Correlation between SPAD and chlorophylls \(a\), \(b\) and total in leaves from \textit{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 \textit{Vaccinium corymbosum} L. cv. Biloxi, Legacy y Victoria en trópico alto

\textbf{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 (\textit{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.

\textbf{Additional key words:} blueberry; cultivars; photosynthetic pigments; physiological status.
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, costoso 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.
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 Tunguavita Experimental Farm of the Universidad Pedagógica y Tecnológica de Colombia 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 °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).

\[
Chl\ a = 12.25A_{663} - 2.79A_{647} \quad (1)
\]
\[
Chl\ b = 21.50A_{647} - 5.10A_{663} \quad (2)
\]
\[
Chl\ total(a+b) = 7.15A_{663} + 18.71A_{647} \quad (3)
\]

**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 \textit{a} concentration presented values that ranged between 0.77 and 1.36 mg g\(^{-1}\) FW, the chl \textit{b} had values that ranged between 0.31 and 0.66 mg g\(^{-1}\) FW, and the chl \textit{a} + \textit{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 \textit{a} had values that ranged between 1.11 and 1.56 mg g\(^{-1}\) FW, chl \textit{b} had values that ranged between 0.40 and 0.76 mg g\(^{-1}\) FW, and the chl \textit{a} + \textit{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 \textit{a} concentration values ranged between 1.20 and 1.67 mg g\(^{-1}\) FW, chl \textit{b} had values that ranged between 0.42 and 1.18 mg g\(^{-1}\) FW, and the chl \textit{a} + \textit{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 \textit{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 \textit{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 \textit{et al.} (2018) observed that, in \textit{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 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 \textit{a}, \textit{b} and \textit{a} + \textit{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 ($n=30$; $P\leq0.001$) 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...
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.

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