01904167.2019.1584215>
- Lichtenthaler, H.K. 1987. Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. Meth. Enzymol. 148(C), 350-382. Doi: [https://doi.org/10.1016/0076-6879\(87\)48036-1](https://doi.org/10.1016/0076-6879(87)48036-1)
- Lim, T.K. 2012. *Vaccinium corymbosum*. pp. 452-464. In: Edible medicinal and non-medicinal plants. Vol. 2. Springer, Amsterdam. Doi: https://doi.org/10.1007/978-94-007-1764-0_60
- Mehrabi, F. and A.R. Sepaskhah. 2022. Leaf nitrogen, based on spad chlorophyll reading can determine agronomic parameters of winter wheat. Int. J. Plant Prod. 16(1), 77-91. Doi: <https://doi.org/10.1007/s42106-021-00172-2>
- Miao, X.R., Q.X. Chen, J.Q. Niu, and Y.P. Guo. 2022. The complete chloroplast genome of highbush blueberry (*Vaccinium corymbosum*). Mitochondrial DNA B: Resour. 7(1), 87-88. Doi: <https://doi.org/10.1080/23802359.2021.2009384>
- Netto, A.T., E. Campostrini, J.G. de Oliveira, and R.E. Bresnan-Smith. 2005. Photosynthetic pigments, nitrogen, chlorophyll a fluorescence and SPAD-502 readings in coffee leaves. Sci. Hort. 104(2), 199-209. Doi: <https://doi.org/10.1016/j.scienta.2004.08.013>
- Padilla, F.M., R. de Souza, M.T. Peña-Fleitas, M. Gallardo, C. Giménez, and R.B. Thompson. 2018. Different responses of various chlorophyll meters to increasing nitrogen supply in sweet pepper. Front. Plant Sci. 871, 1752. Doi: <https://doi.org/10.3389/fpls.2018.01752>
- Padilla, F.M., R. de Souza, M.T. Peña-Fleitas, R. Grasso, M. Gallardo, and R.B. Thompson. 2019. Influence of time of day on measurement with chlorophyll meters and canopy reflectance sensors of different crop N status. Precis. Agric. 20(6), 1087-1106. Doi: <https://doi.org/10.1007/s11119-019-09641-1>

- Parry, C., J.M. Blonquist, and B. Bugbee. 2014. *In situ* measurement of leaf chlorophyll concentration: Analysis of the optical/absolute relationship. *Plant Cell Environ.* 37(11), 2508-2520. Doi: <https://doi.org/10.1111/pce.12324>
- Porra, R.J., W.A. Thompson, and P.E. Kriedemann. 1989. Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophylls a and b extracted with four different solvents: verification of the concentration of chlorophyll standards by atomic absorption spectroscopy. *Biochim. Biophys. Acta (BBA) - Bioenerg.* 975(3), 384-394. Doi: [https://doi.org/10.1016/S0005-2728\(89\)80347-0](https://doi.org/10.1016/S0005-2728(89)80347-0)
- R Core Team. 2022. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. URL <https://www.R-project.org/>
- San Martín, J. 2013. Situación varietal en arándano. pp. 15-21. In: Undurraga, P. and S. Vargas (eds.), *Manual de arándano*. INIA, Chillán, Chile.
- Solarte, M., L. Moreno, and L. Melgarejo. 2010. Fotosíntesis y pigmentos vegetales. pp. 107-122. In: Melgarejo, L. (ed.). *Experimentos en fisiología y bioquímica vegetal*. Universidad Nacional de Colombia, Bogota.
- Stevenson, D. and J. Scalzo. 2012. Anthocyanin composition and content of blueberries from around the world. *J. Berry Res.* 2(4), 179-189. Doi: <https://doi.org/10.3233/JBR-2012-038>
- Uddling, J., J. Gelang-Alfredsson, K. Piikki, and H. Pleijel. 2007. Evaluating the relationship between leaf chlorophyll concentration and SPAD-502 chlorophyll meter readings. *Photosynth. Res.* 91(1), 37-46. Doi: <https://doi.org/10.1007/s11120-006-9077-5>
- Yokoyama, A.H., R.H. Ribeiro, A.A. Balbinot Junior, J.C. Franchini, H. Debiasi, and C. Zucareli. 2018. Índices de área foliar e SPAD da soja em função de culturas de entressafra e nitrogênio e sua relação com a produtividade. *Rev. Cienc. Agrar.* 41(4), 81-90. Doi: <https://doi.org/10.19084/RCA18153>
- You, Q., B. Wang, F. Chen, Z. Huang, X. Wang, and P.G. Luo. 2011. Comparison of anthocyanins and phenolics in organically and conventionally grown blueberries in selected cultivars. *Food Chem.* 125(1), 201-208. Doi: <https://doi.org/10.1016/j.foodchem.2010.08.063>
- Yu, D.J., H. Rho, S.J. Kim, and H.J. Lee. 2015. Photosynthetic characteristics of highbush blueberry (*Vaccinium corymbosum* cv. Bluecrop) leaves in response to water stress and subsequent re-irrigation. *J. Hort. Sci. Biotechnol.* 90(5), 550-556. Doi: <https://doi.org/10.1080/14620316.2015.11668713>
- Zhang, K., Z. Yuan, T. Yang, Z. Lu, Q. Cao, Y. Tian, Y. Zhu, W. Cao, and X. Liu. 2020. Chlorophyll meter-based nitrogen fertilizer optimization algorithm and nitrogen nutrition index for in-season fertilization of paddy rice. *Agron. J.* 112(1), 288-300. Doi: <https://doi.org/10.1002/agj2.20036>