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Evaluation of SPAD Index for Estimating Nitrogen and Magnesium Contents in Three Blueberry Varieties (*Vaccinium corymbosum* L.) on the Andean Tropics

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Abstract: In the Ericaceae family, blueberries are the most commercially important species. Estimating the nutritional and physiological status of plants is a common practice carried out by producers. However, conventional methods are destructive, costly and time consuming. In recent years, methodologies such as measurements with the SPAD chlorophyll index have become available, which has proven to be an easy, fast and non-destructive method for estimating chlorophyll, N and Mg contents in the field. Therefore, this research aimed to estimate variations in SPAD readings between varieties and to determine whether Chlorophyll Index (SPAD) values are associated with Nitrogen and Magnesium contents in blueberry plants (*Vaccinium corymbosum* L.) from the varieties 'Biloxi', 'Legacy' and 'Victoria' in the vegetative phase under the conditions in the municipality of Paipa-Boyacá (Colombia). The varieties presented statistical differences in the SPAD index values, with a linear correlation between the SPAD index and the contents of N and Mg with a coefficient of determination (R^2) greater than 0.8 in the three varieties. This result confirmed the usefulness and importance of using SPAD as a nutrition management tool in the evaluated varieties based on the SPAD readings, with a subsequent relationship with the threshold values of the foliar N and Magnesium status under field conditions.

Keywords: chlorophyll index; mineral nutrition; Biloxi; macronutrient; lineal model

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1. Introduction

Blueberries (*Vaccinium corymbosum* L.) are native to the United States and belong to the Ericaceae family, which has approximately 450 species worldwide [1]. They are perennial fruit shrubs with blue or purple fruits, with a high content of anthocyanins and other antioxidants that provide important benefits for human health, making them attractive to consumers [2,3]. Global production of blueberries doubled between 2010 and 2019 [4]. Colombia, the sixth largest producer in South America [2], has crops between 2200 and 2800 masl [5,6]. Colombia, with its diversity, has a great opportunity to meet global demand for the blueberry market [7].

Within agricultural production systems, mineral nutrition, understood as the adequate supply of nutrients to plants, has the greatest influence on crop yield and quality. Understanding the nutritional needs of plants will allow precise control of nutrient supply, especially if flexible technologies such as fertigation are adopted, and the availability of nutrients in the soil or in the plants is monitored [8].

Each blueberry cultivar has its own response or characteristic adaptation to environments. Therefore, suitable growth conditions vary [9]. For this reason, the demand for nutrients depends on the plant species, the cultivar and the expected yields. Nutrients are comparatively more important in crop production because their deficiency can affect quality characteristics such as firmness and fruit color and can cause postharvest losses [10].

Therefore, the early detection of macronutrient contents in plants facilitates decision-making in the face of deficiency problems for elements such as nitrogen (N) and magnesium (Mg); diagnosis in a timely manner is a fundamental tool for producers [11,12]. Nitrogen is the mineral nutrient most frequently applied to blueberries plants [13], according to Xiong et al. [14] approximately 0.5 to 1.5% of foliar N is assigned to chlorophyll, and between 6% and 5% of the total Mg is bound to chlorophyll [15], depending on the environment, growth or type of the plant. In many species, a greater quantity of foliar N or Mg assigned to chlorophyll-protein complexes has been observed, depending on the light stimulus [15,16]. Mg deficiencies are most frequent in rapid growing blueberry plants or in those with heavier fruit loads [13]. For this reason, it is important to evaluate SPAD readings and their relationship with foliar N and Mg contents when using this method for N management decision-making in agricultural systems [11,14].

The Soil Plant Analysis Development (SPAD-502®) is one of the more widely used instruments in studies to indirectly determine N contents in plants in real time [17,18]. This is a simple and fast portable device that provides non-destructive sampling of the chlorophyll index [19]. Generating interest in the rapid analysis of foliar chlorophyll and nitrogen contents [12,20], because the fertilization of crops is carried out with few technical criteria in Colombia, in some cases only with soil analysis and very rarely with foliar analysis [21].

The SPAD-502® chlorophyll meter has been successfully used for quantifying nitrogen contents in fruit crops such grapevine [22,23] and Guava [12], among others. Because this method is easy, fast and non-destructive.

Therefore, the objectives of this study were: (1) Estimate variations in SPAD readings between evaluated; and (2) Determine whether Chlorophyll Index (SPAD) values are associated with Nitrogen and Magnesium contents for three blueberry varieties grown under Colombian high tropic conditions.

2. Materials and Methods

2.1. Localization

This research was carried out between the months of February and August, 2022 on the farm of the Universidad Pedagógica y Tecnológica de Colombia, which is located in the municipality of Paipa (Boyacá-Colombia), with geographic coordinates 5°44′32.35″ N and 73°06′50.91″ W, at 2525 m above sea level [24]. The average monthly temperature and average monthly relative humidity were quantified using HOBO® MX2305 datalogger (Table 1).

Table 1. Average values of temperature (°C) and relative humidity (RH, %) at the study site during this research.

	February	March	April	May	June	July	August
Temperature (°C)	13.2	15.4	15.0	14.5	14	14.2	13.7
RH (%)	88.8	77.6	79.9	81.1	83.8	80.4	79.8

2.2. Plant Materials

Plants from the Biloxi, Legacy and Victoria varieties were used, from Fallcreek-Farm & Nursery, INC. For each variety, 30 plants were selected, which were 6 months old after transplanting, with an approximate height of 45 cm.

2.3. Crop Management

The plants were under crystal colored anti-hail mesh with a shading percentage of 3%, supplied by AGROPINOS-Colombia, planted in 60 L bags in a rice husk substrate, with pine shavings and black earth, established at 0.45 m between plants and 1.4 m between rows. To guarantee a weekly water supply of 0.8 L-plant, a drip irrigation system was used with a crown arrangement with four 1 L h^{-1} drippers. The mineral nutrition plan was managed according to the requirements proposed by Hirzel [25], with a weekly supply/ha of 1.2 kg of N; 1 kg of P; 1.4 kg of K; 0.3 kg of Ca; 0.2 kg of Mg; 0.2 kg of S, 0.003 kg of B and 0.005 kg of Zn, applied in two fertigation pulses per week.

2.4. Measurement of SPAD, Nitrogen y Magnesium Values

For the quantification of the total chlorophyll index (SPAD), 10 leaves were taken from each of the 30 selected plants from branches located in the middle-third, which were fully expanded. The measurements were taken around 10 AM to avoid photoinhibition effect and guarantee leaves with high photosynthetic activity [9,26]. The measurements were taken with a SPAD-502® (Konica Minolta Camera Co., Osaka, Japan), reporting the average for each plant.

The foliar nitrogen content was determined with the Kjeldahl method, and the foliar magnesium was determined with wet digestion/Atomic Absorption, using a sample of 150 g of dry mass (approximately 15 leaves) plant material per sample unit and the SPAD chlorophyll index. The samples were covered with absorbent paper to eliminate moisture and packed in properly labeled paper bags, which were packed in a Styrofoam cooler. The analyses were carried out by the Laboratorio de Suelos y Aguas of the Universidad Pedagógica y Tecnológica de Colombia, Boyacá-Colombia.

2.5. Statistical Analysis

The data analysis was done with R Core Team software [27]. The assumption of normality was verified by analyzing the residuals of the ANOVA model with the Shapiro-Wilk test ($p > 0.05$), and the assumption of variance homoscedasticity was done with the Bartlett test ($p > 0.05$). Subsequently, to evaluate the SPAD readings between the different varieties, a one-way classification ANOVA and Tukey's multiple comparison test of means ($p < 0.05$) were used with the "agricolae" package. The relationship between the real and adjusted values was estimated using a linear regression model with the "lm" function. A principal component analysis (PCA) was performed using the 'FactoMineR' and 'factoextra' packages to generate two-dimensional biplots. The plots were generated using the "ggplot2" package.

3. Results

The data analysis with the Box plot graph showed that the behavior of the SPAD, nitrogen and magnesium values were adjusted to a normal behavior without atypical data, differentiating between varieties. The Biloxi variety presented chlorophyll values that oscillated between 61.2 and 68.3 SPAD units, foliar nitrogen values that varied between 2.09 and 2.46% and foliar magnesium values that oscillated between 0.17 and 0.33%, the most dispersed values (Figures 1–3).

The Legacy variety had SPAD values that ranged between 68.2 and 73.1, which were the highest values for this variable, foliar nitrogen values that were between 1.86 and 2.09% and foliar magnesium values that varied between 0.13 and 0.19% (Figures 1–3).

The Victoria variety presented SPAD values that fluctuated between 57.4 and 62.7, the lowest values for this variable, nitrogen and magnesium values of 1.93 and 2.32%, and 0.15 and 0.18%, respectively (Figures 1–3).

According to the analysis of variance, there were significant statistical differences ($p < 0.05$) between the varieties for the SPAD readings, foliar nitrogen and magnesium content. The Legacy variety had the highest average SPAD value with 70.59 SPAD units, compared to the Biloxi and Victoria varieties with average values of 64.42 and 60.27 SPAD units, respectively (Figure 4).

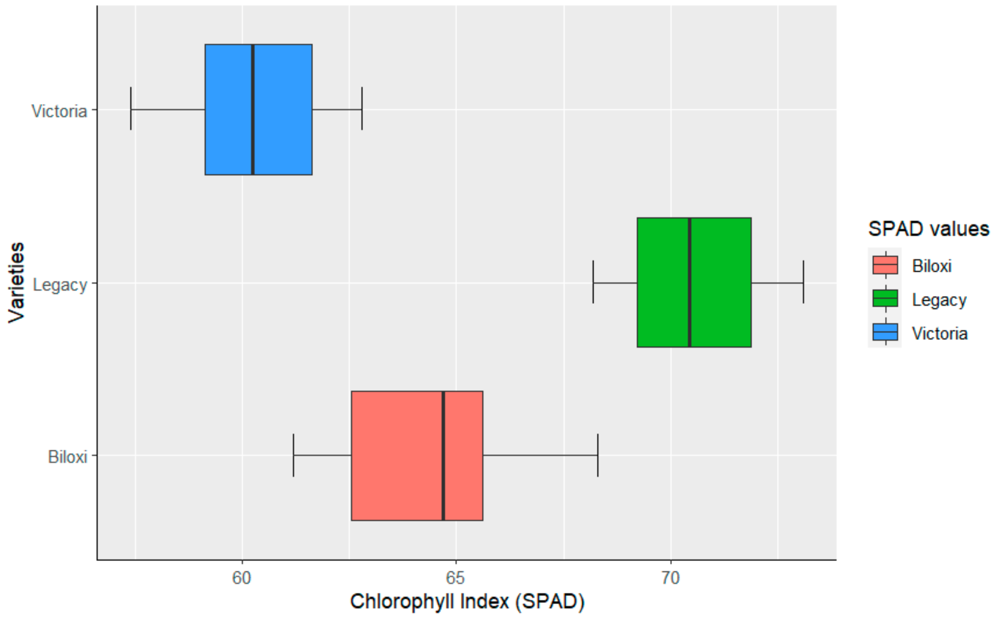

Figure 1. Box-plot of the Chlorophyll index (SPAD) in Biloxi, Legacy and Victoria variety Blueberry plants grown under Colombian high tropic conditions.

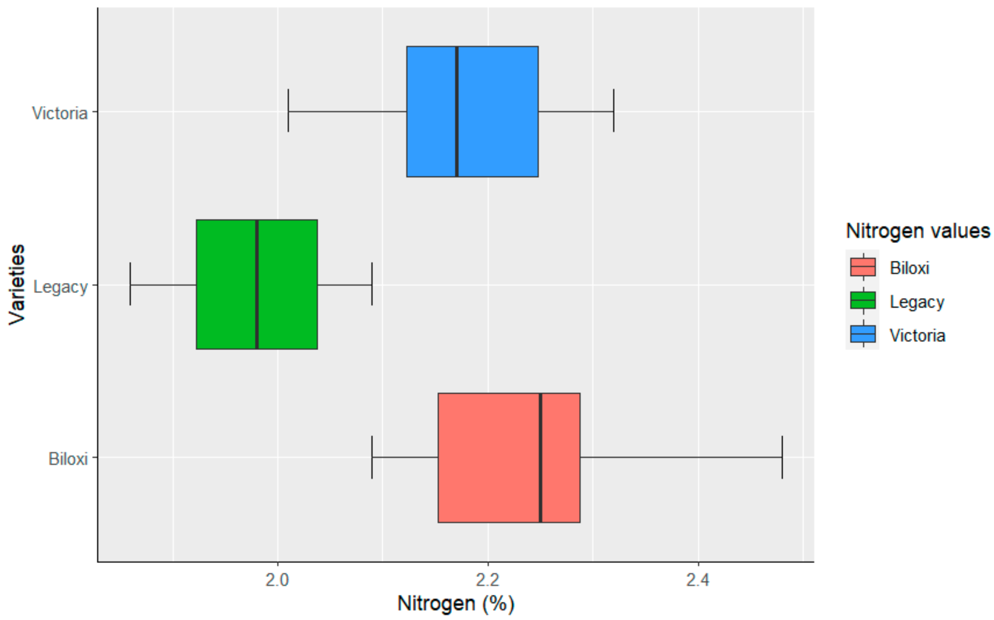

Figure 2. Box-plot of the foliar Nitrogen content in Biloxi, Legacy and Victoria variety Blueberry plants cultivated under conditions of the Colombian high tropics.

The Biloxi variety generated the highest mean values for both foliar nitrogen and magnesium content, with 2.24% and 0.24%, respectively, while the Legacy variety presented the lowest values, with 1.98% and 0.15% for nitrogen and magnesium, respectively (Figures 5 and 6).

The SPAD readings presented a linear relationship with the foliar nitrogen and magnesium contents for the three varieties (Figure 7A,B), and the linear model correctly represented the behavior of the foliar nitrogen and magnesium content towards the total chlorophyll index (SPAD) since an R^2 value greater than or equal to 0.90 (n = 30; $p \leq 0.001$) was obtained for the three varieties (Table 2). This indicated that there was a significant and positive correlation between the destructive method (N and Mg) and the SPAD method.

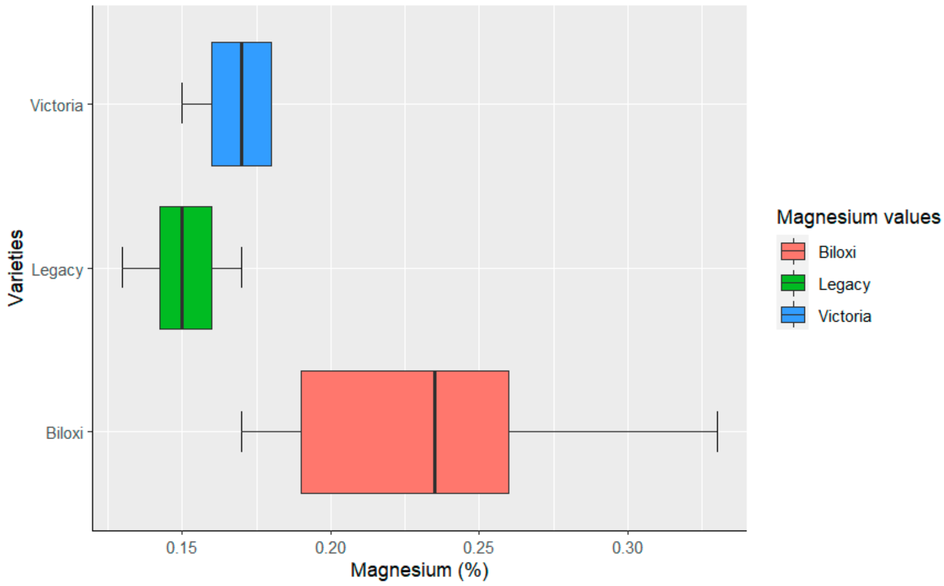

Figure 3. Box-plot of the foliar Magnesium content in Biloxi, Legacy and Victoria variety Blueberry plants cultivated under conditions of the Colombian high tropics.

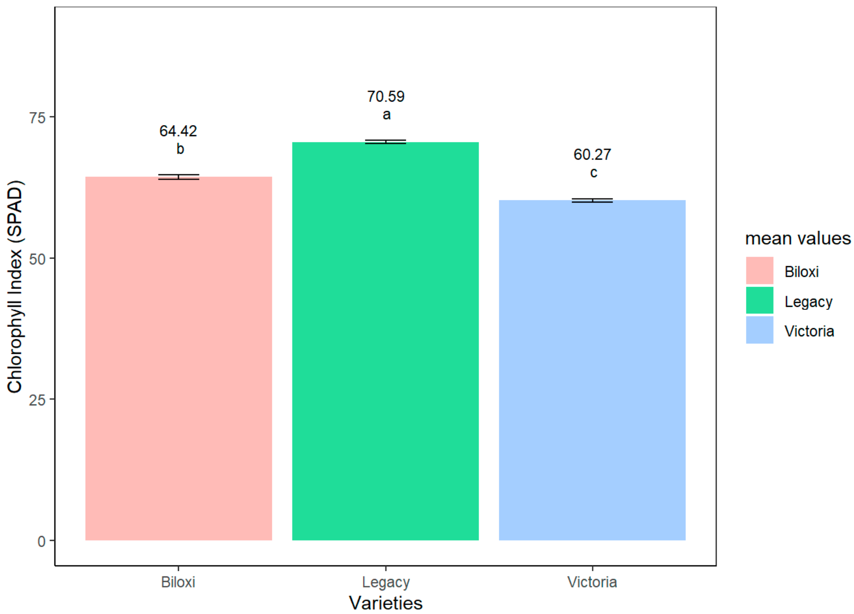

Figure 4. Comparison of SPAD Chlorophyll Index in Biloxi, Legacy and Victoria variety blueberry plants. Different letters indicate significant differences ($p < 0.05$) according to the Tukey's multiple comparison test of means. Vertical bars in each average indicate the standard error (n = 30).

When analyzing the contribution of each variable based on the different cultivars with the principal component analysis (PCA), it was observed that the variability was explained by 63.3% by the first component, where the data for the foliar nitrogen and magnesium contributed the most, with 33.0% and 33.5%, respectively, while the SPAD Chlorophyll Index explained 34.0% of the second component. The biplot shows ellipses for Biloxi and Victoria along the axis of the first dimension, clearly distancing them from Legacy, which formed its ellipse near the second component (Figure 8). The Legacy variety, with the highest SPAD values, was close to this vector, clearly differentiated from Biloxi and Victoria, which were influenced by the foliar nitrogen and Magnesium values. Therefore, Biloxi and Victoria had similar foliar N and Mg contents but differed from the Legacy variety.

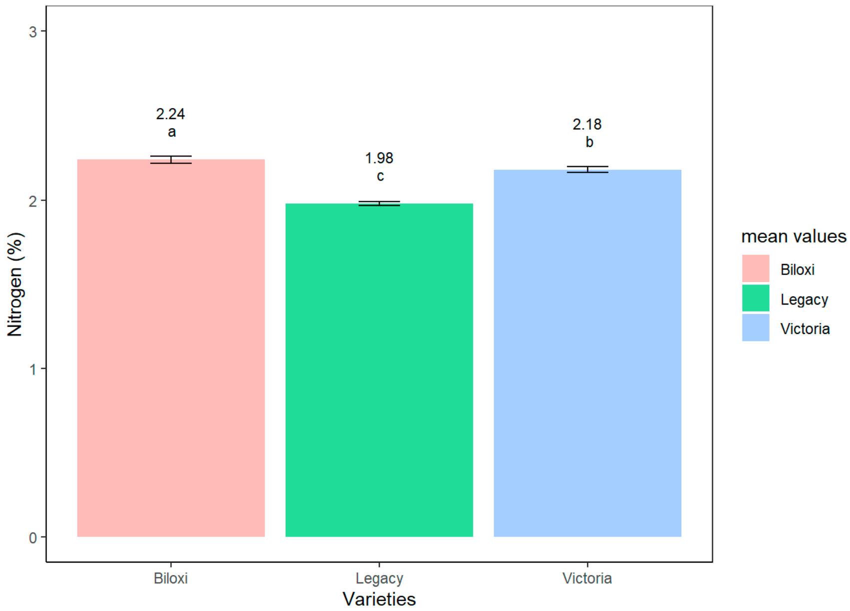

Figure 5. Comparison of the foliar Nitrogen content in Biloxi, Legacy and Victoria variety blueberry plants. Different letters indicate significant differences ($p < 0.05$) according to the Tukey's multiple comparison test of means. Vertical bars in each average indicate the standard error (n = 30).

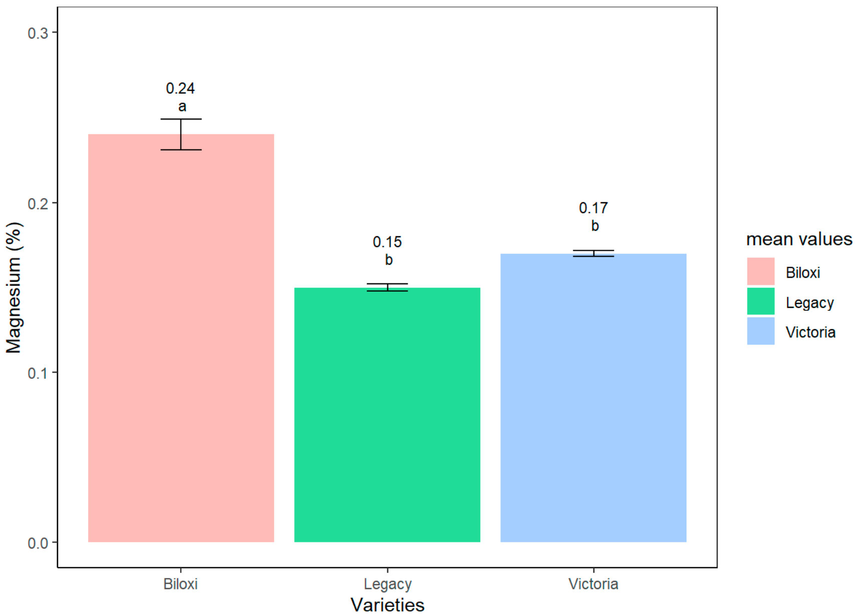

Figure 6. Comparison of the foliar Magnesium content in Biloxi, Legacy and Victoria variety blueberry plants. Different letters indicate significant differences ($p < 0.05$) according to the Tukey's multiple comparison test of means. Vertical bars in each average indicate the standard error (n = 30).

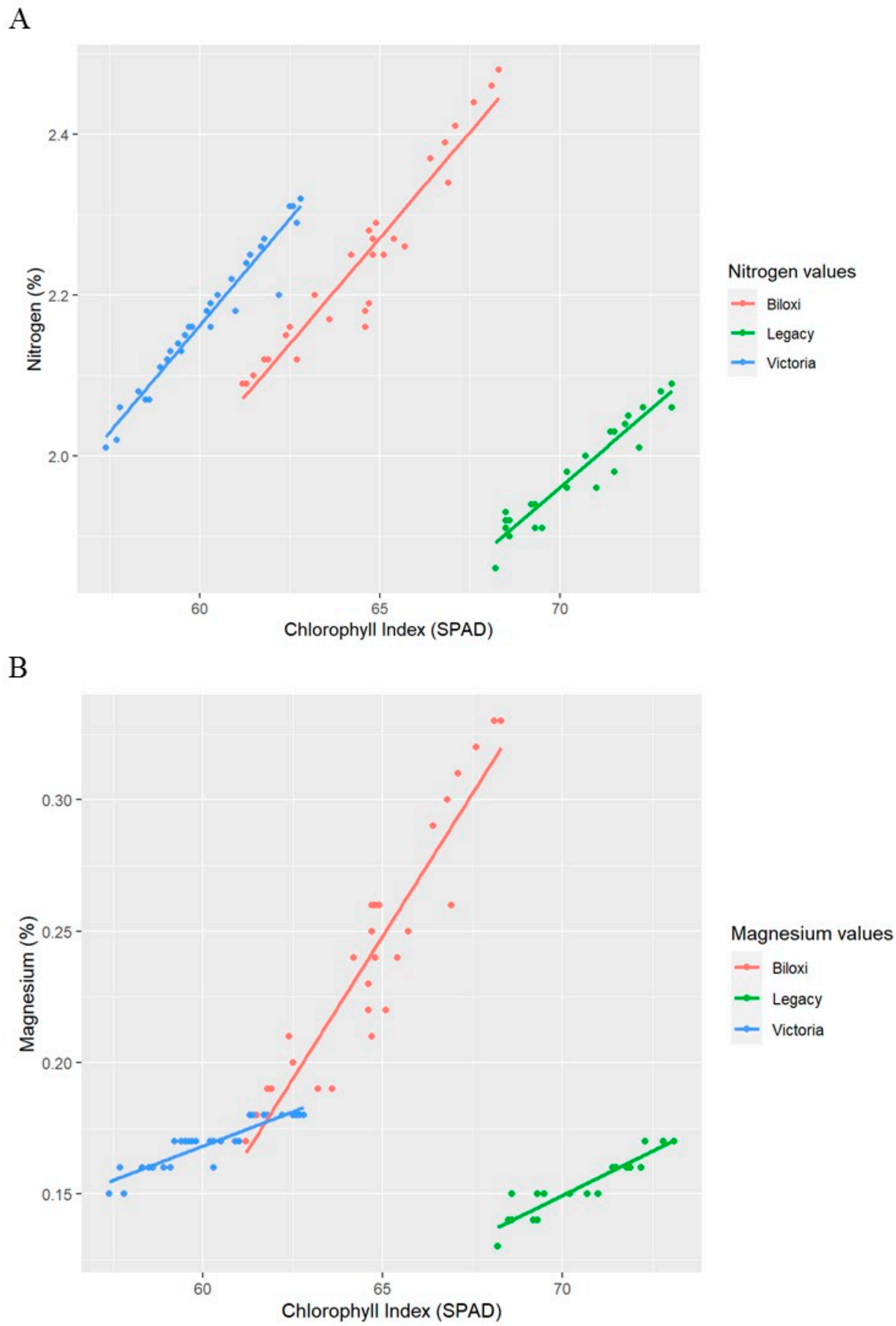

Figure 7. Linear relationship between the SPAD chlorophyll index and the foliar nitrogen content (**A**) and the foliar magnesium content (**B**) in Biloxi, Legacy and Victoria variety blueberry plants.

Table 2. Linear regression parameters between SPAD units and nitrogen and magnesium content in three blueberry cultivars under conditions of the Colombian high tropics.

Variety	Variable	Parameters Linear Regression					
		Intercept	*p*-Value	Slope	*p*-Value	R^2 Adjusted	Equation Lineal Model
Biloxi	Nitrogen	−1.1535	<0.001	0.05269	<0.001	0.91	N = 0.0569 × SPAD − 1.1535
	Magnesium	−1.1637	<0.001	0.02172	<0.001	0.87	Mg = 0.02172 × SPAD − 1.1637
Legacy	Nitrogen	−0.7309	<0.001	0.03846	<0.001	0.9	N = 0.03846 × SPAD − 0.7309
	Magnesium	−0.3226	<0.001	0.00674	<0.001	0.89	Mg = 0.00674 × SPAD − 0.3226
Victoria	Nitrogen	−1.0261	<0.001	0.05313	<0.001	0.94	N = 0.05313 × SPAD − 1.0261
	Magnesium	−0.1448	<0.001	0.005218	<0.001	0.82	Mg = 0.005218 × SPAD − 0.1448

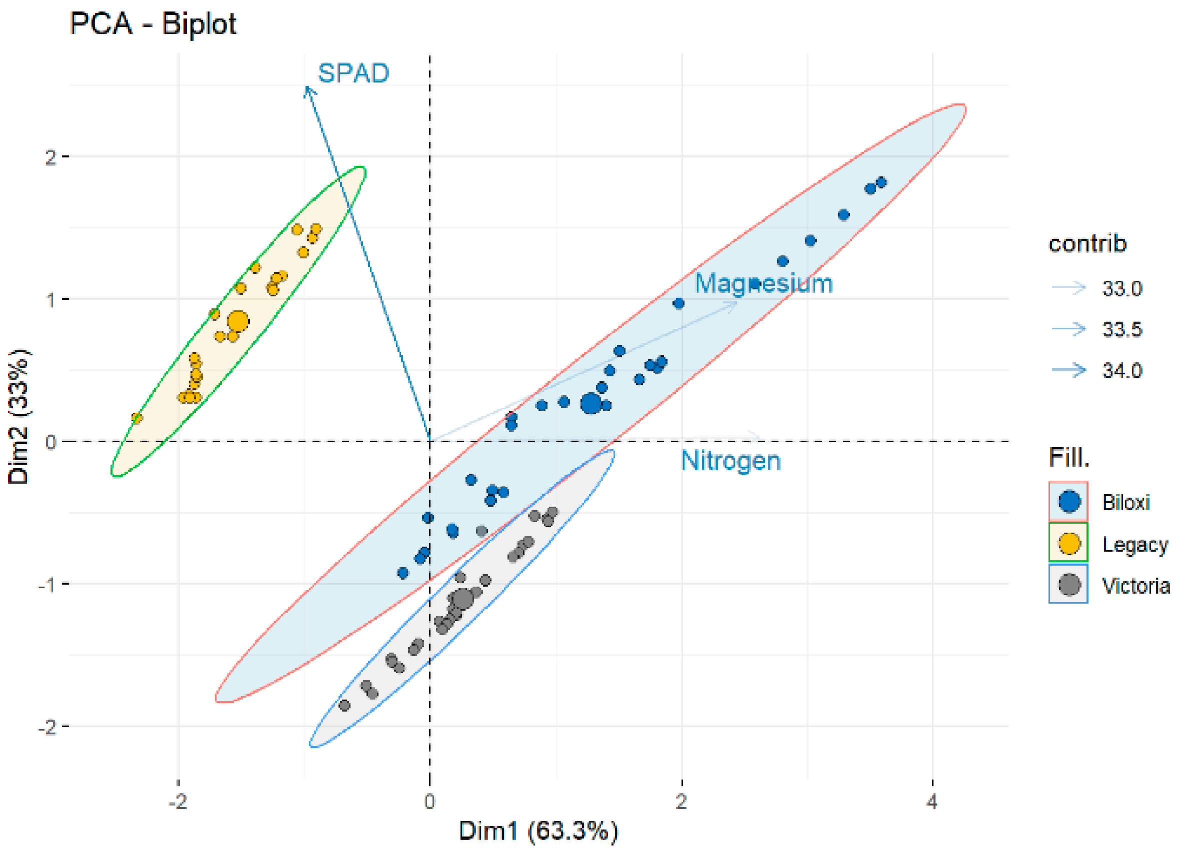

Figure 8. Observational PCA biplot in three blueberry varieties. The PCA biplot shows the scores of the explanatory variables as a vector (arrows in light-blue lines) and individuals of each variety; Biloxi (blue circle), Legacy (yellow circle) and Victoria (grey circle); the colored ellipses (size determined by a probability level of 0.95) emphasize the individuals belonging to each variety.

4. Discussion

In the Biloxi, Legacy and Victoria varieties, linear regressions showed that the chlorophylls a, b and total can be estimated with the SPAD index [17]. This indicates the usefulness of using the SPAD for the non-destructive estimation of chlorophyll contents under field conditions in blueberries. The evaluated varieties presented differences in the SPAD values (Figures 1 and 8), indicating genetic differences for origin. Jiang et al. [28] also found different chlorophyll content indices between the cultivars *Vaccinium ashei* Reade 'Climax' and *V. corymbosum* hybrid 'Chaoyue No.1', being higher in the latter. The Biloxi and Victoria varieties presented low SPAD values. Han et al. [9] found that low SPAD values are typical in evergreen-type materials, where *V. darrowii* is used as a parent for hybridization.

The High SPAD values in Legacy are associated with dark green tones in the leaves because of a greater presence of chlorophylls, as reported previously [17]. This trend of greater greenness associated with a higher value of the SPAD index was also found in grapevine leaves [23]. The chlorophyll content is normally associated with a good nutritional and physiological state; however, in this study, the three varieties were grown under the same conditions, indicating that the differences in SPAD were attributed to genetic differences. Thus, the higher SPAD value in Legacy can be related to higher photosynthetic rates since chlorophylls are pigments in charge of capturing photons to promote the transport of electrons in the photo phase [29]. This may explain why Legacy is a vigorous and productive cultivar [17]. In this regard, it has previously been indicated in blueberries that the chlorophyll content index is highly correlated with biomass, fruit production and photosynthetic efficiency [28].

Biloxi and Victoria were characterized by higher foliar N values (Figure 5); however, the three varieties reported concentrations higher than those found in highbush blueberry (*V. corymbosum* L.) plants, which were between 1.53 and 1.7% [30]. The N in leaves is used for the formation of chlorophylls but also for the synthesis of proteins, hormones and nucleic acids [15,31], essential biomolecules for the normal development of blueberry plants. Bassi et al. [32] reported that nitrogen (N) is a main component of the photosynthetic apparatus.

The three evaluated varieties yielded foliar magnesium concentrations lower than those found in highbush blueberry plants, which were between 0.41% and 0.49% [30], and higher than those found in the Duke, Draper, Aurora and Liberty cultivars, which ranged between 0.13% and 0.14% [33]. Previous studies also agree with the varietal differences found for the tissue Mg, where Biloxi presented higher levels, up to 60% more than Legacy (Figure 6), a very important aspect to take into account in fertigation plans.

Foliar Mg contents could be involved in different physiological functions in blueberry plants, as a central element of the chlorophyll molecule with a final function in photosynthesis, but also in other fundamental processes such as respiration, metabolism of organic acids and carbohydrate partition. Because Mg is an activator of more than three hundred enzymes, including ATPases, phosphatases, and kinases, among others [34,35]. These reports on N and Mg contents in the three varieties can be used in nutrition management programs by producers and technical assistants since they provide a good starting point for decision-making although it is important to indicate that variations in said contents can be found because of specific growth conditions, which include the environment and soil conditions [33].

In perennial crops, it is important to assess the nutritional status so that the appropriate content of each nutrient can be balanced during the growing season [36]. According to the results, the foliar Nitrogen content increased linearly with the SPAD readings in all varieties (Figure 3). Similar results were found in guava plants, where foliar N increased linearly as the SPAD readings were higher [12]. In this study, a good correlation was reported between the foliar N determined by the Kjeldahl method and the chlorophyll content measured with a Minolta® SPAD 502 chlorophyll meter. This showed the usefulness of the method in the non-destructive determination of these nutrients, a good indicator of the chlorophyll concentration and N status in the crop [37]. This was due to the role of N in the formation of the main photosynthetic pigment, chlorophyll [38]. Chlorophyll is a tetrapyrrolic ring, where the central cavity formed by the core four nitrogen atoms hosts the Mg^{2+} ion [39]. Glutamyl-tRNA reductase (GluTR) catalyzes glutamyl-tRNA into glutamate-1-semialdehyde (GSA) and initiates chlorophyll biosynthesis [40].

It has been reported that the SPAD index can be used to predict foliar N concentration in rice but values can be affected by genotype, growth stage, leaf position and measurement point on a leaf [41]. In the blueberries, the variation between the evaluated genotypes was corroborated (Figures 7 and 8), and standardizing the methodology with the other factors that may affect it should continue.

In the three blueberry varieties, the SPAD reading linearly increased as a function of foliar Mg levels (Figure 7) mainly because Mg is a metal ion that is part of the chlorophyll structure. In the chlorophyll biosynthesis process, Mg is inserted into protoporphyrin IX to produce magnesium protoporphyrin IX. This reaction is catalyzed by the enzyme Mg chelatase, a process that also requires a lot of energy in the form of Mg-ATP [42]. The Mg atom has several advantages as the central atom of chlorophyll; most importantly, a coordinatively unsaturated Mg^{2+} acts as the coordination center for axial ligand binding, which constitutes the strongest bond between Chls and polypeptides [39]. Low levels of Mg in tissue can lead to typical symptoms such as interveinal chlorosis, starting with the most mature leaves because Mg has high mobility in plants [15].

Similar results were found in sunflower leaves, where there was a relationship between SPAD values and Mg concentrations, only curvilinearly, mainly because low Mg levels in this species [43]. In the three blueberry varieties, low Mg levels indicated lower SPAD values, as reported for radish [44]. According to Wadas and Dziugieł [45] SPAD values depend mainly on N and Mg contents.

The results provide a nutrition management tool in the evaluated varieties based on the SPAD readings through a subsequent relationship with the threshold values of foliar N and Mg, in accordance with the relationship between SPAD readings and foliar N and Mg contents. In addition, this methodology allows fast and reliable information for decision-making in fertilization plans for the three blueberry varieties and, compared to other rapid methodologies such as spectrometers, chlorophyllometers are low-cost and affordable for farmers. SPAD has been a popular method to determine chlorophylls and N in rice for several years [41]. However, this methodology must continue to be standardized because it can be affected by factors related to plants.

5. Conclusions

Foliar nitrogen values for Biloxi varied between 2.09 and 2.46% and magnesium between 0.17 and 0.33%. For Leagacy, this content were 1.86–2.09% and 0.13–0.19% respectively. While for Victoria N and Mg oscilated of 1.93–2.32%, and 0.15–0.18%, respectively. The Legacy variety presented the highest chlorophyll index (SPAD). A significant correlation was evidenced between the measurement of the total chlorophyll index and the concentration of nitrogen and magnesium quantified in leaves from the middle-third of 'Legacy', 'Victoria' and 'Biloxi' variety blueberry plants in the vegetative phase under conditions found in Paipa-Boyacá. Linear regression equations were obtained between the variables with the chlorophyll index (SPAD). This research confirmed the usefulness and importance of using the SPAD method for the non-destructive determination of nitrogen and magnesium contents in different blueberry varieties under field conditions, being an easy, fast and affordable method for producers.

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