Portable chlorophyll meter in monitoring and management of nitrogen in common bean cultivars

Clorofilómetro portátil en el monitoreo y manejo de nitrógeno en cultivares de frijoles

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Abstract

More sustainable practices involving biological nitrogen fixation (BNF) and the use of portable chlorophyll meter in fertilization management are promising to increase nitrogen (N) use efficiency in the common bean crop. This study aimed to monitor N managements using a portable chlorophyll meter and its effects on agronomic attributes of Carioca-type common bean cultivars with indeterminate and determinate growth habits ('IPR Campos Gerais' and 'IAC Imperador'). The experiment was carried out in a randomized block design (RBD), in a 2 × 9 factorial scheme, with four replicates. The first factor consisted of the cultivars and the second factor of the N management. The data were subjected to analysis of variance by F test and, when necessary, the means were grouped by the Scott-Knott test at 5% probability level. The use of portable chlorophyll meter is a viable alternative for N monitoring and management in common bean, allowing the reduction of top-dressing N application, regardless of the cultivar used.

Keywords
Phaseolus vulgaris L. • chlorophyll index • biological nitrogen fixation • agronomic nitrogen efficiency

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Resumen

Las prácticas más sostenibles que involucran la fijación biológica de nitrógeno (BNF) y el uso del clorofilómetro portátil en el manejo de fertilizantes son prometedoras para aumentar la eficiencia del uso de nitrógeno (N) en el cultivo de frijol común. El objetivo fue monitorear el manejo de N con el clorofilómetro portátil y sus efectos sobre los atributos agronómicos de los cultivares de frijol carioca de crecimiento indeterminado y determinado ('IPR Campos Gerais' e 'IAC Imperador'). El experimento se implementó en un diseño de bloques al azar (DBC), en un esquema factorial 2 x 9, con cuatro repeticiones. El primer factor consistió en cultivares, y el segundo factor fue el manejo de N. Los datos fueron sometidos a análisis de varianza por la prueba F, y cuando fue necesario los medios se agruparon por la prueba de Scott-Knott con un 5% de probabilidad. El uso clorofilómetro portátil es una alternativa viable para el monitoreo y manejo del N en el frijol común, permitiendo la reducción de la aplicación del nutriente en la cobertura, independientemente del cultivar utilizado.

Palabras claves

Phaseolus vulgaris L. • índice de clorofila • fijación biológica de nitrógeno • eficiencia agronómica de nitrógeno

Introduction

The common bean (*Phaseolus vulgaris* L.) has great economic and social importance worldwide. This crop is part of the food base of Brazilians, being one of the most consumed foods in the country (28). Brazil is the world's largest producer of common bean, contributing with approximately 3.12 million t, in an area of 3.17 million ha (8).

The most extracted nutrient by the crop is nitrogen (N), so the proper management of this macronutrient is essential for obtaining high yields. This nutrient is present in soil mainly in organic forms, and only a small part is found in mineral forms such as ammonium (NH_4^+) and nitrate (NO_3^-), which are usable by plants (22). The dynamics of this element in the soil is quite complex, as both forms are subject to various processes such as immobilization, volatilization, leaching and denitrification (11), making nutrient management more challenging. On other hand, the indiscriminate use of N by farmers, in order to avoid reduced yields, increases the risks of environmental contamination (11), in addition to raising production costs.

More sustainable N management practices have been studied in the last years, such as inoculation with symbiotic bacteria that perform biological N fixation (BNF), allowing increments of yield (15). However, symbiosis may have several limiting factors related to edaphoclimatic, genetic and agronomic conditions (14), which make it difficult to completely substitute the use of chemical fertilizers. In addition, the association of rhizobia with growth-promoting bacteria, such as *Azospirillum brasilense*, has promoted production increments, reducing costs and environmental risks (29), such as groundwater contamination.

Aiming to increase N use efficiency, research studies have demonstrated the feasibility of using portable chlorophyll meter to monitor the content of this nutrient and fertilization management in a wide variety of crops, such as: oats (7), maize (5) and common bean (3, 17, 18, 19, 24), among others. Chlorophyll index (CI) readings are used to calculate the N sufficiency index (NSI). This index is obtained by the relationship between CI in samples of the area to be fertilized and in the leaves of plants of a reference area, under high N fertilization (3), in order to avoid nutritional deficiency. The chlorophyll meter allows the indirect estimation of leaf chlorophyll concentration, which is significantly correlated with N concentration, because this nutrient is one of the main elements in the molecular structure of chlorophyll (30).

However, the response to the use of these technologies may vary depending on soil and climatic factors, production system and the genotype used, due to the diversity of cultivars available in the market, with different growth habits, cycles and nutritional requirements, so further studies on these materials under various environmental and management conditions are needed. Given the above, this study aimed to monitor N managements using a portable chlorophyll meter and its effects on agronomic attributes of Carioca-type common bean cultivars with indeterminate and determinate growth habits ('IPR Campos Gerais' and 'IAC Imperador').

Materials and Methods

The experiment was conducted in the agricultural year of 2016/2017, in the winter cropping season (3rd cropping season), in the municipality of Jaboticabal/SP, Brazil (21° 14' 59" S latitude South and 48° 17' 13" W longitude West at 575 m altitude). The climate is classified as Aw, according to Köppen's classification (humid tropical with rainy season in the summer and dry winter). The soil was as Latossolo Vermelho eutroférrico (Oxisol) (9), with clayey texture, and the relief is gently undulating, with 6% slope.

The experimental area was in its first year of no-tillage system (NTS). In order to implement the NTS, the soil was scarified and limed to correct acidity, with subsequent incorporation using a disc plow and two passes of a leveling harrow. ADR-300 millet (*Pennisentum americanum* L.) was sown in December 12, 2016, at density of 14 kg ha^{-1} with 0.45 m spacing between rows, aiming at straw formation. Desiccation was performed at 60 days after emergence (DAE) of the seedlings, using potassium glyphosate at dose of 1.3 g ha^{-1} of the active ingredient. Then, the crop was crushed using a mechanized straw crusher, generating 5.1 t ha^{-1} of straw dry matter.

Soil fertility chemical attributes and particle size were determined in the surface layer (0.00-0.20 m), prior to common bean sowing. The results were pH ($CaCl_2$) = 5.5; OM = 25 g dm^{-3}; P (resin) = 45 mg dm^{-3}; K = 4 $mmol_c$ dm^{-3}; Ca = 22 $mmol_c$ dm^{-3}; Mg = 14 $mmol_c$ dm^{-3}; S = 8 mg dm^{-3}; B = 0.2 mg dm^{-3}; Cu = 0.8 mg dm^{-3}; Fe = 25 mg dm^{-3}; Mn = 2.8 mg dm^{-3}; Zn = 0.2 mg dm^{-3}; H+Al = 16 $mmol_c$ dm^{-3}; CEC = 55.8 mmolc dm^{-3}; base saturation (V) = 72%; aluminum saturation (m) = 1%; clay = 540 g kg^{-1} (54%); silt = 230 g kg^{-1} (23%) and sand = 230 g kg^{-1} (23%).

The experiment was carried out in a randomized block design (RBD), in a 2 × 9 factorial scheme, with four replicates. The first factor consisted of the following cultivars from the Carioca commercial group: 'IPR Campos Gerais', of Type II indeterminate growth habit and medium cycle, and 'IAC Imperador', of Type I determinate growth habit and early cycle, according to the descriptions of the cultivars. The second factor consisted of the N managements presented in table 1. *Rhizobium tropici* was applied through seed inoculation, whereas *Azospirillum brasilense* was applied by foliar spraying during the V_4 stage. The N dose of 90 kg ha^{-1} at V_4 was adopted according to the high expected response and due to the previous cultivation of grasses and presence of irrigation (2). For the reference management (M_{Ref}), double the recommended dose was used (3), in order to avoid N deficiency. The N dose of 30 kg ha^{-1} and NSI of 90% were defined based on the results of Maia *et al.* (2017).

Seeds of the N managements with inoculation of *R. tropici* (M_{Rhz}, $M_{Rhz+Chl}$ and $M_{Rhz+Chl+Azo}$) were inoculated on the night before sowing, with liquid inoculant containing the strains SEMIA 4077, SEMIA 4080 and SEMIA 4088. The recommended dose of 200 mL for every 50 kg of seeds was used in order to meet the technical recommendation based on the Brazilian legislation, 1.2 × 10^6 viable cells per seed. Foliar application of *A. brasilense* was performed in the $M_{Rhz+Chl+Azo}$ management at 31 DAE, at V_4 stage (third trifoliate leaf completely expanded). A pressurized costal sprayer with application rate of 200 L ha^{-1} was used to apply a commercial liquid inoculant at the recommended dose of 200 mL ha^{-1}. The product contained the AbV5 strain at the concentration of 1 × 10^8 viable cells per mL of inoculant, and the application was performed in the morning.

In each experimental unit, 6 rows of common bean with 5 m length were planted, and the 4 central rows were considered as the usable area, disregarding 0.50 m on each end. Polymer-coated urea (Kimcoat®) containing 45% N was used as source of top-dressing N, applied in a continuous strip at 10 cm away from the crop row and incorporated by an irrigation water depth.

Furrowing and fertilization at sowing were mechanically performed on June 27, 2017, using 250 kg ha^{-1} of the 08-28-16 formulation, supplying 20 kg ha^{-1} of N, 70 kg ha^{-1} of P_2O_5 and 40 kg ha^{-1} of K_2O.

Table 1. Description of N managements evaluated.

Tabla 1. Descripción de manejos N evaluados.

N management	Description
M_C	Control without inoculation and without top-dressing N fertilization
M_{Rhz}	Control inoculated with *R. tropici* without top-dressing N fertilization
$M_{Rhz+Chl}$	*R. tropici* + fertilization with 30 kg·ha^{-1} of N when NSI < 90%
$M_{Rhz+Chl+Azo}$	*R. tropici* + 30 kg·ha^{-1} of N when NSI < 90% + *A. brasilense* at V_4
M_{Chl}	Fertilization with 30 kg ha^{-1} of N when NSI < 90%
M_{45}	45 kg ha^{-1} of N at V_4
M_{90}	90 kg ha^{-1} of N at V_4
M_{135}	135 kg ha^{-1} of N at V_4
M_{Ref}	Splitting of 90 kg·ha^{-1} of N at V_3 + 90 kg·ha^{-1} of N at V_4

The common bean cultivars were manually sown on June 28, 2017, under millet straw, during the morning. A 0.45 m spacing between rows with 15 seeds per meter was used. The experimental units were previously demarcated, and small paper bags were used to separate the amount of seeds needed for each row, in order to avoid the contact of seeds from different treatments. Plastic gloves were used to handle the seeds inoculated with *R. tropici*, to prevent contamination of the treatments without inoculant.

Weeds, pests and diseases were controlled with the application of products recommended for the crop. Irrigation was applied using a conventional sprinkler, with variable intervals, according to the need of the crop. The accumulated water depth was approximately 550 mm.

A portable chlorophyll meter (ClorofiLOG 1030, Falker, Brazil) was used for CI determination and NSI monitoring. The device analyzes 3 bands of wave frequency, two emitters in the red band (one close to the type of chlorophyll A and the other for chlorophyll B) and one in the near-infrared band. Readings were taken at 29, 36, 43 and 50 DAE, in the third completely expanded trifoliate leaf from the apex, as recommended by Maia *et al.* (2017). The first reading (29 DAE) was performed 11 days after fertilizing the plots referring to the reference management (M_{Ref}). Readings were performed in the morning, shading the device to avoid interference of sunlight in the readings. Five plants were randomly selected in the usable area of the plot and one reading was taken per leaflet of each trifoliate leaf, totaling 15 readings per experimental unit. The NSI of each treatment was obtained by calculating the ratio between the means of the chlorophyll meter readings obtained in each plot and in the reference plot (M_{Ref}), according to the following equation: (1)

$$NSI(\%) = \left(\frac{Mean\ of\ the\ treatment}{Mean\ of\ reference\ treatment}\right)x100$$

In situations in which the obtained NSI was higher than 90%, top-dressing N was not applied. When the value was below 90%, the N dose of 30 kg ha^{-1} was applied, according to the recommendation of Maia *et al.* (2017), in the N managements $M_{Rhz+Chl}$, $M_{Rhz+Chl+Azo}$ and M_{Chl}. After the CI was determined, the sampled leaves were collected, washed with distilled water, packed in paper bags and subjected to drying in a forced air ventilation oven at 65 °C, until constant weight. Subsequently, the material was subjected to chemical analysis to determine N concentrations (20).

The full flowering (R_6) of the cultivars 'IAC Imperador' and 'IPR Campos Gerais' occurred at 45 DAE and 53 DAE, respectively. In order to determine shoot dry mass (SDM), five consecutive plants were collected in the usable area of each plot during the stage R_6. These plants were washed, dried in a forced air circulation oven at 65 °C until constant weight and weighed. After the plants reached physiological maturity (R_9), ten consecutive plants were collected to determine the following yield components: number of pods per plant (NPP), number of grains per pod (NGP) and hundred-grain weight (HGW).

Harvest was performed by manually uprooting the plants present in two rows of the usable area of each plot. After drying in the sun, these plants were mechanically threshed. In order to obtain the yield, grain moisture was determined in kg ha^{-1}, after standardization to 0.13 kg kg^{-1} on wet basis. Agronomic efficiency of nitrogen fertilization (AE) was obtained by the following equation: (2)

$$AE = \frac{GY_F - GY_{NF}}{QN}$$

Where:

GY_F = grain yield with N fertilizer;

GY_{NF} = grain yield without N fertilizer;

QN = quantity of N applied, with results expressed in kg kg^{-1}.

The data were subjected to analysis of variance by F test ($p < 0.05$) and, when necessary, the means were grouped by the Scott-Knott test ($p < 0.05$). When F was significant for the interaction between factors, it was further analyzed using the program AgroEstat®.

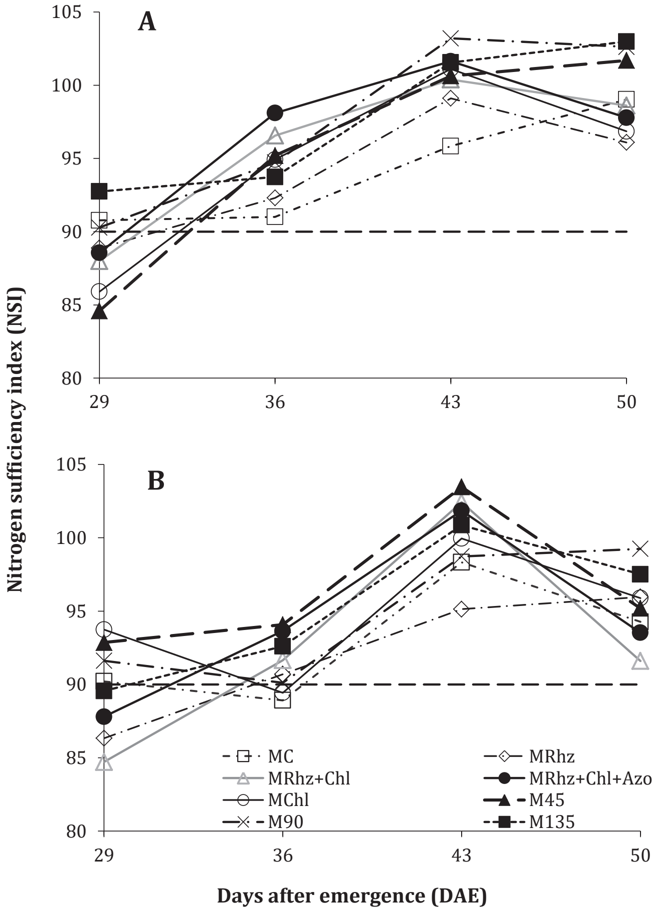
Results and Discussion

Figure 1 (page 70) shows the N sufficiency index (NSI) for the N managements as a function of evaluation periods and common bean cultivars. From the curves of NSI obtained in 4 periods (29, 36, 43 and 50 DAE), it was possible to monitor the need for top-dressing N fertilization. In the first evaluation, at 29 DAE, all N managements defined for the use of chlorophyll meter ($M_{Rhz+Chl}$, $M_{Rhz+Chl+Azo}$ and M_{Chl}) of the cultivar 'IPR Campos Gerais' showed NSI below 90% (figure 1A, page 70), and top-dressing N fertilization was performed on the day after evaluation using the established N dose of 30 kg·ha^{-1}. For the cultivar 'IAC Imperador' (figure 1B, page 70), only M_{Chl} did not show NSI below 90% on this date. However, in the following week, at 36 DAE, it was verified that the NSI was less than the established, and top-dressing N application of 30 kg ha^{-1} was carried out on the following day.

The NSI values of both common bean cultivars for each N management were increasing along the evaluation periods, reaching their maximum at 43 DAE and then stabilizing or decreasing (figure 1, page 70). This tendency may be justified by the redistribution of N to reproductive structures and by the increase in shoot dry matter mass, called "dilution effect" (10). More pronounced reduction of NSI in N managements of the cultivar 'IAC Imperador' (figure 1B, page 70) is attributed to its shorter cycle, for being an early cultivar, hence ahead in the phenological scale compared to 'IPR Campos Gerais' (figure 1A, page 70).

During the common bean cycle, the NSI (figure 1, page 70) and chlorophyll index (CI) (table 2, page 71) of the managements M_C and M_{Rhz} also increased, despite the absence of top-dressing N fertilization. This behavior could be explained by the contribution of nitrogen from the mineralization of plant residues from the previous crop (millet) and by the BNF promoted by N-fixing bacteria naturally present in the soil, because the experimental area has a history of common bean cultivation, and inserted by inoculation in the case of the M_{Rhz} management. BNF is favored under conditions of lower availability of this nutrient, because the supply of mineral N in soil can result in the reduction of BNF (23). According to Barros *et al.* (2013), fertilization at sowing with 20 kg ha^{-1} of N associated with inoculation does not inhibit nodulation and leads to an increment in SDM and yield, compared to the managements only with inoculation or only with fertilization at sowing.

Leaf N contents showed similar trends to that of CI, with increment in the values over time, reaching the maximum at 43 DAE for both cultivars, at the beginning of the reproductive stage (table 2, page 71). In the last evaluation, there was also a reduction in the contents of almost all managements, due to the effect of dilution and redistribution of the nutrient to other plant tissues. The N contents of the M_C and M_{Rhz} managements, despite the absence



M_C: control without top-dressing fertilization; M_{Rhz}: control inoculated with *R. tropici*; $M_{Rhz+Chl}$: inoculation with *R. tropici* + fertilization of 30 kg ha^{-1} of N when NSI < 90%; $M_{Rhz+Chl+Azo}$: inoculation with *R. tropici* + fertilization with 30 kg ha^{-1} of N when NSI < 90% + foliar application of *A. brasilense* (V_4); M_{Chl}: fertilization with 30 kg ha^{-1} of N when NSI < 90%; M_{45}: 45 kg ha^{-1} of N (V_4); M_{90}: 90 kg ha^{-1} of N (V_4); M_{135}: 135 kg ha^{-1} of N (V_4); M_{Ref}: reference management with 90 kg ha^{-1} of N (V_3) + 90 kg ha^{-1} of N (V_4).

M_C: Testigo sin fertilizar; M_{Rhz}: Testigo inoculado con *R. tropici*; $M_{Rhz+Chl}$: Inoculación con *R. tropici* + fertilización de 30 kg ha^{-1} de N cuando ISN < 90%; $M_{Rhz+Chl+Azo}$: Inoculación con *R. tropici* + fertilización de 30 kg ha^{-1} de N cuando ISN < 90% + aplicación de *A. brasilense* por fertilización foliar (V_4); M_{Chl}: fertilización con N de 30 kg ha^{-1} cuando ISN < 90%; $M_{45:}$ 45 kg ha^{-1} de N (V_4); M_{90}: 90 kg ha^{-1} de N (V_4); M_{135}: 135 kg ha^{-1} de N (V_4); M_{Ref}: manejo de referencia con 90 kg ha^{-1} de N (V_3) + 90 kg ha^{-1} de N (V_4).

Figure 1. Nitrogen sufficiency index (NSI) of the cultivars 'IPR Campos Gerais' (A) and 'IAC Imperador' (B) in different evaluation periods as a function of nitrogen (N) management.

Figura 1. Índice de suficiencia de nitrógeno (NSI) de los cultivares 'IPR Campos Gerais' (A) e 'IAC Imperador' (B) en diferentes tiempos de evaluación en función del manejo del nitrógeno (N).

Table 2. Comparison of chlorophyll index (CI) and leaf nitrogen content (N) in the different evaluation periods and N managements for the cultivars 'IPR Campos Gerais' and 'IAC Imperador'.

Tabla 2. Comparación del índice de clorofila (CI) y el contenido de nitrógeno en la hoja (N) (g kg^{-1}) en diferentes períodos de evaluación de N y cobertura para los cultivos 'IPR Campos Gerais' y 'IAC Imperador'.

Variables	**CI**				**Leaf N content (g kg^{-1})**			
Period	29 DAE	36 DAE	43 DAE	50 DAE	29 DAE	36 DAE	43 DAE	50 DAE
Cultivar								
IPR Campos Gerais	38.0aC	38.1aC	44.2bA	39.7aB	31.3C	39.8B	44.1A	40.3B
IAC Imperador	37.6aB	38.2aB	45.2aA	38.3bB	31.6C	38.1B	42.5A	39.1B
N management								
M_{Ref}	41.9aB	40.7aB	44.6aA	39.9aB	41.2aA	44.1aA	46.3aA	43.8aA
M_{135}	38.2bB	37.9bB	45.1aA	40.0aB	30.7bD	36.5bC	47.1aA	43.5aB
M_{90}	38.1bC	37.6bC	45.0aA	40.3aB	30.9bD	36.6bC	44.9aA	40.3bB
M_{45}	37.1bB	38.5bB	45.5aA	39.3aB	32.3bC	36.8bB	44.5aA	39.1bB
M_{Chl}	37.6bB	37.5bB	44.8aA	38.5aB	30.7bC	38.5bB	44.9aA	38.8bB
$M_{Rhz+Chl+Azo}$	36.9bB	39.0aB	45.4aA	38.2aB	30.7bC	42.8aA	42.2bA	37.0bB
$M_{Rhz+Chl}$	36.2bB	38.3bB	45.2aA	38.0aB	29.3bC	42.4aA	42.1bA	36.8bB
M_{Rhz}	36.7bB	37.2bB	43.3aA	38.3aB	29.8bB	37.1bA	40.4bA	39.7bA
M_C	37.9bB	36.6bB	43.3aA	38.6aB	27.7bB	35.4bA	37.6cA	38.3bA
CV (%)	5.3				9.0			
Overall mean	39.9				38.4			

Means followed by the same uppercase letter in the row and lowercase letter in the column do not differ by Scott-Knott test at 5% probability level. *Significant by F test ($p < 0.05$); **Significant by F test ($p < 0.01$). DAE: days after emergence; CV: coefficient of variation. M_C: control without top-dressing fertilization; M_{Rhz}: control inoculated with *R. tropici*; $M_{Rhz+Chl}$: inoculation with *R. tropici* + fertilization with 30 kg·ha^{-1} of N when NSI < 90%; $M_{Rhz+Chl+Azo}$: inoculation with *R. tropici* + fertilization with 30 kg ha^{-1} of N when NSI < 90% + foliar application of *A. brasilense* (V_4); M_{Chl}: fertilization with 30 kg ha^{-1} of N when NSI < 90%; M_{45}: 45 kg ha^{-1} of N (V_4); M_{90}: 90 kg ha^{-1} of N (V_4); M_{135}: 135 kg ha^{-1} of N (V_4); M_{Ref}: reference management with 90 kg ha^{-1} of N (V_3) + 90 kg ha^{-1} of N (V_4).

Los promedios seguidos de la misma letra mayúscula en la fila y la letra minúscula en la columna no difieren entre sí en la prueba de Scott-Knott con una probabilidad del 5%. *Significativo por la prueba F ($p <0{,}05$); **Significativo por la prueba F ($p <0{,}01$). DAE: días después de la emergencia; CV: coeficiente de variación. M_C: Testigo sin fertilizar; M_{Rhz}: Testigo inoculado con *R. tropici*; $M_{Rhz+Chl}$: Inoculación con *R. tropici* + fertilización de 30 kg ha^{-1} de N cuando ISN < 90%; $M_{Rhz+Chl+Azo}$: Inoculación con *R. tropici* + fertilización de 30 kg ha^{-1} de N cuando ISN < 90% + aplicación de *A. brasilense* por fertilización foliar (V_4); M_{Chl}: fertilización con N de 30 kg ha^{-1} cuando ISN < 90%; $M_{45:}$ 45 kg ha^{-1} de N (V_4); M_{90}: 90 kg ha^{-1} de N (V_4); M_{135}: 135 kg ha^{-1} de N (V_4); M_{Ref}: manejo de referencia con 90 kg ha^{-1} de N (V_3) + 90 kg ha^{-1} de N (V_4).

of top-dressing fertilization, were also increasing until the second evaluation, with subsequent stabilization. For M_{Ref}, the maintenance of leaf N content until the last period can be attributed to the high top-dressing N dose (180 kg ha^{-1} split into two applications), making available a large quantity of the nutrient to plants, which was able to counterbalance the dilution effect.

There was also an increase in leaf N contents in response to the N managements within each evaluation period (table 2, page 71). At 29 DAE, N managements did not cause differences in leaf N content, and only M_{Ref} was superior to the others, with a mean of 41.2 g kg^{-1}, due to the early fertilization with 90 kg ha^{-1} at 18 DAE at V_3 phenological stage. From 36 DAE, the N managements $M_{Rhz+Chl}$ and $M_{Rhz+Chl+Azo}$ were equal to the reference management, justified by the top-dressing fertilization performed in the previous week.

Prior to the third evaluation, fertilization was performed in M_{Chl} (only for 'IAC Imperador') and in the conventional managements (M_{45}, M_{90}, M_{135} and M_{Ref}), leading to a significant increase in the mean N contents of these managements at 43 DAE (table 2, page 71). The control M_C, without top-dressing fertilization and without seed inoculation, had the lowest mean. The inoculated control (M_{Rhz}) was significantly superior to M_C at this time, possibly because of the response to seed inoculation with *R. tropici*. In the last evaluation period, at 50 DAE, all N managements had the same leaf N content, except for those which received the highest top-dressing N doses (M_{135} and M_{Ref}). It is worth pointing out that both cultivars reached values within the range recommended for the crop, from 30 to 50 g kg^{-1} (2).

There were some differences between the results of leaf N contents with CI in relation to the N managements (table 2, page 71), especially in the last two periods (43 and 50 DAE). In the third evaluation, at 43 DAE, all CI values were statistically similar, whereas the N contents were significantly different. The control management (M_C) showed the lowest leaf N content, being outperformed by the management with rhizobium inoculation without top-dressing fertilization (M_{Rhz}), indicating that the rhizobium promoted increment in leaf N content, with no difference from the managements with fertilization $M_{Rhz+Chl}$ and $M_{Rhz+Chl+Azo}$. The other managements were significantly superior. The M_{Chl} management was statistically equal to those with higher N doses because the fertilization of this treatment in the cultivar 'IAC Imperador' was performed one week after the other treatments managed with chlorophyll meter, since the NSI was less than 90% in the second evaluation period. It can be noted in table 2 (page 71) that M_{Chl} had lower N content in the previous week, at 36 DAE, in comparison to $M_{Rhz+Chl}$ and $M_{Rhz+Chl+Azo}$, because fertilization had already been performed in both cultivars in these managements.

The CI values of all managements did not differ statistically at 50 DAE, whereas the N contents of M_{135} and M_{Ref} were significantly higher, as previously discussed. In the comparison of CI values of the N managements within each period, there were no significant differences between M_C and M_{Rhz} and almost all managements that received top-dressing fertilization, unlike results for leaf N content. This may have occurred because the chlorophyll meter is based on a technique of indirect measurement of chlorophyll content.

The cultivar 'IPR Campos Gerais' showed higher SDM (table 3, page 73), since it is a cultivar of normal growth habit,

Table 3. Weight of shoot dry mass (SDM), number of pods per plant (NPP), number of grains per pod (NGP), hundred-grain weight (HGW), grain yield (GY) and agronomic efficiency of nitrogen fertilization (AE) of common bean as a function of cultivars and nitrogen (N) managements.

Tabla 3. Peso de la masa seca aérea (SDM), número de vainas por planta (NPP), número de granos por vaina (NGP), cien granos de masa (HGW), rendimiento de grano (GY) y eficiencia agronómica de la fertilización nitrogenada (AE) de plantas de frijol en función de cultivares y manejo de nitrógeno (N).

Variables	SDM (g plant^{-1})	NPP	NGP	HGW (g)	GY (kg ha^{-1})	AE (kg kg^{-1})
Cultivar						
IPR Campos Gerais	7.1a	11.0a	4.8a	24.2a	2,933a	15.4a
IAC Imperador	4.3b	11.7a	4.7a	21.6b	2,120b	12.2a
N management						
M_{Ref}	6.8a	13.7a	5.0a	23.8a	3,160a	7.0b
M_{135}	5.8a	13.1a	4.7a	23.5a	2,648b	5.5b
M_{90}	5.9a	13.6a	4.8a	22.5a	2,605b	7.7b
M_{45}	5.9a	12.0a	4.9a	22.9a	2,769b	19.1a
M_{Chl}	6.5a	9.7b	4.7a	22.6a	2,423b	17.1a
$M_{Rhz+Chl+Azo}$	5.7a	10.5b	4.9a	22.7a	2,526b	20.6a
$M_{Rhz+Chl}$	5.1a	10.4b	4.6a	23.0a	2,493b	19.5a
M_{Rhz}	4.7a	9.2b	4.8a	22.2a	2,202c	-
M_C	4.9a	9.6b	4.7a	23.0a	1,909c	-
CV (%)	26.7	21.1	6.5	5.4	16.4	85.5
Overall mean	5.7	11.3	4.8	22.9	2,526	-

Means followed by the same uppercase letter in the row and lowercase letter in the column do not differ by Scott-Knott test at 5% probability level. * Significant by F test ($p < 0.05$); ** Significant by F test ($p < 0.01$). CV: coefficient of variation. M_C: control without top-dressing fertilization; M_{Rhz}: control inoculated with *R. tropici*; $M_{Rhz+Chl}$: inoculation with *R. tropici* + fertilization with 30 kg·ha^{-1} of N when NSI < 90%; $M_{Rhz+Chl+Azo}$: inoculation with *R. tropici* + fertilization with 30 kg ha^{-1} of N when NSI < 90% + foliar application of *A. brasilense* (V_4); M_{Chl}: fertilization with 30 kg ha^{-1} of N when NSI < 90%; M_{45}: 45 kg ha^{-1} of N (V_4); M_{90}: 90 kg ha^{-1} of N (V_4); M_{135}: 135 kg ha^{-1} of N (V_4); M_{Ref}: reference management with 90 kg ha^{-1} of N (V_3) + 90 kg ha^{-1} of N (V_4).

Los promedios seguidos de la misma letra mayúscula en la fila y la letra minúscula en la columna no difieren entre sí en la prueba de Scott-Knott con una probabilidad del 5%. *Significativo por la prueba F ($p <0,05$); **Significativo por la prueba F ($p <0,01$). DAE: días después de la emergencia; CV: coeficiente de variación. M_C: Testigo sin fertilizar; M_{Rhz}: Testigo inoculado con *R. tropici*; $M_{Rhz+Chl}$: Inoculación con *R. tropici* + fertilización de 30 kg ha^{-1} de N cuando ISN < 90%; $M_{Rhz+Chl+Azo}$: Inoculación con *R. tropici* + fertilización de 30 kg ha^{-1} de N cuando ISN < 90% + aplicación de *A. brasilense* por fertilización foliar (V_4); M_{Chl}: fertilización con N de 30 kg ha^{-1} cuando ISN < 90%; $M_{45:}$ 45 kg ha^{-1} de N (V_4); M_{90}: 90 kg ha^{-1} de N (V_4); M_{135}: 135 kg ha^{-1} de N (V_4); M_{Ref}: manejo de referencia con 90 kg ha^{-1} de N (V_3) + 90 kg ha^{-1} de N (V_4).

type II, which continues to produce new vegetative structures after the beginning of the reproductive stage, unlike early cultivars, which interrupt the production of new branches and leaves, besides the longer cycle. SDM is associated with yield and the absorption and accumulation of nutrients (11). Between the N managements, there were no significant differences for this variable.

For the yield components (table 3, page 73), the number of pods per plant (NPP) reached mean values of 11.0 and 11.7 for the cultivars 'IPR Campos Gerais' and 'IAC Imperador', respectively. Despite the different growth habits and the interruption of the formation of new nodes at the beginning of the reproductive stage of early cultivars, such as 'IAC Imperador', no statistical differences were observed, demonstrating good performance of the early cultivar in relation to pod formation.

Regarding the N managements, those without top-dressing fertilization (M_C and M_{Rhz}) and with fertilization established by the chlorophyll meter ($M_{Rhz+Chl}$, $M_{Rhz+Chl+Azo}$ and M_{Chl}) did differ statistical for this variable, being outperformed by the other managements with top-dressing N doses ranging from 45 to 180 kg ha^{-1} (table 3, page 73).

The mean NGP of both cultivars was close to 5 and was not influenced by the N managements. This variable is of high genetic heritability, being usually resistant to environmental influences and management, such as N fertilization (25). For HGW, difference was only found between the cultivars, and 'IPR Campos Gerais' stood out with heavier grains, but there were no statistical differences within the managements. This result can be attributed to the low influence of the environment in this attribute (26).

The most productive cultivar was 'IPR Campos Gerais', followed by 'IAC Imperador', with means of 2,993 and 2,120 kg ha^{-1}, respectively. This variable was significantly influenced by the N managements. The managements without top-dressing fertilization (M_C and M_{Rhz}) showed the lowest values (1,909 and 2,202 kg ha^{-1}, respectively) and did not differ statistically. These mean yields are higher than the average of 1,137 kg ha^{-1} for the autumn-winter common bean of the country (8). The fact that both managements result in satisfactory yields can be justified by the genetic potential of the cultivars, high soil fertility of the experimental area, contribution of organic matter, mineralization of plant residues and contribution of BNF, due to previous cultivation of common bean. High yields in this crop can be achieved even without top-dressing fertilization, in no-tillage system (27).

Seed inoculation with the commercial dose of *R. tropici* and foliar application of *A. brasilense* (M_{Rhz} and $M_{Rhz+Chl+Azo}$, respectively) did not promote significant increment in any of the variables shown in table 3, page 73. Field experiments have demonstrated gains with sustainability with the use of *R. tropici* and *A. brasilense* (15, 29). However, symbiosis may have several limiting factors related to the edaphoclimatic, genetic and agronomic conditions (14), which restrict its efficiency.

Rhizobia are free-living microorganisms, and there is signaling between symbionts, infection and the development of nodules under conditions with N limitation. Nitrogenase, the catalytic enzyme of BNF, also demands great energy investment (30). Therefore, the greater N availability in soil due to the fertility of the experimental area may have limited biological fixation, leading to greater energy saving. Fertilizer application reduces N contribution by BNF (31).

Another limiting factor may have been the occurrence of low temperatures during the vegetative stage, when the minimum temperature reached values lower than 15 °C, with averages of 12.9 and 13.2 °C for the cultivars 'IAC Imperador' and 'IPR Campos Gerais', respectively. Possibly because of the presence of straw and irrigation, the temperatures in soil reached lower values than those recorded in the air. Lower temperatures hinder infection, nodule development and BNF (13). In addition, native rhizobia can also promote yields equivalent to those obtained with the inoculation of rhizobium strains (12), which may compete for occupying the sites of nodular infection.

There are also variable responses for the use of *A. brasilense* in agriculture, which may depend on the cultivar used, mode of application and environmental conditions (6). An explanation for the absence of response may be related to the phenological stage of *A. brasilense* application, which was performed later, at V_4. Seed inoculation and foliar application at previous stages (V_2, V_3) could lead to a different result, favoring the initial growth of the roots. Therefore, more studies are needed under several experimental conditions, with doses and forms of application in order to better indicate the adoption of this technology in each system of production and management for the rural producer.

The yields of managements based on the chlorophyll meter ($M_{Rhz+Chl}$, $M_{Rhz+Chl+Azo}$ and M_{Chl}) were significantly superior to N managements without top-dressing fertilization, being equivalent to the N dose of 135 kg ha^{-1} (M_{135}) (table 3, page 73). Management with chlorophyll meter reduced the need for chemical fertilizer application, without significant reduction of yield, saving 105 kg ha^{-1} of N.

The average agronomic efficiency (AE) of the N applied as top-dressing for the two cultivars was very similar, with greater variation as a function of the managements (table 3, page 73). The managements $M_{Rhz+Chl}$, $M_{Rhz+Chl+Azo}$, M_{Chl} and M_{45} had higher AE. This is an important criterion to define fertilization management, which can be influenced by the N doses and production system (1). Monitoring with portable chlorophyll meter proves to be a useful tool to define the moment of N application, avoiding unnecessary fertilization, promoting higher efficiency and lower production costs and environmental impacts (19).

The increase in fertilizer dose leads to gains of yield, and the highest value was obtained by the M_{Ref} management, with 180 kg ha^{-1} of N (table 3, page 73), but the cost-benefit ratio should be taken into account, since most N fertilizers are imported.

Although the portable chlorophyll meter can indicate the time of N application and is not influenced by the "luxury consumption" of N by the plant in the form of nitrate (5), the device has limitations because it does not consider the effect of dilution due to the production of fresh matter (21). Studying the maize crop, Hurtado *et al.* (2011) state that the adoption of a fixed NSI throughout the cycle may not be the most appropriate strategy. Silveira and Gonzaga (2017), using the chlorophyll meter SPAD-502, proposed that for every 0.1% increase in the NSI value to reach the adequate level defined in their study (95%), it would be necessary to apply from 1.1 to 1.5 kg ha^{-1} of top-dressing N. Despite the promising results, further studies are needed to define the most adequate indices and doses of N for different genotypes cultivated in several production systems in order to improve the use of this technology.

Conclusions

The use of portable chlorophyll meter is a viable alternative for N monitoring and management in common bean, allowing the reduction of top-dressing N application, regardless of the cultivar used.

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