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Volume 13 - No. 2 - 2019

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EDITORIAL NOTE

DIEGO MIRANDA LASPRILLA
Chief editor
Revista Colombiana de Ciencias Hortícolas

The institutions Sociedad Colombiana de Ciencias Hortícolas (SCCH), Universidad Pedagógica y Tecnológica de Colombia (UPTC) and Universidad Francisco de Paula Santander (UFPS), editors of the Revista Colombiana de Ciencias Hortícolas (Category B in Publindex - Colciencias), inform our authors and readers that the economic and administrative problems we faced in 2019 have been dealt with, and it is our desire to carry on with our editorial role and continue to strengthen all sections of our journal.

As such, it is our pleasure to introduce to our readers volume 13 number 2 of 2019, with 16 articles, published completely in English, with a digital format that allows us to publish articles individually and restructure pagination when the design and diagrams are finished. However, a print version of this edition will be maintained for legal and administrative purposes.

This issue has contributions to the Fruit Section that include research on the physiological responses of a banana crop to the use of biostimulants with an evaluation of the use of exogenous cytokinins and their effect on growth and development in this important fruit species and an evaluation of plastic covers for the protection of banana bunches and their effect on quality. We also included an article on genetics (evaluation of half-sibling families) in the cultivation of Creole melon grown on the equatorial coasts. Another article analyzed dwarf anacardium peduncles for use in food as fresh and processed products. This section ends with a timely topic related to the resilience of citrus agroecosystems with a sociological focus.

The Vegetable Section begins with the results of research on the molecular characterization of pea genotypes using SSR markers and continues with an article on molybdenum nutrition in sweet corn cultivation at different doses and application frequencies. In addition, there is a paper on the effect of organic and mineral nutrition on the growth and development of crisp lettuce. Another article relates an evaluation of some pumpkin introductions based on the nutritional quality of the seeds. This section also includes an article on the physiological response of the tomato to a mineral compound in the soil and another one on weed control in corn crops with organic management.

The Ornamentals Section contains an article on the use of diazotrophic bacteria in micropropagated ornamental pineapples and ends with an article on carnation cultivation productivity in response to the application of modified nutritional solutions.

Finally, the Section on Aromatic, Medicinal and Seasoning Herbs includes an article on the species *Lippia alba* that deals with the use of phosphorus and luminosity gradients in terms of seedling quality. A second article evaluates the behavior of stevia genotypes in response to long days.

We have used this content to publish distinct articles from areas of horticulture that suffered from the management and administrative difficulties that the Sociedad Colombiana de Ciencias Hortícola, owner of this journal, was going through, for which we apologize to our readers and reiterate that our goal with the journal is to share the knowledge gained for various horticultural species with a view towards contributing to the solution of current horticultural problems with science and technology.

To conclude, we remind our authors to continue to adjust the content of their articles to our publication standards in order to streamline and update our volumes.

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Effect of biostimulants on dry matter accumulation and gas exchange in plantain plants (*Musa* AAB)

Efecto de bioestimulantes sobre la acumulación de materia seca e intercambio de gases en plantas de plátano (*Musa* AAB)

DIANA MATEUS-CAGUA[1, 2]
GUSTAVO RODRÍGUEZ-YZQUIERDO[2, 3]

'Hartón' plantain plants in greenhouse, Granada (Colombia).

Photo: D. Mateus-Cagua

ABSTRACT

Biostimulants can potentially improve plant growth and development, modifying physiological processes. This study evaluated the effect of four biostimulants on the growth of 'Hartón' plantain plants and the leaf gas exchange during the vegetative phase. This experiment was developed on a plantain farm's nursery in Fuente de Oro (Colombia) with a randomized complete block design with four replicates. The treatments were the biostimulants: Bactox WP®: *Bacillus subtilis* (*Bs*); Baliente®: *Bacillus amyloliquefaciens* (*Ba*); Tierra Diatomeas®: silicon dioxide (Si); Re-Leaf®: salicylic acid (SA) and the control (water). All products had a positive effect on the accumulation of total dry matter (DM) (between 58.4 and 21.9%) and on the photosynthetic activity (a maximum of 110 and 24.3% in first and second evaluation), as compared to the control, while no differences were found ($P>0.05$) for the foliar emission rate and chlorophyll content between the treatments. The plants treated with *Bs* had the greatest DM accumulation at the end of the study and a constant, high photosynthetic activity. All the while *Bs, Ba* and Si managed to stimulate greater early photosynthetic activity. According to the results, the use of these biostimulants during the vegetative phase had an effect on the physiological processes that enhance DM accumulation in plantain plants, which could be potentially useful for the transplanting stage and increase the reserves used during their establishment and development in the field.

Additional key words: photoassimilate distribution; 'Hartón' plantain; plant growth promoting rhizobacteria (PGPR); *Bacillus*; silicon; salicylic acid.

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RESUMEN

Los bioestimulantes son productos que potencialmente pueden mejorar el crecimiento y desarrollo de las plantas al modificar procesos fisiológicos. En este estudio se evaluó la influencia de cuatro bioestimulantes en el crecimiento de plantas de plátano 'Hartón' e intercambio de gases en un periodo de la fase vegetativa. El experimento se desarrolló en el vivero de una finca productora de plátano en Fuente de Oro (Colombia), en un diseño de bloques completos al azar generalizados con cuatro repeticiones. Los tratamientos correspondieron a los bioestimulantes Bactox WP®: *Bacillus subtilis* (*Bs*); Baliente®: *Bacillus amyloliquefaciens* (*Ba*); Tierras de diatomeas®: dióxido de silicio (Si); Re-Leaf®: ácido salicílico (As) y el control (agua). Todos los productos mostraron tener un efecto positivo en la acumulación de materia seca (MS) total (entre 58,4 y 21,9%) y en la actividad fotosintética (en un máximo de 110 y 24,3% en primera y segunda evaluación) respecto al control, mientras que en ritmo de emisión foliar y contenido de clorofila no se encontraron diferencias ($P>0,05$) entre tratamientos. Plantas tratadas con *Bs* tuvieron la mayor acumulación de MS al finalizar el estudio y una alta actividad fotosintética constante. Mientras que *Bs*, *Ba* y Si lograron estimular una mayor actividad fotosintética temprana. De acuerdo con los resultados el uso de estos bioestimulantes durante esta fase vegetativa tiene efecto sobre procesos fisiológicos que mejoran la acumulación de MS en plantas de plátano, lo que podría capacitarlo para enfrentar la etapa de trasplante y aumentar las reservas para ser utilizadas durante su establecimiento y desarrollo en campo.

Palabras clave adicionales: distribución de fotoasimilados; plátano 'Hartón'; rizobacterias promotoras de crecimiento (PGPR); *Bacillus*; silicio; ácido salicílico.

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INTRODUCTION

Biotic and abiotic factors affect crop production around the world. The presence of any of these can alter growth and development or completely interrupt the productive cycle, affecting physiological processes; for this reason, currently, strategies are being sought to prevent and minimize the risk of loss in production systems with a low investment, without affecting safety or quality (Halpern *et al*., 2015; Posmyk and Szafrańska, 2016). Several biostimulants have been evaluated and recommended as an alternative that prevents damage from these limitations, optimizing the growth of plants through different mechanisms (Saa *et al*., 2015; Wang *et al.,* 2016; Magalhães *et al*., 2016), promoting quality and indirectly decreasing the use of agrochemicals (Yakhin *et al.,* 2017).

According to du Jardin (2015), a plant biostimulant is any substance or microorganism applied to plants with the aim to enhance nutrition efficiency, abiotic stress tolerance and/or crop quality traits, regardless of its nutrients content. These substances can regulate physiological processes and perform directly on the metabolism, affecting development and productivity (Bulgari *et al.,* 2015; Yakhin *et al.,* 2017). Different categories of biostimulants have been proposed (Calvo *et al.,* 2014); these include products containing hormones (Kauffman *et al.,* 2007), inorganic compounds (such as Se y Si), and bacteria (mutualistic endosymbiont and plant growth-promoting rhizobacteria -PGPR-) (du Jardin, 2015), etc. Positive results have been found for different conditions and crops uses. Thus, silicon applications have been reported that improve plant growth and development under stress (Kurabachew and Wydra*,* 2014; Aucique-Pérez *et al*., 2017; Helaly *et al*., 2017) and non-stress conditions (Lavinsky *et al*., 2016). Likewise, PGPR applications have been shown to impact plant growth (Lavakush *et al*., 2014; Calvo *et al*., 2017), photosynthetic activity (Stefan *et al*., 2013), accumulation of dry matter, quality and yield of the final product (Mena-Violante and Olalde-Portugal, 2007; Ul Hassan and Bano, 2015).

Plantains and bananas are fruits crop with critical importance to global food security. The estimated annual global production exceeds 147 million t (FAOSTAT, 2016). However, these species are considered crops with high demand for agricultural inputs in order to obtain outstanding production and minimize risk from external factors, which in addition to being expensive may have an impact on the environment (Mia *et al*., 2010a). Biostimulants based on PGPR have been shown to enhance banana plant growth and nutrient uptake. Applications in a nursery can influence subsequent physiological stages, as well as in the final production, showing potential for use as an alternative in integrated management and cost reduction (Kavino *et al*., 2010; Mia *et al.,* 2010a, 2010b). These reports indicate that the application of biostimulants (PGPR or another source) in the nursery phase could be useful for obtaining a greater accumulation of dry biomass for the critical stages of the species.

In this study, four biostimulants were evaluated: two PGPRs (*Bacillus subtilis* (*Bs*), *Bacillus amyloliquefaciens* (*Ba*)), silicon dioxide (Si) and salicylic acid (As). The objective was to determinate their influence on growth variables and gas exchange in 'Hartón' plantain plants in the nursery phase. In this case, it was found that the use of these products promoted a greater accumulation of dry matter; furthermore, *Bs* and Si stimulated an early and higher photosynthetic rate, improving it significantly.

MATERIALS AND METHODS

This study was conducted in an outdoor nursery on a plantain production farm in Fuente de Oro, Meta (Colombia, 2017-2018) (3°25'39" N and 73°37'12" W, altitude 307 m a.s.l.) from November to February. The climate of the region was tropical humid with an annual average rainfall of 2,621 mm (in a monomodal pattern) and a mean temperature of 25.6°C. Plantain corms from cv. 'Hartón' (*Musa* AAB) were used from a farm with ICA registration to obtain the plant material. The whole corm technique was used for macropropagation (Njukwe *et al*., 2007; Buah and Tachie-Menson, 2015), which were established in wooden propagators for a month and a half. Then, shoots were carefully excised from the corm and transplanted as individual plantlets in 1.0 × 7.0 m propagators containing rice husk. Each nursery bed was irrigated to maintain a 23±3% substrate moisture content; the plants were fertilized biweekly with a mixture of diammonium phosphate, urea, potassium chloride and micronutrients. This experiment was established in a randomized complete block design (simple factorial), with four repetitions and six plants per treatment. The treatments were the biostimulants (Bactox WP®: *Bacillus subtilis* (*Bs*); Baliente®: *Bacillus amyloliquefaciens* (*Ba*); Tierra Diatomeas®: silicon dioxide (Si); Re-Leaf®: salicylic acid (SA)) and control (current volume of water). The products were applied based on the recommended doses: 5 g L^{-1}, 2.5 and 1.25 cc L^{-1}, and 4 g L^{-1} every 21 d from 0 to 12th week. First, two biostimulants were applied as a substrate drench, SA by spraying the leaf tissue and by drenching and foliar spraying in the last two.

'Hartón' plantain plantlet growth

The pseudostem length was determined with a measuring tape, from the ground level to the insertion of the last leaf (youngest leaf); basal diameter was recorded with a Vernier caliper, and measured at 2 cm above the surface of the substrate. The foliar emission rate (FER) was determined by counting the number of fully expanded leaves emerged on each plant (Galán-Saúco and Robinson, 2013). These measurements were carried out in three plants per experiment unit every 15 d.

At the end of the experiment, plants were carefully removed from the propagators, detached and weighed for the different organs (leaves, pseudostem, corm and roots). A sample of each one was taken to estimate the dry matter (DM) content. Likewise, the maximum root-length was measured with a tape measure. The collected plant samples were dried for 72 h in an oven at 65°C, then the dry biomass was recorded.

Gas exchange and SPAD units

The leaf gas exchange were measured using a LI-6400XT portable photosynthesis system (LICOR, Inc., Lincoln, NE), equipped with a LED light source (6400-02B). The assessments were taken between 9:00 and 11:30 am at a photosynthetic photon flux density of 300 μmol m^{-2} s^{-1} with a previous light saturation curve, while the CO_2 concentration was adjusted to 400 μmol m^{-2} s^{-1}; the data were recorded when the coefficient of variation was less than 5%. Parameters such as net photosynthesis rate (A), transpiration rate (Tr), stomatal conductance (Gs) and intercellular CO_2 concentration (Ci) were evaluated. The instantaneous carboxylation efficiency was calculated as ratio of A to Ci (A/Ci). A portable chlorophyll meter (SPAD 502, Konica Minolta, Tokyo) was used to determinate the chlorophyll content based on the SPAD index. Data were collected from eight plants per treatment in the last four weeks, corresponding to 10th through 12th week; the third youngest fully expanded leaf (from the top) was measured for each plant (Galán-Saúco and Robinson, 2013).

Statistical analysis

A variance analysis followed by Tukey's multiple comparison test was carried out using statistical software R. Statistical significance at $P \leq 0.05$ was used to determine significant differences between the treatments. The data for length, diameter of pseudostem and number of emitted leaves were analyzed in a design with repeated measurements where time was considered the intra-subject factor. The means were determined with 12 plants per treatment in these variables.

RESULTS

The 'Hartón' plantain plantlets were treated with four commercial biostimulants for 12 weeks to identify their effect on physiological variables.

Plantain plantlet growth

There were no significant differences ($P > 0.05$) caused by the interaction (treatment and time) or treatments in the length, pseudostem diameter or number of emitted leaves, although there was effect of time from fourth week (Fig. 1). Despite the absence of differences caused by the treatments, there was a slight reduction in the increase of pseudostem length and diameter starting with the sixth week, mainly in the treatments *Ba*, *Bs* and Si; however, the trend was to increase (Fig. 1A and 1B). At the end of the experiment, the average length was 53.3 cm, the pseudostem diameter was 4.23 cm and the number of emitted leaves was 12.73 (Fig. 1C). The mean FER was between 0.60 and 0.77, with no differences between the treatments (Tab. 1).

All the plants submitted to treatment with biostimulants had a higher total DM accumulation than the control at 12 weeks after initiation of treatments (Tab. 1) although significant differences were found with *Bs*. The highest accumulation in the leaves, pseudostem, corm and root were stimulated with *Bs* while the control treatment maintained the lowest accumulation in the leaves, pseudostem and root. The maximum root length was achieved with the application of *Bs* and Si while As and *Ba* resulted in the lowest values. In general, the results suggested a positive effect from the biostimulants on the plantain plant growth.

Dry matter distribution between the plant organs; leaves had the greatest DM accumulation (percentage) during the vegetative phase in all treatments, followed by the pseudostem, corm and roots (Fig. 2).

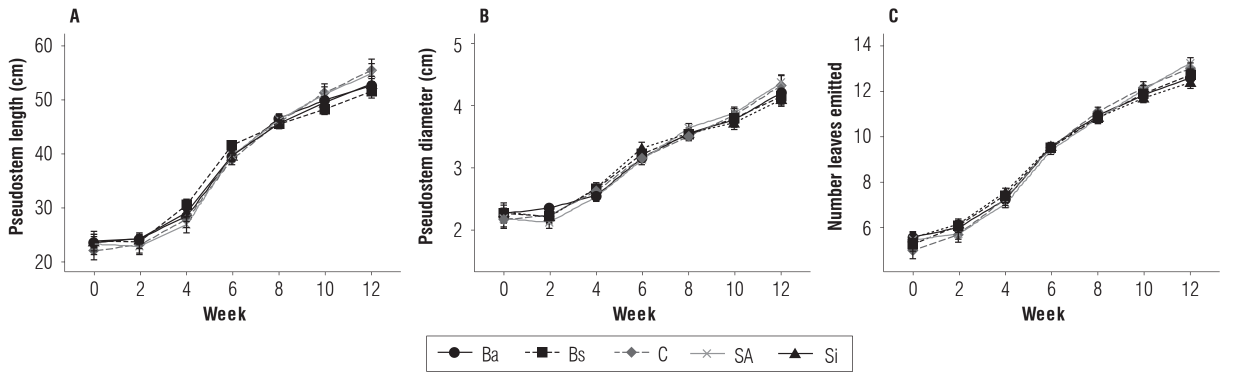

Figure 1. **Pseudostem length (A) and diameter (B); number of leaves (C) emitted in 'Hartón' plantain seedlings treated with biostimulant (*Bs*: *B. subtilis*, Si: silicon dioxide, SA: salicylic acid, C: control, *Ba*: *B. amyloliquefaciens*) in biweekly samplings from corm independence. Bars correspond to the standard error considering intra-subject variables (*n*=12).**

Table 1. Influence of biostimulants on accumulation of dry matter, maximum root length and foliar emission rate (FER) of 'Hartón' plantain plants 12 weeks after detachment from the corms.

Treatments	Dry matter (g)					Root length (cm)	FER
	Leaves	Pseudostem	Corm	Root	Total		
Bs	27.30 b	18.92 b	12.23 b	6.26 b	64.71 b	50.05 b	0.66 a
SA	20.83 ab	13.01 ab	9.85 ab	6.23 b	49.91 a	43.06 a	0.77 a
Ba	20.58 ab	17.57 a	8.90 a	4.88 ab	51.92 ab	42.48 a	0.67 a
Si	20.01 ab	18.04 a	9.59 ab	6.14 ab	53.78 ab	50.99 b	0.60 a
C	17.23 a	9.69 a	9.30 a	4.74 a	40.95 a	44.69 ab	0.72 a

Means with different letters indicate significant differences after the Tukey test ($P \leq 0.05$) ($n=12$). *Bs*, *B. subtilis;* As, salicylic acid; *Ba*, *B. amyloliquefaciens;* Si, silicon dioxide; C, control.

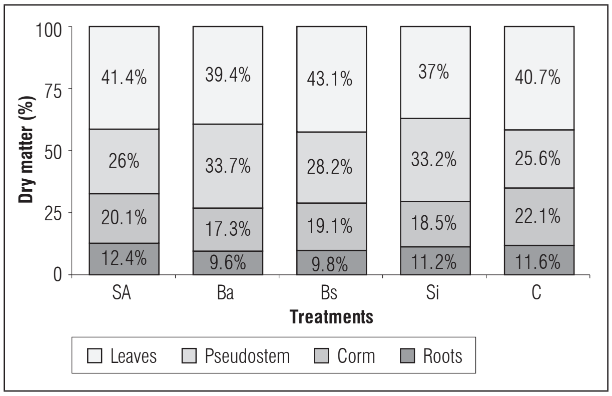

Figure 2. Dry matter distribution in 'Hartón' plantain plants 12 weeks after being independent from the corm. *Bs*: *B. subtilis*, Si: silicon dioxide, SA: salicylic acid, C: control (water), *Ba*: *B. amyloliquefaciens*.

Photosynthetic activity

Gas exchange variables were measured to validate the biostimulant effect on photosynthetic activity. The assessments were carried out at the 10th and 12th week (Fig. 3). The differences between the treatments were significant ($P \leq 0.05$) for A, Tr and carboxylation efficiency (Fig. 3). Three of the four biostimulants increased the net CO_2 assimilation rate (A), as compared to the control: *Bs*, *Ba* and Si, while SA was in the same data range as the control plants. These three treatments had 110, 81 and 63% higher photosynthetic performance than the control in first evaluation. The results obtained with Gs and Tr had a similar trend. *Bs* induced the highest values at the first sampling (10th week) in A and Tr, resulting in a 2-fold increase as compared to the control plants. For the subsequent evaluation (12th week), the difference between the non-treated, SA treated plants and the remaining treatments was reduced. Silicon dioxide reached the values of *Bs* in A and A/Ci; these two treatments had the highest values for the measured parameters, with a significant difference in A/Ci.

No significant differences were found in Ci between the treatments, while the instantaneous carboxylation efficiency had the same tendency as the photosynthetic rate, with significant differences. There were no differences in the chlorophyll content (SPAD index) between the treatments ($P>0.05$); the average content was between 38.88 and 43.88 SPAD units in both the first and second evaluation (Fig. 3).

DISCUSSION

The use of biostimulants has been shown to promote growth in different plant species (Gemin *et al*., 2019; Saia *et al*., 2019; Agarwal *et al*., 2019). Musaceas such as plantains and banana plants require a high and fast dry matter accumulation and distribution to produce high-quality bunches (Turner, 1998; Chaves *et al*., 2009). Because these plants have a determinate growth, the development of the root system and leaves occurs during the vegetative phase and ceases with floral differentiation (Turner, 1998; Chaves *et al*., 2009). In this stage, the leaves, being the main source of assimilates, allocate part of the dry matter to form a strong pseudostem, while the corm is the main storage organ (Turner, 1998; Martínez and Cayón, 2011). Since stored photoassimilates can be remobilized in the next phases, an adequate dry matter accumulation at this phase could improve the source-sink relationship. The results revealed that the *Bs*, Si, *Ba* and SA applications increased the total dry matter accumulation in the 'Hartón' plantain plants, with values of 58.3, 31.3, 26.8 and 21.9%,

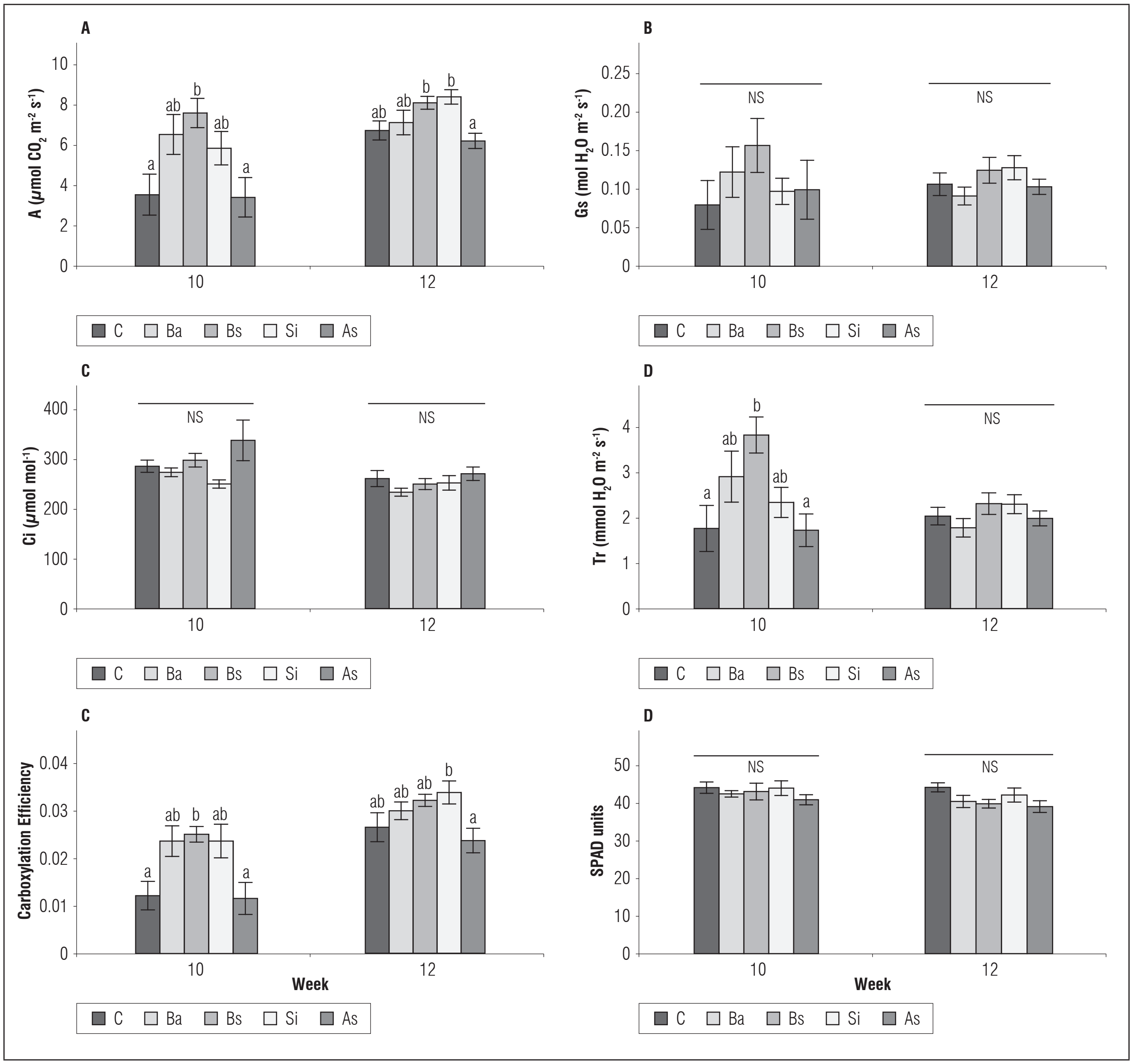

Figure 3. Influence of biostimulants on parameters of gas exchange in 'Hartón' plantain plants, 10 and 12 weeks after being independent of the corm. A: photosynthesis; Gs: stomatal conductance; Ci: intercellular CO_2 concentration; Tr: transpiration; carboxylation efficiency (A/Ci): μmol m^{-2} s^{-1}; NS: not significant. Bars correspond to the standard error. Means with different letters per week of evaluation indicate significant differences between treatments after the Tukey test ($P \leq 0.05$) ($n = 8$). *Bs*: *B. subtilis*, Si: silicon dioxide, SA: salicylic acid, C: control, *Ba*: *B. amyloliquefaciens*.

respectively, as compared with the control plants during the vegetative phase. Although this improvement occurred through different modes of action, the results indicated that these mechanisms directly or indirectly improved the photosynthetic activity, as can be observed with *Bs*, *Ba* and Si (Fig. 3).

B. subtilis was the treatment (*Bs*) that induced the highest response of biomass production and photosynthetic activity in the vegetative period. This PGPR has been reported as promoting DM accumulation through direct mechanisms: phytohormone production such as auxins and gibberellins (involved in processes such as cell elongation and division) and enhancing plant nutrient absorption (Mohamed and Gomaa, 2012; Ahmad *et al*., 2017; Jang *et al*., 2018). In addition, *Bs* has been shown to increase photosynthetic efficiency, chlorophyll content and sugar accumulation in several studies (Zhang *et al*., 2008; Ul Hassan and Bano, 2015). According to Zhang *et al*. (2008), *Bs* can repress hexokinase-dependent glucose signaling (HXK), which limits the photosynthetic inhibition induced by the end-product and stimulates photosynthetic activity. The high activity found in this experiment was accompanied by an increase in Gs at the 10th week (Fig. 3), which coincided with the reports of Zhang *et al*. (2008) and Mahomed and Gomaa (2012), who stated that *Bs* mediates the reduction of abscisic acid levels, the hormone involved in stomatal closure. *Bs* could promote an increase in endogenous hormones, improving the efficiency of nutrient acquisition, with better root growth evidenced in DM accumulation (Tab. 1) and an increase in the photosynthetic rate (Fig. 3), which would explain the outstanding feature.

The plants treated with *B. amyloliquefaciens* (*Ba*) showed an intermediate behavior in both A and DM accumulation. In comparison with *Bs* (also a PGPR), *Ba* stimulated leaf and pseudostem growth more than root growth although the pseudostem length and diameter (Fig. 1) were similar, suggesting its effect as a growth promoter, which could be related to its action on the hormonal balance as reported by Asari *et al*. (2017). That promotion was also observed in the evaluation of the photosynthetic activity. The treatments did not show differences in the chlorophyll content (SPAD index); the data were similar to reports for Musaceae (Hooks *et al*., 2008; Anusuya, 2014).

The effects of exogenous applications of SA on the physiological processes of plants, on the other hand, are controversial. Some studies have reported better growth, photosynthetic activity and photosynthetic pigment contents with its use in stressed plants (Jalal *et al*., 2012; Nazar *et al*., 2015; Cao *et al*., 2015; Elhakem, 2019), while other research results have indicated a negative effect on these parameters (Mancheva *et al*., 1996). Therefore, these assays suggest that there is a species-specific response and a relationship with the applied proportion (Janda *et al*., 2014). In this study, the SA application enhanced the dry matter accumulation of the 'Hartón' plantain plants in comparison with the control plants (although without statistical differences) by a different mechanism that increased photosynthetic activity (Fig. 3).

Silicon has been the subject of multiple studies. Its main positive effect has been found in alleviating biotic or abiotic stress (Zanetti *et al*., 2016; Maghsoudi *et al*., 2016a). However, under the non-stress conditions of the present study, the results showed that Si induced a higher photosynthetic rate than the control plants on the two evaluation dates although there was a statistical difference. Similar results were reported for wheat, corn, and rice by Maghsoudi *et al*. (2016b), Xie *et al*. (2014), and Detmann *et al*. (2012), respectively. The stimulated photosynthetic activity could be related to the stability in the Tr values because, despite increases in Gs between the first and second evaluation, the Tr rates remained constant and the water use efficiency improved (data not shown). This response could be related to Si deposition mainly in epidermal cells, which would maintain transpiration and plant water potential, as has been reported for rice and corn (Agarie *et al*., 1998; Gao *et al*., 2006). The carboxylation efficiency was greater in the Si treated plants than in the control, suggesting a higher CO_2 fixation as a result of the increase in Gs; the total DM accumulation was also superior to the control plants, especially at the leaf and pseudostem level. In addition, according to Xu *et al*. (2016), an increase in photosynthesis without differences in Ci (between the first and second evaluation) could suggest that Si has an effect on stomatal and non-stomatal factors, as has been reported in rice plants subjected to stress, in which an Si application increased the transcription of genes involved in photosystem efficiency and electron transport chain (Song *et al*., 2014).

In banana, Mia *et al*. (2010b) reported than PGPR applications, in combination with fertilizer-N (after transplant to hydroponic system), significantly increased root growth, total biomass, photosynthetic rate and bunch yield. Similarly, the use of bio-formulations containing PGPR in different stages of the productive cycle improved plant biometric parameters and had a cumulative effect on yield and fruit quality attributes (Kavino *et al*., 2010). On the other hand, the effect of hormones and inorganic compounds (such as SA and Si) on musaceas plants has been researched mainly with a phytopathological approach.

According to the background and results obtained in the vegetative phase, it could be expected that the use of the evaluated biostimulants (*Bs* and Si) has a potential effect on subsequent phases of the productive cycle since they improved growth (DM accumulation) through various mechanisms. Therefore, these products could be an important technological alternative in integrated plantain management. Additionally, their active ingredients have been reported to reduce the disease symptoms caused by *Ralstonia solanacearum* in different species. Since it is one of the most important production-limiting diseases in the plantain crop system throughout the domestic production area, it would be important to determine their influence on *disease progression.*

CONCLUSIONS

All biostimulants applied in the evaluated vegetative phase stimulated a greater DM accumulation than in the control through different mechanisms (in a range between 58.3 and 21.9%) although only the *Bs* treatment had a significant difference. This DM was assigned mainly to the leaves and pseudostem, indicating the formation of a strong structure that will physically support production and will serve as storage for the reproductive and productive phases. On the other hand, *Bs*, *Ba* and Si stood out because of their outstanding action on the early photosynthetic activity and DM accumulation, showing potential for evaluation under field conditions.

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Effect of *trans*-zeatin riboside application on growth of banana (*Musa* AAA Simmonds) cv. Williams in the juvenile phase

Efecto de la aplicación de *trans*-zeatina ribósido sobre el crecimiento de banano (*Musa* AAA Simmonds) cv. Williams en etapa juvenil

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Banana plantlets grown in nursery before field experiment with cytokinin applications.

Photo: L.G. Schiller

ABSTRACT

Cytokinins are physiologically active adenine derivatives that are vital to the regulation of various developmental processes in plants, such as vegetative growth and flower induction. The objective of this study was to understand how the application of cytokinin *trans*-zeatin riboside affects growth in banana (*Musa* AAA Simmonds) cv. Williams plants during the vegetative phase on two farms located in the production zone of the Magdalena province, Colombia. The effect of *trans*-zeatin riboside applications at doses of 0.00, 0.05, 0.25, or 0.45 mg L^{-1} via foliar spraying was evaluated, with 15 days between the applications, starting with transplant to the field and lasting until 10 weeks of growth. The treatment 0.05 mg L^{-1} of cytokinin resulted in increases in the plant growth variables, such as height in V, pseudostem diameter, leaf width, and leaf area.

Additional key words: hormone; cytokinin; meristem; Musaceae.

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[*] Article based on the MSc thesis of the first author “Efecto de la aplicación de *trans*-zeatina ribósido sobre el crecimiento y la diferenciación floral en banano (*Musa* sp.) variedad Williams”.

RESUMEN

Las citoquininas son compuestos fisiológicamente activos derivados de adenina, vitales para la regulación de varios procesos de desarrollo en plantas, tal como el crecimiento vegetativo y la inducción floral. El objetivo de este trabajo fue conocer como la aplicación de citoquinina *trans*-zeatina ribósido afecta el crecimiento vegetativo de plantas de banano (*Musa* AAA Simmonds) cv. Williams en dos fincas ubicadas en la zona bananera del departamento de Magdalena, Colombia. Se evaluó el efecto de la aplicación de *trans*-zeatina ribósido en dosis de 0,00; 0,05; 0,25 o 0,45 mg L^{-1} vía foliar, con espacio entre aplicación de 15 días iniciando en el momento de trasplante de plantas al sitio definitivo y hasta 10 semanas de crecimiento. El tratamiento de plantas con 0,05 mg L^{-1} de citoquinina resultó en incrementos en variables del crecimiento, tales como altura a la V, diámetro del seudotallo, ancho foliar y área foliar.

Palabras clave adicionales: hormona; citoquinina; meristemo; Musaceae.

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INTRODUCTION

In Colombia, banana cultivation occupied 85,700 ha in 2016, of which 8,635 ha were in the municipalities of the banana production zone, Magdalena province, which generated 290,428 t, corresponding to 14.2% of domestic production. This production had an approximate value of 2,093,985 million US dollars (Agronet, 2018). One of the tasks necessary for a better yield is adequate edaphic and foliar fertilization of the crop that is done properly. Phytohormones could be used as a complement to mineral nutrition, acting on the metabolic functions of plants (Albán, 2014). Among the main phytohormones, cytokinins stimulate plant growth and development (Mok, 2018). Within the group of cytokinins, agricultural applications in the field exist for kinetin, zeatin, zeatin riboside, *trans*-zeatin, *cis*-zeatin, dihydrozeatin, 6-benzylaminopurine, and other natural and synthetic compounds (Sánchez and Mira, 2013). The physiological benefits of cytokinin applications to plants include stimulation of leaf growth, regulation of shoot and root development, stimulation of respiration and photosynthesis, flower induction, and fruit growth (Jordán and Casaretto, 2006; Bar and Ori, 2014; Mok, 2018).

In Musaceae, according to the BBCH scale of development, the vegetative meristem has a characteristic arrangement of leaves with a spiral form, absence of lateral shoots and absence of internode growth (Nalina *et al*., 2006). The reproductive phase in this crop begins with changes occurring in the vegetative meristem, which undergoes metabolic shifts that transform it into the floral bud (Sánchez and Mira, 2013; Landrein *et al*., 2018). In banana, flower induction depends on the number of emerged leaves (Rodríguez *et al*., 2012), which, in turn, relays on external factors and internal regulators, such as hormone activity (Chaurasia *et al*., 2017).

An important phase in the life cycle of banana plants is inflorescence development, where climatic conditions including temperature, wind, and precipitation can delay flower differentiation and affect bunch formation (Galán *et al*., 2012). Air temperature influences the transpiration rate, as well as duration of life cycle and bunch weight in banana plants through the control that it exerts on leaf metabolism (Robinson and Galán, 2012). Precipitation is one of the main climatic factors that determine banana crop development (Santos *et al*., 2018); plants can quickly adapt to a water deficit in soil, which would further severely reduce the rates of leaf emission and bunch development (Galán *et al*., 2012). The effects of edaphoclimatic conditions on flower induction and differentiation in Musaceae could be explained, at least in part, by the hormonal interactions in plants (Robinson and Galán, 2012).

According to previous studies, applications of plant growth regulators to Musaceae might shorten the juvenile phase of growth and accelerate flower differentiation. Thus, the application of auxins at a rate 100 mg L^{-1} have stimulated floral differentiation in banana; however, treatments with naphthalene acetic acid with doses exceeding 500 mg L^{-1} have caused fruit malformations (Lima *et al.*, 2016). At the same time, applying gibberellins at doses of 500 to 1,000 mg

L^{-1} has caused elongation of the pseudostem (Lima *et al*., 2016). Likewise, during vegetative growth, spraying brassinolide at 3-6 g L^{-1} on banana (*Musa* sp. cv. Berangan) plantlets significantly has increased plant height, pseudostem diameter, leaf number, leaf area, and fresh and dry weight of plants (Zakaria *et al*., 2018). Applications of cytokinins, such as benzyladenine, at doses of 20 to 100 mg L^{-1} have increased the growth of corm in banana plants (Lahav and Gottreich, 1984). According to Muriel (2012), cytokinins influenced cell division, retarded senescence, and increased growth, fruit weight, and number of exportable hands of banana.

In general, the effects of cytokinin applications on banana growth in the field are poorly studied. López (2014) evaluated applications of cytokinins, comparing two fertilizer alternatives injected into the pseudostem of *Musa* sp. These treatments had no positive effect on the plant growth; one of the treatments contained kinetin at 1 g L^{-1} (López, 2014). In the vegetative propagation of *Musa* sp. with 6-benzylaminopurine (BAP) and indoleacetic acid (IAA), the best treatment was 30 mg L^{-1} BAP, which positively influenced shoot formation in each studied variety, followed by the concentration 30 mg L^{-1} BAP + 10 mg L^{-1} IAA (Canchignia *et al*., 2008). Albán (2014) found positive effects from cytokinin applications on leaf emission, increasing the pseudostem diameter in cv. Grand Naine; in addition, foliar applications of algae extracts rich in cytokinins were a source of hormones and essential carbohydrates, which resulted in better yield and harvest quality (Albán, 2014). Aspiazu (2014) compared the applications of various hormones, concluding that gibberellins (20 mg L^{-1}) and brassinosteroids (2 mg L^{-1}) positively influenced plant growth and increased leaf length in banana. However, when 20 mg L^{-1} gibberellin, 20 mg L^{-1} cytokinin, and 2 mg L^{-1} brassinosteroids were jointly applied, no significant increases were found in the pseudostem diameter,number/weight of roots or leaf width (Aspiazu, 2014). Langford *et al*. (2017) studied the macropropagation of banana plants in a nursery with BAP at two concentrations: 10^{-2} M and 5 x 10^{-3} M, with the following treatments: immersion of corms in BAP solutions for 30 min, immersion of corms in coconut water for 30 min, and placing the corms in a substrate of rice husk. The treatments with BAP and coconut water (a natural source of cytokinins) induced sprouting in the corms and a loss of the apical dominance (Langford *et al*., 2017).

The use of phytohormones gibberellins, brassinosteroids, and cytokinins, individually or mixed, is mainly studied in the propagation of *Musa* sp., such as in the asexual multiplication of corms planted in the field (Canchignia *et al*., 2008). However, no reports were found in the literature on the use of *trans*-zeatin riboside as a possible factor increasing vegetative growth in banana. The objective of the present research was to evaluate the effect of applications of *trans*-zeatin riboside (cytokinin) on the vegetative growth of banana cv. Williams in the Magdalena Province of Colombia.

MATERIALS AND METHODS

Experiment locations

This field study was carried out on two farms: El Polo located at 10°53'39.905'' N and 74°11'58.214'' W and La Paz 1 located at 10°53'37.17'' N and 74°11'54.623'' W, both at an altitude of 20 m a.s.l. in the town El Mamey (Colombia), and characterized with a tropical dry climate according to Holdridge (Aguirre, 2012). This study was conducted between October 2016 and January 2017. The duration of the field experiment was 13 weeks.

The climatic data in the field were obtained from the meteorological station El Enano of the Colombian meteorological institute INAT (El Mamey). The average air temperature was 26.8°C, with monthly precipitation of 12.8 mm and an average wind speed of 14.54 km h^{-1}. In general, the climatic conditions were typical for areas of commercial banana production on the Atlantic coast of Colombia, which are adequate for the development of banana plants (Sánchez and Mira, 2013). The two farms, where the plants were established, differed in physical and chemical characteristics of the soil. The soil type on both was Inceptisoles (Soil Survey Staff, 2010). The marked differences between the two locations were in soil texture (La Paz 1: Loamy, El Polo: Sandy Loam); pH (La Paz 1: 5.8, El Polo: 7.7), C/N ratio (La Paz 1: 11, El Polo: 12.2), and cation exchange capacity (La Paz 1: 16.9, El Polo: 9.4 meq 100 g^{-1}) of the soil arable layer.

Field crop management

Banana (*Musa* AAA Simmonds) cv. Williams plants, propagated *in vitro,* were employed. Prior to transplant to the field, the plants were hardened in a shade house, in which water and mineral nutrients were supplied as edaphic and foliar applications for 6 weeks.

The plants were established in the field at week zero with 4 leaves and 25 cm maximum height at the time of transplant; the planting density was 2.40 m between the plants and 2.40 m between the rows. In the sowing sites, 60 g per plant of Rafos® (Yara, Colombia) edaphic fertilizer were applied. The plant management practices employed by Torres (2016) for conventional banana produced for export in Colombia were used.

The irrigation with sprinkling was done between weeks 0 and 13 according to the needs of the crop. Between weeks 0 and 6 after planting, 6 mm ha^{-1} of irrigation water were applied daily, divided in two irrigation periods. Starting from week 7 after planting, 5 mm ha^{-1} water were applied per day; for this, a sub-foliar spray system was used, with 2014 Senninger® sprinklers spaced at 10 m by 10 m.

Every 15 d during weeks 0-12, edaphic fertilizers were applied at a rate of 60-120 g/plant. On the La Paz 1 farm, Rafos® was applied at 60 g/plant (week 0), Ammonium sulfate at 90 g/plant (week 2), Amidas® (Yara, Colombia) at 90 g/plant (week 4), Calcium Nitrate at 90 g/plant (week 6), and Aboteck® (Yara, Colombia) at 90 g/plant (weeks 8 and 10) and 120 g/plant (week 12). On the El Polo farm, the same products were used but the doses were different: 60 g/plant (week 2), 60 g/plant (week 4), and 90 g/plant (week 12). Every 15 d starting from week 1, foliar fertilizers (Wuxal®, Bayer, Colombia) were applied using a 20 L mechanical back pump, prepared with 100 cm^3 of molasse water.

The mechanical weed control (Quintero-Pertúz and Carbonó-Delahoz, 2015) was done every 15 d. Every week, the lower leaves affected by Black Sigatoka (*Mycosphaerella fijiensis*) were eliminated until week 5. Starting from week 6, only the fraction of the leaf affected by Black Sigatoka was removed. The products applied to control Black Sigatoka were Sico-Dithane® (3rd week), Siganex-Dithane® (5th week), Opus-Dithane® (8th week), and Voley-Dithane® (12th week). The suckers were cut off the plants every 6th week.

Applications of *trans*-zeatin riboside

The hormone *trans*-zeatin riboside (t-ZR), 95% purity, was obtained from Sigma® laboratories (Sigma-Aldrich, St. Louis, MO), stored according to the manufacturer recommendations at -20°C, and diluted in type 1 water before the applications to reach the dose required in each treatment. Four treatments were tested on the plants via foliar spraying: water (control, 0 mg L^{-1} *trans*-zeatin riboside), 0.05 mg L^{-1}, 0.25 mg L^{-1}, or 0.45 mg L^{-1} of *trans*-zeatin riboside. Applications were made every 15 d (Fig. 1) using a manual back pump with a 21 pound pressure regulator and yellow nozzle (GEF-REPCar, 2011), spraying the plant leaves and pseudostem. The treatments started at planting in the field and continued until completing 6 applications of the hormone in each plant. The applications of cytokinin ended at week 10 after transplant (Fig. 1).

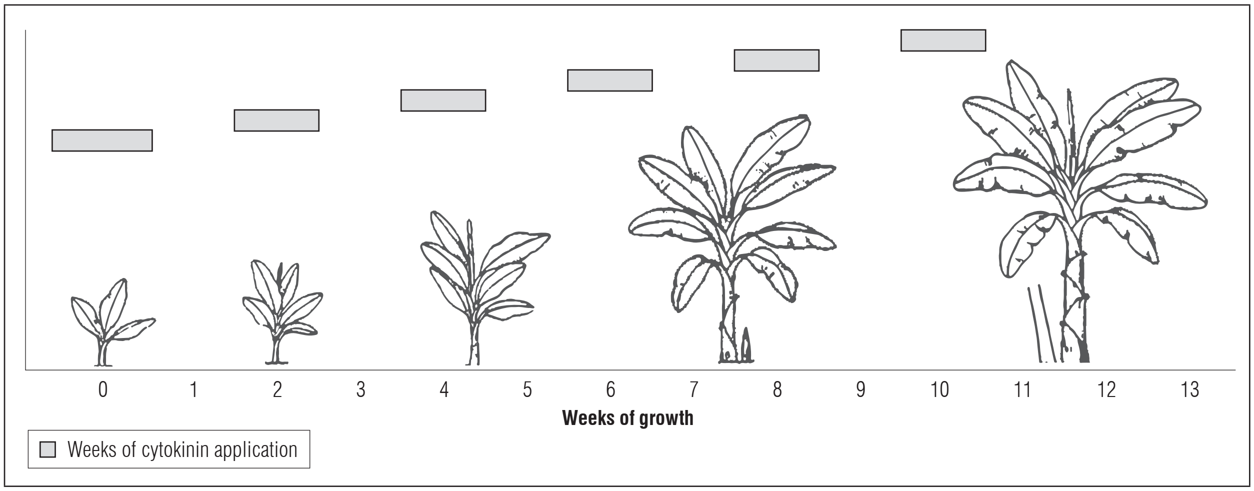

Figure 1. Development of banana plants and the time of cytokinin applications.

Data collection in the field

The experiment had a bi-factorial randomized block design with six replicates (one plant per replicate) on the La Paz 1 and El Polo farms. A total of 360 plants were planted; in each location, 20 plots were arranged, of which, 15 plots had 6 plants and the other five plots had 18 plants.

From week 1 and up to week 13, in each research plot, biometric data were collected weekly on each plant: leaf length (in each leaf), leaf width (in each leaf), pseudostem diameter, and plant height. From the ground to the bifurcation point of the pseudostem, the height of the plant (cm) denominated as "height in V" was measured with a tape measure. The diameter of the pseudostem was measured with a caliper, 5 cm from the ground. The area of each leaf was calculated with the following equation (1) (Martínez *et al*., 2015):

$$LA = \Sigma\, L \times A \times 0.80 \qquad (1)$$

where LA is leaf area (cm^2), L is length of the leaf blade from the apex of the leaf to its base (cm), and A is width of the leaf blade in the middle part (cm).

Statistical analysis

The statistical analysis used one-way analysis of variance, with a Tukey test at a confidence level of 95%. For all cases, the assumption of normality of the Shapiro-Wilk residuals and Bartlett's variance equality was tested. The area under the curve was calculated using the AUDPS function of the Agricolae® package (Simko and Piepho, 2012). To identify statistically significant differences between the means of the variables, the Student t test ($P<0.05$) was used.

RESULTS AND DISCUSSION

Plant height

On the farm La Paz 1, treatments with 0 and 0.05 mg L^{-1} *trans*-zeatin riboside resulted in a significantly larger plant height in V (height of the plant from the ground level up to point of the pseudostem bifurcation), as compared to the treatments with 0.25 and 0.45 mg L^{-1} cytokinin (Fig. 2A). The tallest plants were obtained in the treatment with 0.05 mg L^{-1} cytokinin, with a 46.3 cm average height (average value of measurements between weeks 1 and 13) (Fig. 2A). On El Polo, the four treatments had different heights, with the tallest plants, 46.9 cm average height, obtained after applications of 0.05 mg L^{-1} cytokinin (Fig. 2B). Apparently, this indicated that *trans*-zeatin riboside, at a rate of 0.05 mg L^{-1} , stimulated cell growth (cell division or/and cell elongation), which resulted in an increased height. These results are consistent with the research of Ortiz *et al*. (2013), in which cytokinin applications generated a faster growth in *Musa* sp. In banana, the pseudostem diameter and height-to-circumference ratio (HCR) for tall cultivars as well as HCR for medium-height cultivars are known as good predictors of inflorescence emergence since these variables have exhibited linear or quadratic

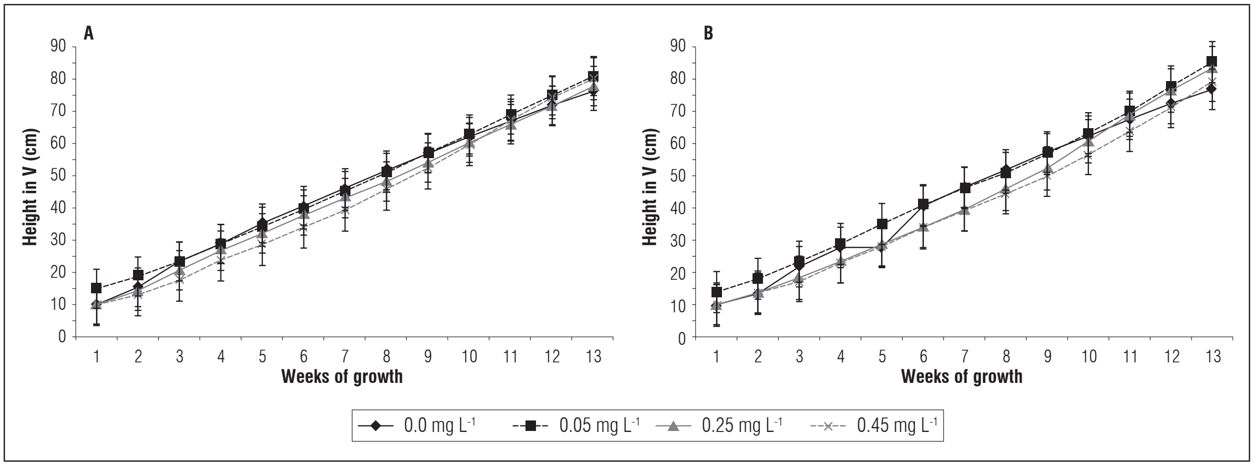

Figure 2. Height in V of banana plants cv. Williams sprayed with different doses of *trans*-zeatin riboside during vegetative growth on the farms La Paz 1 (A) and El Polo (B). The results are presented as mean ± standard error.

relationships with the number of days from planting to inflorescence emergence (Vinson *et al*., 2018).

Pseudostem diameter

The diameter of the pseudostem was assessed as a variable of plant development; all treatments increased the pseudostem diameter on the farms La Paz 1 and El Polo over time. However, the plants grown on La Paz 1 (Fig. 3A) differed in the confidence intervals for the treatments 0 and 0.05 mg L^{-1} , as compared to the treatments 0.25 and 0.45 mg L^{-1}; the treatment 0.05 mg L^{-1} yielded the highest average pseudostem diameter, 4.6 cm (average value of measurements between 1 and 13 weeks). On the El Polo farm, the plants treated with 0.05 mg L^{-1} cytokinin had the largest average pseudostem diameter, 4.6 cm (Fig. 3B). These results indicate that the best treatment in both locations was 0.05 mg L^{-1} *trans*-zeatin riboside, which was the lowest dose. The increase in pseudostem diameter could indicate a higher cellular activity, which implies increases in dry mass, higher demand for nutrients, and a higher meristematic activity (Sánchez and Mira, 2013). In *Musa* sp., a large pseudostem diameter correlates with a higher plant resistance to breakage by wind and reflects a plant's ability to sustain the bunch (Gonçalves *et al*., 2018), as well as correlates with a higher storage capacity in the pseudostem for water and carbohydrates (Sánchez and Mira, 2013; Shivashankar *et al*., 2016).

Leaf growth variables

The leaf length did not present significant differences between the treatments (Fig. 4), while on La Paz 1, the leaves tended to be the longest with the 0.25 mg L^{-1} cytokinin application, with an average length value of 58.5 cm (average value of measurements between 1 and 13 weeks) and, on El Polo, the best treatment was 0.45 mg L^{-1}, with a length value of 63.1 cm (Fig. 4 A and B).

At the same time, the leaf width significantly differed between the treatments (Fig. 5). On La Paz 1, the widest leaves were obtained in the treatment with 0.25 mg L^{-1} cytokinin, where the average leaf width reached 28.3 cm (average value of the measurements between 1 and 13 weeks) (Fig. 5A). On El Polo, the largest leaf width was recorded in the 0.05 mg L^{-1} treatment, with a 29.9 cm average value (Fig. 5B). As a result, the plants on the El Polo farm presented the widest leaves. This effect from the cytokinins was consistent with the results of Bar and Ori (2014), who indicated that leaf blade development depends on light and cytokinins for maintaining balance with auxins and stimulation of meristem activity. Cytokinins are known to stimulate cell division and cell expansion in leaves (Mok, 2018). In a review carried out by Landrein *et al*. (2018) on *Arabidopsis*, expression of the *CYCD3* gene is required for development of new leaves until reaching the leaf size typical of this species.

The leaf area reflected a relationship between leaf length and width, presenting no significant differences between the locations or between the treatments on La Paz 1 and El Polo. The highest average leaf area on La Paz 1 was reached with 0.25 mg L^{-1} cytokinin, 1473 cm^2 (average value of measurements between weeks 1 and 13) and, on the El Polo farm, the 0.05 mg L^{-1} cytokinin treatment resulted in the

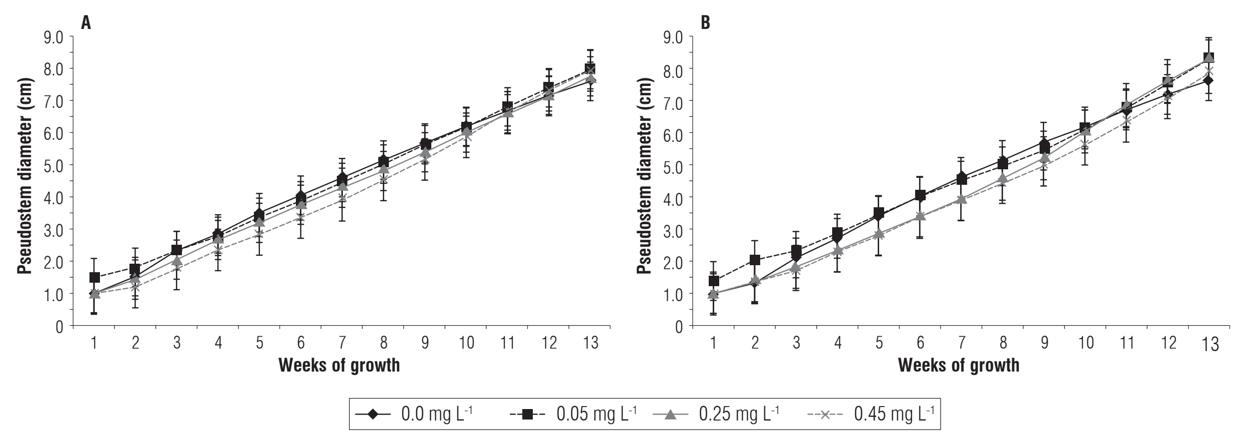

Figure 3. Pseudostem diameter of banana plants cv. Williams sprayed with different doses of *trans*-zeatin riboside during vegetative growth on the farms La Paz 1 (A) and El Polo (B). The results are presented as mean ± standard error.

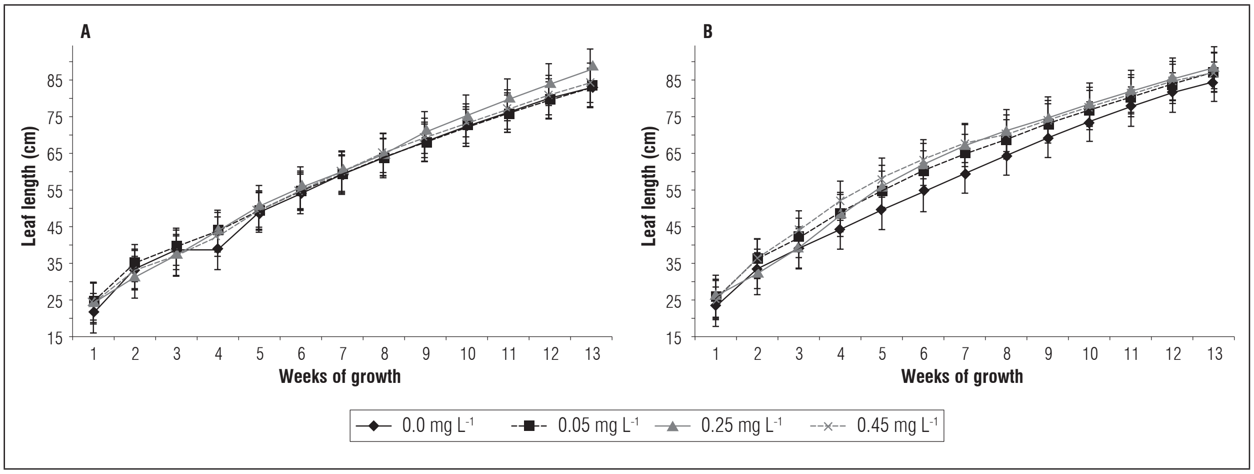

Figure 4. Leaf length of banana plants cv. Williams sprayed with different doses of trans-zeatin riboside during vegetative growth on the farms La Paz 1 (A) and El Polo (B). The results are presented as mean ± standard error.

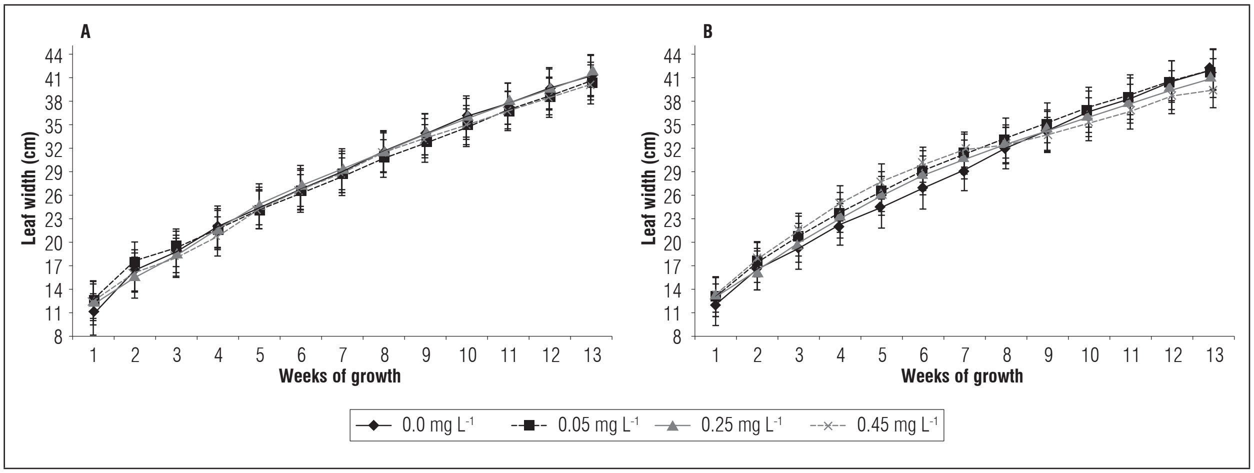

Figure 5. Leaf width of banana plants cv. Williams sprayed with different doses of *trans*-zeatin riboside during vegetative growth on the farms La Paz 1 (A) and El Polo (B). The results are presented as mean ± standard error.

highest average leaf area, 1616 cm^2 (Fig. 6A and B). It can be speculated that the phytohormone spraying on the leaves/pseudostem affected expression of the *LOG* genes in the apical meristem region of the stem, where the stem cells reside. In *Arabidopsis*, the *LOG4* gene is expressed in the L1 layer of the vegetative meristem; *LOG* genes encode enzymes that convert inactive cytokinin ribosides into active forms, providing a localized source of active cytokinin in the vegetative meristem (Landrein *et al*., 2018). Cytokinins could increase leaf size because of the high rate of cell expansion, yielding a higher shoot biomass (Skalák *et al*., 2019) and delayed leaf senescence (Gan, 2014). In banana, direct relationships between leaf area and yield were previously established (Robinson and Galán, 2012); these data are important since the growth and formation of banana bunches depends on the number and physiological activity of functional leaves (Rodríguez *et al*., 2012).

The results of the present study indicated that the application of *trans*-zeatin riboside positively influenced the development of banana plants without a reduction in growth or presenting deformations in plant morphology, such as the ones reported by Albán (2014). In our research, the treatment that generated, on average, the highest leaf expansion was 0.05 mg L^{-1} *trans*-zeatin riboside, as compared with

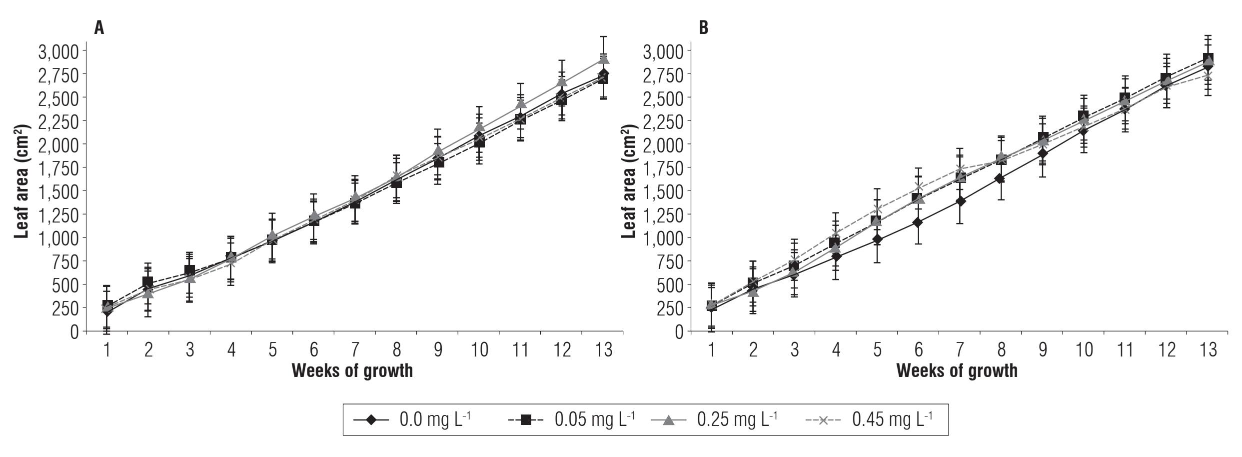

Figure 6. **Leaf area of banana plants cv. Williams sprayed with different doses of *trans*-zeatin riboside during vegetative growth on the farms La Paz 1 (A) and El Polo (B). The results are presented as mean ± standard error.**

the other evaluated treatments. These findings agree with the data reported for applications of other rates/ formulae of cytokinins, which tended to increase the growth rate of plants, resulting in increases in height, pseudostem diameter, leaf length and leaf width in *Musa* sp. plants (Albán, 2014; Aremu *et al*., 2014; Aspiazu, 2014).

The practical importance of faster vegetative growth in Musaceae at the commercial level includes a reduction of the juvenile phase of growth, which favors a change from vegetative to reproductive growth, accelerates flower differentiation, and reduces the number of weeks required for flowering and initiation of bunch formation. The applications of *trans*-zeatin riboside on *Musa* sp. plants during vegetative development could be explored for possibly counteracting the effect of stress caused by biotic and abiotic factors (Schäfer *et al*., 2015; Miller *et al*., 2017), which might further increase production levels on commercial plantations.

CONCLUSIONS

The growth variables height in V, leaf width, leaf area, and pseudostem diameter presented statistically significant differences, depending on the level of *trans*-zeatin riboside sprayed on the banana plants cv. Williams during 10 weeks of growth in the field. The low doses of *trans*-zeatin riboside, such as 0.05 mg L^{-1}, generated the best results for plant height at V, pseudostem diameter, leaf width and leaf area.

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Conflict of interests: this manuscript was prepared and reviewed with the participation of the authors, who declare that there is no exists conflict of interest that puts the validity of the presented results at risk.

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Production and quality of banana ‘BRS Conquista’ bagged with different colored polypropylene bags

Producción y calidad de frutos del banano ‘BRS Conquista’ empacados en bolsas con polipropileno de diferentes colores

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‘BRS Conquista’ banana bunch bagged with blue polyethylene.

Photo: R.C. Martins

ABSTRACT

Worldwide, the second most traded fruit is banana, which is highly appreciated by Brazilian consumers. Moreover, new technologies have been used to improve fruit quality during cultivation. This study aimed to assess the influence of plastic bag colors on the production and quality of banana ‘BRS Conquista’. The treatments consisted of the use of commercial polypropylene bags e colored white, black, red or blue, in addition to the control (non-bagged). This study used a randomized complete block design with five treatments, four replicates and four plants per plot, totalling 80 plants. The assessments consisted of bunch mass; rachis mass; fruit total mass; mean cluster mass; number of fruits per bunch and per cluster; and fruit length and diameter; along with fruits physicochemical traits, such as soluble solids, titratable acidity, pulp/peel ratio and maturation index. The results indicated that no interference was obtained from the different plastic bag colors in the productive variables. However, a greater content of soluble solids was observed in the non-bagged bunches.

Additional key words: *Musa* sp.; bunch cover; yield; postharvest.

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RESUMEN

La banano es la segunda fruta con mayor volumen comercializado en el mundo. Por el hecho de ser una fruta muy apreciada por el consumidor brasileño, en los últimos años surgieron diversas tecnologías que ayudan al cultivo del plátano, principalmente relacionadas a la calidad de los frutos. En este contexto, el trabajo tuvo como objetivo evaluar la influencia de la coloración de las bolsas plásticas empleadas en el embolsado de racimos del banano 'BRS Conquista' sobre la producción y calidad de los frutos. Los tratamientos consistieron en el uso de bolsas de polietileno comerciales, en los colores blanco, negro, rojo y azul, además del testigo (sin embolsado). El diseño experimental fue en bloques casualizados, con cinco tratamientos, cuatro repeticiones y cuatro plantas por parcela, totalizando 80 plantas. Se evaluó la masa del racimo, masa de la raquis, masa del los frutos, número de frutos por racimo, número de pencas por racimo, masa de las pencas, número, longitud y diámetro de frutos de la segunda penca, y las características físico-químicas de los frutos, como sólidos solubles, acidez titulable, relación pulpa/cáscara e índice de maduración. Se verificó que el embolsado de los racimos con bolsas plásticas de diferentes colores no interfirió en las variables productivas. Sin embargo, en los racimos que no recibieron embolsado se observaron mayores contenidos de sólidos solubles que en los racimos empacados.

Palabras clave adicionales: *Musa* sp.; ensacado de racimos; productividad; poscosecha.

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INTRODUCTION

The banana tree belongs to the Musaceae family and is considered one of the main crops of economic interest in the fruit market. Also, banana is the second most traded fruit in the world (Perrier *et al*., 2011) and is grown in 80 tropical countries, with a production of 106 million tons. India, China, the Philippines and Brazil are the largest producers. The largest exporting countries are Costa Rica and Ecuador, with Ecuador accounting for 30% of world trade (FAO, 2017).

Since it is a highly appreciated fruit, innovations and technologies have appeared to optimize the productive process in recent years, mainly related to the improvement of fruit quality.

However, research on the quality aspects of banana fruits is of extreme importance because they are mainly destined for direct consumption.

Considered an ecological practice, fruit bagging is a very effective and old technique for protecting fruits from attack by pests and diseases (Teixeira *et al*., 2011). In banana cultures, in addition to phytosanitary factors, studies have indicated that bagging can improve fruit quality. Thus, in temperate areas, it is common to cover. The fruits, while they are growing, improve yield and fruit quality. However, this technique is also used in warmer countries during the coldest seasons to protect fruits (Sakai, 2015).

Although it is an expensive technique, it protects the bunches from physical damage, such as wind action, low temperatures, fruit contact with the leaves, diseases and hail (Alves *et al*., 1999; Rodrigues *et al*., 2001; Costa *et al*., 2002; Silva Filho and Moreira, 2005; Moreira, 2008; Euleuterio *et al*., 2010; Sakai, 2015).

The method of bagging fruits is a cultural practice adopted by large-scale banana growers. Questions that have not been resolved refer to the existence of differences in the types of materials used for bagging bunches, along with bag color here are differences between fruit bagging recommendations around the world, based on preferences for color and material (Surajit *et al*, 2016; Santos *et al*., 2017).

There is a need for studies on bagging materials (Azevedo *et al*., 2016; Coelho *et al*., 2008) information and on how to perform bagging in different situations (Costa *et al*., 2002). Sarkar *et al*. (2016), in an experiment in India, evaluated the use of polypropylene bags and found that bagged fruits presented less insect damage and higher mass. However, Kutinyu (2014), in a study with different cluster protection materials, found that bagging did not provide significant results for fruit weight in Grande Naine. Sakai (2015) obtained similar results, evaluating the physical protection of 'Nanicão' bunches, and observed that the bunch mass, second bunch mass, fruit density and pulp pH were not influenced by bagging.

Therefore, studies that evaluate the effect of color of the plastic used to cover banana bunches are crucial since they can subsidize information for the productive sector to demystify and elucidate the role of this method in the production and quality of fruits. This study aimed to assess the influence of plastic bag colors on the production and quality of banana 'BRS Conquista'.

MATERIAL AND METHODS

The current study took place at the Sao Paulo State University (Unesp), School of Agriculture, Botucatu, São Paulo, Brazil. According to the Köppen classification, mesothermic temperate type (Cfa) was the predominant climate in the region, that is, humid and with a mean temperature of the hottest month close to 22ºC (Cunha and Martins, 2009). The soil of the experimental area was classified as Red Nitosol (Santos *et al*., 2018).

Prior to the experiment installation, a soil analysis was performed with soil samples at depths of 0-20 cm and 20-40 cm. Based on this analysis, liming and fertilization were performed according to Raij *et al*. (1999), i.e. using 1.89 t ha^{-1} of dolomitic limestone, 300 g/plant of urea and 241.5 g/plant of potassium chloride. During the experiment, other cultural treatments were carried out in the orchard according to the technical recommendations for the crop.

Two-year-old banana 'BRS Conquista' plants were spaced at 2 x 2.5 m and evaluated in an agricultural cycle in 2017.

The treatments consisted of polypropylene bags with different colors (i.e. white, black, red and blue), plus a control (non-bagged bunches). The bag dimensions were 150×75 cm, open at the ends, with lateral perforations of 0.5 cm to allow gas exchange between the fruits and the outside air. The experiment design used randomized blocks, with five treatments, four replications and four plants per plot, totalling 80 plants. The bagging was done shortly after the inflorescence issued, before the opening of the bracts. The bags were tied at the top.

Starting with the second cycle, cluster emission is not simultaneous among plants; therefore, fruit development occurs under different conditions between plants. To standardize the harvest point, the bunches were harvested when they reached the thermal accumulation degree-days of 3,200.33°C after inflorescence emission. To assess this thermal accumulation pattern, non-useful plants in the area were previously selected, and the thermal accumulation between inflorescence emission and harvest was calculated; the basal temperature was lower than 15°C and higher than 37ºC, according to Figueiredo *et al.* (2006).

The evaluations of plant productive performance was done right after harvesting, when the fruits presented a completely green peel (PBMH and PIF, 2006). The variables included production or bunch mass (kg), rachis mass (kg), fruit total mass (kg) and mean cluster mass (kg), which were measured in scale; number of fruits per bunch and per cluster; and yield (t ha^{-1}), for which the cluster mass was considered with a stand of 2,000 plants/ha.

The evaluations of yield performance considered five fruits from the second cluster of the bunch. The following variables were evaluated: fruit number per length and length (cm) and diameter (mm) of the fruits; the latter was measured using a digital caliper for evaluations of the physical and physicochemical characteristics of the ripe fruits. The leaves were kept on shelves at room temperature until they were completely yellow (PBMH and PIF, 2006). The following variables were evaluated: fruit number per cluster; fruit length (cm) and fruit diameter (mm); the latter was measured using a digital calliper.

For the physical and physicochemical characteristics of the ripe fruits, clusters were kept on shelves at room temperature until they were completely yellow (PBMH and PIF, 2006). The following evaluations were carried out on the five fruits of the second ripen cluster: pulp/peel ratio, with the fruits and peels weighed on a semi-analytical balance. The fruit mass was determined with the peel divided by peel mass; the values were expressed in grams. The titratable acidity (TA) was obtained according to the analytical standards of Instituto Adolfo Lutz (Lutz, 2005), titrated with sodium hydroxide (NaOH) to 0.1 N, using 5 g of homogenized pulp, diluted in 100 mL of distilled water and 0.3 mL of phenolphthalein. The content of soluble solids (SS) was measured with the aid of a digital refractometer (Atago 3405 PR-32a Palette), expressed in °Brix. The maturation index (SS/TA) was calculated with the ratio of the soluble solids and the titratable acidity.

The data were submitted to analysis of variance, and, when there was significance, the means were compared with the Scott-Knot test at 5% probability level. All analyses were performed in the program SISVAR 5.0 - Program of Statistical Analysis and Planning of Experiments of the Federal University of Lavras (Ferreira, 2011).

RESULTS AND DISCUSSION

In terms of productive performance, the analysis of variance did not detect any significant effect of treatments on the variables (Tab. 1).

The fact that there was no difference between the color of the polypropylene bags for bunch mass indirectly resulted in the absence of effect on the rachis and fruits mass since bunch mass is composed of rachis and fruit mass. Moreira (2008) evaluated banana bunch mass in the presence and absence of bagging in the State of Amazonia and did not found any significance in the variation of the treatments for cultivars Prata Zulu, FHIA 18, Nanicão 2001 and Thap Maeo.

In an experiment using blue plastic bags to cover banana bunches, cv. Williams did not present any significant effect on bunch mass, according to Muchui *et al*. (2010). Also, Sakai (2015) evaluated bagging in two fruit development seasons (i.e. summer and winter) and did not notice any statistical difference for bunch mass.

The mean cluster mass was also unaffected by fruit bagging, regardless of color (Tab. 1). These results corroborate with those found by Rodrigues *et al.* (2001), who aimed to verify the influence of bagging banana bunches on the production of cv. 'Prata-anã' and did not verify statistical differences for cluster mass, fruit mass and number of clusters. Sakai (2015) evaluated bagging bananas in two fruit development seasons (i.e. Summer and Winter) did not report any statistical difference for cluster mass.

It was evident that the environmental conditions of Botucatu, State of Sao Paulo, the microclimate of the treatments, did not impact the productive aspects.

Fruit bagging only changes the amount of radiation, humidity and temperature within the cluster area. All other parts of the plants, i.e. leaves, are still exposed to environmental conditions and variations. Nevertheless, it is worth emphasizing that leaves are the main photosynthetic organs that produce carbohydrates that will later be translocated to fruits; also, photosynthesis is not efficient enough in green fruits. Thus, these traits may have resulted in the absence of effects of bagging bunches on the productive factors.

However, Costa *et al.* (2002) studied bagged bunches during winter, summer and autumn seasons and reported an increase in bunch mass of cv. Grand Naine bagged in the summer. Soto (2015) verified an increase in bunch yield, as a function of bagging in the summer. The highest yield of the bunches was attributed to the increase in temperature inside the bags, which created a more uniform microclimate (Alves, 1999). Another relevant aspect is the banana's ability to emit leaves more frequently in the summer, which leads to a greater photosynthetically active area and, consequently, greater production.

Table 1. Values of the F test, degrees of freedom (DF), coefficients of variation (CV) and mean bunch, rachis mass, fruit mass, hand mass, number of fruits per clusters and number of clusters per bunch of banana 'BRS Conquista' in Botucatu, State of Sao Paulo, 2018.

F test	DF	Bunch mass (kg)	Rachis mass (kg)	Fruit mass (kg)	Hand mass (kg)	Fruit number per clusters	Cluster number per bunch	Yield (t ha $^{-1}$)
Block	3	4.99*	3.43*	4.55*	1.44^{NS}	2.02^{NS}	2.78^{NS}	4.99*
Treatment	4	1.15^{NS}	0.91^{NS}	1.21^{NS}	0.96^{NS}	2.72^{NS}	0.61^{NS}	1.15^{NS}
CV (%)		12.12	14.29	12.72	14.10	12.23	6.77	12.12
Mean		19.26	1.56	17.71	1.34	22.15	13.18	19.26

^{NS} = not significant; * = significant at $P \leq 0.05$.

For the number of fruits, cluster and yield, the color of the polypropylene bags showed no differences, indicating that any of the evaluated colors could be recommended for bagging banana bunches in the field. Costa *et al.* (2002) did not find any significant difference for the yield of bagged and non-bagged bunches in a study on cv. Prata-anã and Grande Naine.

In an experiment on banana bunches covered with polyethylene bags, Moreira (2008) found that the bags did not affect the number of fruits or clusters; also, the differences were only observed in the cultivars. Plant behaviour related to the number of fruits and clusters and the productive capacity is mainly due to genetic and edaphoclimatic factors.

Another important factor is the moment of floral differentiation since any disturbances, such as unfavourable climatic conditions, may reflect on bunch formation and development (Karamura *et al.,* 2011).

When the physical characteristics were evaluated in the second cluster with green fruits, no significant difference was observed for the number, length and diameter of the fruits (Tab. 2).

Table 2. Values of F test, degrees of freedom (DF), coefficients of variation (CV) and mean fruit number, length and diameter of fruits of the second cluster of banana 'BRS Conquista' in Botucatu, State of Sao Paulo, 2018.

F test	DF	Fruit number	Length (cm)	Diameter (cm)
Block	3	1.03^{NS}	1.03^{NS}	0.10^{NS}
Treatment	4	1.06^{NS}	0.18^{NS}	1.36^{NS}
CV (%)		24.08	8.46	6.91
Mean		23.80	14.15	34.77

^{NS} = not significant at $P<0.01$ and $P\leq0.05$ by F test.

The values found for fruit length and diameter were similar to those found by Rodrigues *et al.* (2001). Likewise, similar values were found by Aquino *et al*. (2017), who observed length and diameter values of approximately 14.00 and 38 cm in plants of the 'Prata' group, respectively. An experiment in Kenya evaluated the effect of blue-colored polyethylene bags and of non-bagging on cv. Williams, Muchui *et al*. (2010) and did not find any significant differences for length. Rodrigues *et al.* (2001) also did not obtain statistical difference for fruit length and diameter for bagged bunches in cv. Prata anã.

According to the classification proposed for cv. Prata by Abanorte by the Fruit Growers' Central Association of North Minas Gerais - (MI *et al*., 2000), the fruits from 'BRS Conquista' fit the best quality type since they presented values higher than 14 cm in length and larger than 32 mm in diameter. Independent of the bag color, the fruits presented within the stated size range for 'Prata' in the current study. Therefore, the bunch bagging method did not impair fruit development.

It is noteworthy that length, diameter and fruit number are not only related to the bunch bagging method but also to abiotic factors during floral differentiation to bunch harvest and to cultivar genetic characteristics.

For the post-harvest characteristics of the ripe fruits, there was no statistical difference between the treatments for pulp/peel ratio, acidity, and maturation index time (Tab. 3), but there was a significant effect for soluble solids (Tab. 4).

The pulp/peel ratio is linked to the genetic characteristics of the cultivar and fruit ripening phase; therefore, during the maturation process, there was an increase in pulp mass and, consequently, a decrease in peel mass because of the osmotic transfer of moisture from the peel to the pulp and water loss from the peel to the environment. Thus, there are two factors that may interfere in the pulp/peel ratio that did not occur in the current study (Payasi and Sanwl, 2005).

Table 3. Values of F test, degrees of freedom (DF), coefficients of variation (CV) and means of pulp/peel ratio, acidity, soluble solids and maturation index of 'BRS Conquista' in Botucatu, State of Sao Paulo, 2018.

F test	DF	Pulp/peel ratio	Acidity (%)	Soluble solids (ºBrix)	Maturation index
Block	3	0.54^{NS}	0.24^{NS}	2.26^{NS}	0.24^{NS}
Treatment	4	2.24^{NS}	1.20^{NS}	5.99**	1.49^{NS}
CV (%)		19.02	13.97	2.89	15.91
Mean		100.72	0.66	23.24	36.15

^{NS} = not significant; ** = significant at $P<0.01$ by F test.

Silva Filho and Moreira (2005) also verified that bagged or non-bagged bunches in different types of cultivars did not alter the pulp/peel ratio. Muchui *et al*. (2010) did not find any significant relationship between the pulp/peel ratio of cv. Williams in an experiment with different bag colors and perforation sizes.

These authors also found no statistical differences for titratable acidity in fruits, as observed by Silva Filho and Moreira (2005) in an experiment with several varieties using the bunch bagging method in the State of Amazonia.

For the fruit maturation index, there was no significant difference between the treatments. The maturation index is the most widely used method to evaluate fruit flavor, which is the most representative isolated measurement of acidity and sugars (Chitarra and Chitarra, 2005). A ripe banana is a great example of a high SS/TA ratio because it has high levels of sugar and a low acid content.

There was a difference in the content of soluble solids in the fruits. The non-bagged fruits presented the highest average, 24.65° Brix (Tab. 4). Green bananas have high levels of starch; however, as a fruit ripens, starch is broken down into sugars to be used in the respiratory process, consequently increasing the soluble solid contents in the pulp (Chitarra and Chitarra, 2005). This result can be related to the fact that the non-bagged fruits were more exposed to radiation, which, even in small proportions, may have allowed greater photosynthesis and sugar production.

Table 4. Soluble solids values of the second cluster of the bunch of 'BRS Conquista' submitted to different polypropylene bag colors in Botucatu, State of Sao Paulo, 2018.

Treatment	Soluble solids (°Brix)
Non-bagged	24.65 a
White polypropylene	22.94 b
Black polypropylene	22.73 b
Red polypropylene	23.24 b
Blue polypropylene	22.64 b
CV (%)	2.89

Means with different letters indicate significant differences according to the Scott-Knot test ($P \leq 0.05$).

The non-bagged fruits may have presented a lower respiration rate than the bagged ones because, inside the bags, the mean temperature was much higher that outside, which in turn promoted an increased respiratory rate and, therefore, an increased sugar consumption.

During the respiratory process, substrates are consumed but mainly organic acids, sugars and starch are consumed (Chitarra and Chitarra, 2005), so the lower the respiration rate, the lower the substance consumption, causing higher soluble solids contents, as observed in the non-bagged fruits. Costa *et al*. (2002) observed a low soluble solids content in bagged bunches in cv. Nanicão, in Tietê, State of Sao Paulo. Likewise, Silva Filho and Moreira (2005) found higher soluble solids in non-bagged bunches than in bagged ones.

CONCLUSIONS

Regardless of the bag color, the banana bunch bagging method for 'BRS Conquista' did not promote changes in the plant productive performance.

The non-bagged fruits presented a higher soluble solids content than the bagged ones although the maturation index and the other physicochemical traits were not affected.

Conflict of interests: this manuscript was prepared and reviewed with the participation of the authors, who declare that there is no exists conflict of interest that puts the validity of the presented results at risk.

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Selection of half-sib families of creole melon (*Cucumis melo* L.) on the Ecuadorian coast

Evaluación de familias de medios hermanos de melón criollo (*Cucumis melo* L.) en la costa ecuatoriana

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Creole melon fruit.

Photo: F. Espinosa

ABSTRACT

The creole melon cantaloupe is a horticultural species, whose fruits are used for human nutrition. However, no research has been reported on their genetic variability for starting breeding processes. The objective of this study was to take advantage of the variability of creole melons for genetic improvement. 20 creole melon half-sib families were selected and evaluated for production characteristics and fruit quality in order to obtain improved populations. The study was carried out in Palenque canton, Ecuador. Seeds from 20 half-sib families were used, arranged in a randomized complete block design with three replications. The differences between the families were significant for all the studied characteristics. Family 93 presented higher averages for fruit weight, fruit diameter and production (per hectare), and family 196 did so for soluble solids. The phenotypic correlations were positive and significant between the fruit weight and fruit diameter, fruit length, pulp thickness, fruit cavity, soluble solids and production. The coefficients of genetic variation, in general, were lower than the coefficients of environmental variation. The estimates of heritability broadly had a moderate magnitude (30-60%) for the 10 evaluated characteristics, and the magnitude of the expected genetic progress was low (<10%) for the fruit weight, production, and soluble solids, so the selection for high production should focus on fruit weight and soluble solids.

Additional key words: slice melon; genetic variability; heritability; genetic gain.

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RESUMEN

El melón criollo de tajada cantalupo es una especie hortícola, cuyos frutos son utilizados en la alimentación humana. Sin embargo, no se han reportado investigaciones de su variabilidad genética para iniciar procesos de mejoramiento. El objetivo de esta investigación fue aprovechar la variabilidad de los melones criollos para mejorarlos genéticamente, mediante selección. Se seleccionaron y evaluaron 20 familias de medios hermanos de melón criollo de tajada por características de producción y calidad del fruto, con el fin de obtener poblaciones mejoradas. El estudio se realizó en el cantón Palenque, Ecuador. Se utilizó semilla de 20 familias de medios hermanos, dispuestas en un diseño de bloques completos al azar con tres repeticiones. Las diferencias entre familias fueron significativas para todos los caracteres estudiados. La familia 93 presentó mayores promedios para peso de fruto, diámetro de fruto y producción (por hectárea) y la familia 196 para sólidos solubles. Las correlaciones fenotípicas fueron positivas y significativas entre el peso de fruto y el diámetro de fruto, longitud del fruto, espesor de la pulpa, cavidad del fruto, sólidos solubles y producción. Los coeficientes de variación genética, en general, fueron menores que los coeficientes de variación ambiental. Las estimaciones de heredabilidad en sentido amplio fueron de magnitud moderada (30-60%) para los 10 caracteres evaluados y la magnitud del progreso genético esperado fue baja (<10%) para peso de fruto, producción, y sólidos solubles, por lo que se considera que la selección para melones de alta producción debe enfocarse en peso del fruto y sólidos solubles.

Palabras clave adicionales: melón de tajada; variabilidad genética; heredabilidad; ganancia genética.

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INTRODUCTION

The genus *Cucumis*, which includes melon (*Cucumis melo* L.), has numerous wild species in Africa, which is its origin (Sebastian *et al*., 2010), from where it dispersed to the rest of the world, and, nowadays, it is cultivated under tropical and subtropical climatic conditions (Reddy *et al*., 2013a). According to the FAO (2014), melons and watermelons, from the commercial point of view, are horticultural crops because they are temporary crops.

In 2010, 1,074,558 ha of melon were grown worldwide. Production in that year reached 25.0 million metric tons, with China being the largest producer, accounting for 45% of global production (Monge-Pérez, 2013). The data published by FAOSTAT, the statistical agency of the Food and Agriculture Organization of the United Nations (FAO), for 2014 indicated that global melon production was 29.62633 billion tons, over an area of 1,189,565 ha, with an average yield of 2.49 kg m^2. The data from (FAO, 2018), based on the imputation methodology, indicated that, in Ecuador, 2,036 ha were cultivated in 2016, with an average yield of 2.43 kg m^2, which represented a total production of 49,201 t. The cultivated area had a high percentage of hybrid melon seeds from transnational companies.

On the Ecuadorian coast, there are creole melons, grown by small farmers, which are highly appreciated by local consumers because of their aroma and flavor. The Ecuadorian creole melon is a little explored cultivar. This melon exhibits a wide phenotypic diversity for fruit traits, including fruit ripening, fruit shape, size, flesh color, texture, sweetness and aroma (Latrasse *et al*., 2017). Preliminary tests showed that the yields of these creole varieties were below 4 t ha, much lower than the production of imported hybrids that are commercially cultivated. This is possibly due to the poor quality of the creole variety seeds, which are obtained from the previous harvest or purchased in the local market (UPOV, 2008).

In the provinces of Manabí, Los Ríos and Guayas, creole melon crops have been identified, within which there is the melon known as ‘Slice Melon’ (Fig. 1), which is characterized by having a large fruit that can reach weigh more than 4 kg, with a rounded, oval or flattened shape, thick, smooth bark and segmentations or grooves arranged as meridians (apostille), pale yellow to intense yellow color, and white, pink or orange broad pulp, with a characteristic fragrant odor and sweet flavor, possibly belonging to *C. melo* var. Naud cantaloupensis, which, according to (Guis *et al*., 1998), have fruits with a round shape, medium size, smooth surface, marked ribs, orange flesh, aromatic and sweet flavor. The intraspecific classification of *C. melo* has been reviewed several times, the most recent performed by Pitrat, in 2008, included within this species are two subspecies, *melo* and *agrestis* and 15 varieties: Cantalupensis and inodorus varieties have greater commercial interest (Pavan *et al.,* 2017) and are the most cultivated in the world (Abraham *et al*., 2018).

Figure 1. Creole melon fruit (photo: F. Espinosa).

The use of creole melon varieties depends on the availability of seeds and access for farmers to other production resources such as land, water, fertilizers and work capacity (Chacón and García, 2016). To maintain and improve the competitiveness of the creole melon, it is necessary to promote innovation in varieties and crop management technologies. New varietal options that offer better productive and organoleptic characteristics must be sought (Monge-Pérez, 2013). Previous research showed the productive potential of the creole melon, which creates the need to identify the ability to achieve advances in the production and organoleptic quality of the sliced melons with a formal procedure. The aim of this research was to evaluate 20 half-sib families of slice creole melon (*Cucumis melo* L.) on the Ecuadorian coast with characteristics of production and quality of the fruits.

MATERIALS AND METHODS

This study was conducted in Palenque, Provincia de Los Ríos, Ecuador, located at latitude -638455 and longitude -9841885. The main climate characteristics are presented in (Tab. 1).

Table 1. Climatological characteristics in Palenque, Provincia de Los Ríos, Ecuador. 2018.

Parameters	Annual average
Temperature (ºC)	24 - 25
Relative humidity (%)	75 - 80
Annual heliofania (hours light)	1,011.9
Precipitation (mm year^{-1})	1,500 - 2,000
Ecological zone	Tropical dry forest

Source: GADCQ (2014), INAMHI (2016).

From a massal population that represented the creole melon collection, made between August 2015 and April 2016, on the Ecuadorian coast, 20 half- sib families were selected for further evaluation. Seeds from the massal population were used as a control treatment.

The 20 half-sib families and the control treatment were evaluated in a randomized complete block design with three repetitions. In each repetition, the families were located randomly. The experiment units were linear plots with 5 plants, the net plot had 3 plants, with 3 m between rows and 1.50 m between plants, according to the traditional form of cultivation of creole melons; and the population density was 2,222 plants/ha.

In each plant, characteristics related to the commercial fruit were evaluated: fruit weight (FW) using an electronic scale Jadever JWP with a capacity of 6,000.0±0.1 g, fruit diameter (FD), fruit length (FL), pulp thickness (PT) (Wang *et al*., 2016), bark thickness (BT) and fruit cavity (FC) (Aragão *et al*., 2015) measured with a digital electronic caliper brand Uberman, pulp firmness (PF) quantified with a fruit hardness meter model FHT-804 with a capacity of 24±0.1 kg cm^{-2} (Monge, 2016), (SS) soluble solids (°Brix) measured with a RHB-50ATC refractometer, scale of 0.0-50.0±0.2% (Aragão *et al*., 2015), pulp acidity (PA) quantified with a digital pH electronic meter Biocharge ATC and production per hectare calculated with the average weight of the fruits in the population density of 2,222 plants/ha.

Each characteristic was submitted to an analysis of variance under the experiment block design, randomly with three repetitions. In the case of significant differences between the families, the comparison of means was made with the Tukey test ($P \leq 0.05$) (Abraham *et al.,* 2018). A correlation analysis was carried out for the evaluated characteristics. The results were processed with the statistical software SAS 9.4.

The environmental (1), genetic (2) and phenotypic (3) variances were determined using the analysis of variance, according to the equations below:

$$\text{Environmental variance } (\sigma^2_E) = CMe \qquad (1)$$

$$\text{Genetic variance } (\sigma^2_G) = \frac{CMf - CMe}{r} \qquad (2)$$

$$\text{Phenotypic variance } (\sigma^2_P) = \sigma^2_G + \sigma^2_E \qquad (3)$$

where *CMf* is mean square of the families, *CMe* is mean square of the experimental error, and *r* is number of repetitions (Pistorale *et al*., 2008).

The coefficients of genetic (4) and environmental (5) variation were calculated according to:

Genetic variation coefficient

$$\text{GVC} = \frac{\sqrt{\sigma^2 G}}{\bar{X}} \times 100 \qquad (4)$$

Environmental variation coefficient

$$\text{EVC} = \frac{\sqrt{\sigma^2 E}}{\bar{X}} \times 100 \qquad (5)$$

Broad heritability (6) for each variable was estimated classically, as described below:

$$\text{Broad heritability } h^2_A = (\sigma^2_G/\sigma^2_P) \times 100 \qquad (6)$$

Generic progress expected with the classic formula: $\Delta g = ds \times h^2_A$, where ds = differential of selection.

The values of the phenotypic variation coefficients (PVC), genotypic variation (GVC) and genetic progress were low (<10%), moderate (10-20%) and high (>20%). The heritability values were low (<30%), moderate (30-60%) and high (>60%), as used by (Reddy *et al*., 2013a).

The establishment of the plots was done with direct sowing, depositing a seed previously treated with aluminum tris-o-ethylphosphonato per site. The crop received fertilization at 150 kg ha^{-1} of the formula 8-20-20 plus 100 kg ha^{-1} of urea N at 46%, with two applications. Pre-emergence weed control was carried out with an application of glyphosate, along with a post-emergence application of paraquat dichloride 276 g L^{-1} between the plants and an application of haloxyfop-R-methyl ester, 108 g. Additionally, two manual cleansings were performed. The pest control, mainly trips (*Frankliniella occidentalis*) and aphids (*Aphididae*), was carried out with six rotating applications of abamectin, imidacloprid and triflumuron. The control of fungal diseases was carried out in a preventive manner with eight rotating applications of fosetil aluminum and fludioxonil 2.5 g + metalaxil-M 1.0 g. The soil remained at field capacity with a drip irrigation system installed with drippers that supplied water at a rate of 2 L h^{-1}. Fruit harvesting began 93 d after sowing, when the fruits had the characteristic yellow color of maturity.

RESULTS AND DISCUSSION

The mean squares of the 10 characteristics evaluated in the 20 half-sib families of creole melon (Tab. 2) presented statistically significant differences ($P \leq 0.05$) for fruit cavity (FC) and highly statistically significant ($P \leq 0.01$) differences for the other nine characteristics, showing high variability between the melon families. The coefficient of variation (CV) was higher than 30% for fruit weight (FW), pulp firmness (PF) and production; the other evaluated characteristics had a (CV) less than 23%. These results indicated that the creole melon massal population contained broad reserves of genetic variation and adaptation to different environmental conditions (Eguiarte *et al*., 2013), in agreement with the results obtained by Reddy *et al*. (2013a), who stated that the highly significant differences between the genotypes indicated the presence of a sufficient amount of variability in the germplasm, facilitating selection possibilities for modifying the studied characteristics.

The averages, maximum, and minimum values presented significant difference for the characteristics of the 20 melon half-sib families (Tab. 3). Family 93 showed the highest average fruit weight (5,201.80 g), fruit diameter (179.59 mm) and production (11,560.00 kg ha^{-1}), family 176 showed the highest average for fruit length (213.78 mm), family 117 did so for the thickness of the pulp (38.62 mm), family 21 did so for the thickness of the bark (1.07 mm), family 68 did so for fruit cavity (103.60 mm), family 47 did so for pulp firmness (5.11), (the minimum acceptable firmness is 3.1 kg cm^2 for Cantaloupe melon and Orange Flesh (Monge, 2016)), family 123 did so for acidity of pulp (7.04) and family 196 (8.44) did so for soluble solids. These values were higher than those reported by (Reddy *et al*., 2013a) in his research on 35 lines of cantaloupe cross-linked melon, who obtained an average fruit weight of (g) 416.57±17.94, fruit diameter of (cm) 8.56±0.38, fruit length of (cm) 10.70±0.48, pulp thickness of (cm) 1.58±0.11, bark thickness of (mm) 1.70±0.14, fruit cavity length of (cm) 6.26±0.33, fruit cavity width of (cm) 4.65±0.25, total soluble solids of (°Brix) 6.70±0.15 and fruit production of (kg/plant) 1.16±0.15. Monge

Table 2. Mean squares of the analysis of variance and coefficients of variation for the 10 fruit characteristics of the creole melon (*Cucumis melo* L.) cantaloupe. Palenque, Provincia de Los Ríos, Ecuador. 2018.

Source	DF	FW (g)	FD (mm)	FL (mm)	FT (mm)	BT (mm)
Blocks	2	31473929.10**	9429.66**	10952.60**	63.64^{NS}	0.03^{NS}
Families	20	6037165.10**	796.04**	2257.00**	91.86**	0.09**
Blocks × Family	40	1809207	352.26	511.15	0.04	0.04
CV		39.51	11.82	13.28	16.15	22.87
Source	**DF**	**FC (mm)**	**PF (kg cm^{2})**	**PA (pH)**	**SS (°Brix)**	**P (kg ha^{-1})**
Blocks	2	3011.42**	2.61^{NS}	0.30^{NS}	10.24**	155426521.00**
Families	20	422.09*	3.73**	0.43**	2.91**	29813106.90**
Blocks × Family	40	228.09	1.72	0.14	0.80	8934337.00
CV		16.71	32.69	5.63	12.39	39.51

FW, fruit weight; FD, fruit diameter; FL, fruit length; PT, pulp thickness; BT, bark thickness; FC, fruit cavity; PF, pulp firmness; PA, pulp acidity; SS, soluble solids (°Brix); P, production (kg ha^{-1}). DF, degrees of freedom; ** significance level $P\leq0.01$; *significance level $P\leq0.05$ of probability; ^{NS}: non-significant.

Table 3. Averages, maximum values, minimum values and significant minimum difference for the characteristics of 20 half-sib families of creole melon (*Cucumis melo* L.) cantaloupe. Palenque, Provincia de Los Ríos, Ecuador. 2018.

Families	FW (g)	FD (mm)	FL (mm)	FT (mm)	RT (mm)	FC (mm)	PF (kg cm^{-2})	PA (pH)	SS (°Brix)	P (kg ha^{-1})
21	2662.90	167.03	198.17	35.02	1.07	98.07	4.64	6.67	7.78	5918.00
43	2900.80	153.52	179.67	36.01	0.78	91.59	3.96	6.69	6.89	6446.00
44	3588.20	163.02	174.41	35.68	0.81	88.15	4.33	6.32	7.11	7974.00
47	3205.00	158.65	169.12	32.89	0.78	89.46	5.11	6.39	7.56	7122.00
61	3864.80	165.20	175.33	37.40	0.81	96.89	4.34	6.71	7.78	8588.00
68	4743.70	165.67	167.17	35.03	0.73	103.60	3.57	6.43	6.89	10541.00
86	2745.30	150.78	155.12	30.29	0.84	78.23	3.37	6.88	7.33	6101.00
89	4087.30	154.64	173.50	33.80	0.73	86.95	4.55	6.56	7.44	9083.00
93	5201.80	179.59	189.57	38.49	0.82	94.77	3.49	6.85	7.78	11560.00
97	3740.70	150.50	161.59	35.91	0.82	83.77	3.77	6.15	6.67	8313.00
109	2516.30	150.75	161.04	30.32	0.97	86.30	3.88	6.55	7.11	5592.00
110	3160.70	151.67	157.35	33.43	0.64	87.71	3.76	6.74	6.67	7024.00
117	3891.20	171.70	167.33	38.62	0.80	99.52	4.36	6.49	7.78	8647.00
123	3147.80	168.03	178.89	36.30	0.84	84.36	4.27	7.04	7.22	6995.00
138	3106.10	162.43	150.93	34.98	0.84	93.86	4.50	6.62	6.89	6902.00
140	4454.00	156.67	163.00	26.52	0.92	84.81	2.46	6.47	7.11	9898.00
149	2421.90	159.29	172.11	32.46	0.87	86.49	4.57	6.59	6.78	5382.00
176	2874.70	163.97	213.78	35.07	0.85	97.59	2.83	6.61	7.89	6388.00
177	3560.80	154.75	154.55	32.31	0.90	93.63	4.75	6.47	7.11	7913.00
196	3794.80	149.68	164.66	28.74	0.99	93.85	3.98	6.99	8.44	8433.00
testigo	1815.50	137.05	148.57	30.21	0.69	77.90	3.68	6.74	5.83	4035.00
Average	3404.01	158.79	170.28	33.79	0.83	90.36	4.01	6.62	7.24	7564.52
Maximum	5201.8	179.59	213.78	38.62	1.07	103.6	5.11	7.04	8.44	11560.00
Minimum	1815.5	137.05	148.57	26.52	0.64	77.9	2.46	6.15	5.83	4035.00
LSD	2300.7	32.1	38.67	9.33	0.33	25.83	2.24	0.64	1.53	5112.7

FW, fruit weight; FD, fruit diameter; FL, fruit length; PT, pulp thickness; RT, rind thickness; FC, fruit cavity; PF, pulp firmness; PA, pulp acidity; SS, soluble solids (°Brix); P, production (kg ha^{-1}). LSD = Least significant difference Tukey ($P\leq0.05$).

(2016) evaluated 70 greenhouse melon genotypes and found inferior results for fruit weight and pulp firmness and higher results for percentage of total soluble solids; in addition, the characteristics showed wide variability among the genotypes in terms of average fruit weight (268.7-1279.4 g), production per area (0-70.85 t ha^{-1}), fruit firmness (0.5-4.8 kg cm^{-2}) and percentage of total soluble solids (9.9-17.1°Brix).

The correlation coefficients between the characteristics in the 20-half-sib-families of melon (Tab. 4) showed positive significant phenotypic associations between the fruit weight (FW) with the fruit diameter (FD), fruit length (FL), pulp thickness (PT), fruit cavity (FC), soluble solids (SS, °Brix) and production (kg ha^{-1}), indicating that the selection for a greater fruit weight would lead to an increase in the thickness of the pulp, soluble solids and production per hectare. A negative, non-significant correlation was found between the fruit weight (FW) and the rind thickness (RT), pulp acidity (PA) and pulp firmness (PF). There were significant and positive phenotypic correlations between the (SS) soluble solids (°Brix) and the fruit diameter (FD), fruit length (FL), pulp thickness (PT), fruit cavity (FC) and production (kg ha^{-1}). All significant correlations were positive except for the correlation between the pulp acidity (PA) and the pulp firmness (PF) and production (kg ha^{-1}). The correlation can be attributed to the presence of pleiotropic effects of the genes, physiological relationship and development, environmental effect or a combination of all the above (Reddy *et al*., 2013b). The correlation coefficient associations, of great or little value, could be found for an improvement program through selection.

The values of the coefficients of genotypic variation (GVC) for the characteristics evaluated in the 20 half-sib families, in general, were lower than the values of the coefficients of environmental variation (EVC). The parameters of heritability (h^2_A) were moderate, and the genetic advance (Δg) had a low magnitude (Tab. 5).

For the fruit weight (FW), the (GVC) was 34.87%, and the (EVC) was 39.51%, indicating that both the genetic and environmental components had similar variations in the phenotypic manifestation of the characteristic. These results differ with that reported by Reddy *et al*. (2013b), who stated that the average fruit weight registered high EVC and GVC values, which indicated the presence of a high degree of variability and a better scope for the improvement of these characteristics through selection. The soluble solids (SS) presented a GVC equal to 11.57% and a EVC equal to 12.39%, indicating little genetic and environmental variation in the expression of this characteristic. Reddy *et al*. (2013b) reported that the total soluble solids showed low to moderate EVC and GVC values, which indicated the presence of a high degree of variability and, therefore, a greater margin of selection. The production (kg ha^{-1}) presented a GVC of 34.87% and a EVC of 39.50%, indicating similar variations in the manifestation of this characteristic.

Table 4. Correlation coefficients (r) among the characteristics evaluated in 20 half-sib families of creole melon (*Cucumis melo* L.) cantaloupe. Palenque, Provincia de Los Ríos, Ecuador. 2018.

Characteristic	FD (mm)	FL (mm)	PT (mm)	BT (mm)	FC (mm)	SS (°Brix)	PA (pH)	PF (kg cm^{-2})	P (kg ha^{-1})
FW (g)	0.54**	0.32**	-0.04^{NS}	0.22**	0.47**	0.36**	-0.12^{NS}	-0.12^{NS}	1.00**
FD (mm)		0.64**	0.02^{NS}	0.51**	0.66**	0.51**	0.09^{NS}	0.09^{NS}	0.556**
FL (mm)			0.15*	0.46**	0.45**	0.49**	0.06^{NS}	0.08^{NS}	0.32**
PT (mm)				-0.03^{NS}	-0.02^{NS}	0.11^{NS}	0.05^{NS}	0.07^{NS}	-0.04^{NS}
BT (mm)					0.30**	0.24**	0.03^{NS}	0.09^{NS}	0.23**
FC (mm)						0.36**	-0.13^{NS}	0.18*	0.47**
SS (°Brix)							0.18^{NS}	0.12^{NS}	0.36**
PA (pH)								-0.18*	-0.12**
PF (kg cm^{-2})									-0.12^{NS}

FW, fruit weight; FD, fruit diameter; FL, fruit length; PT, pulp thickness; BT, bark thickness; FC, fruit cavity; SS, soluble solids; PA, pulp acidity; PF, pulp firmness; P, production.

** significance level $P \leq 0.01$; * significance level $P \leq 0.05$ of probability; ^{NS}: non-significant.

Table 5. **Averages, genetic variation coefficient, environmental variation coefficient, heritability in a broad sense and expected genetic progress (expressed in percentage) of the characteristics evaluated in 20 half-sib families of creole melon (*Cucumis melo* L.) canteloupe. Palenque, Provincia de Los Ríos, Ecuador. 2018.**

Characters	Averages	GVC (%)	EVC (%)	h^2A (%)	Δg	Δg (%)
FW (g)	3404.01	34.87	39.51	43.79	228.55	6.71
FD (mm)	158.79	7.66	11.82	29.57	2.14	1.35
FL (mm)	170.28	14.17	13.28	53.24	6.31	3.71
PT (mm)	33.79	13.47	16.15	41.01	0.77	2.28
BT (mm)	0.83	16.24	22.87	33.52	0.01	0.67
FC (mm)	90.36	8.9	16.71	22.09	1.36	1.51
PF (kg cm^{-2})	4.01	20.44	32.69	28.12	-0.06	-1.46
PA (pH)	6.62	4.71	5.63	41.17	0.05	0.71
SS (°Brix)	7.24	11.57	12.39	46.59	0.32	4.44
P (kg ha^{-1})	7564.52	34.87	39.51	43.79	507.36	6.71

GVC, genetic variation coefficient; EVC, environmental variation coefficient; $h^2_{A,}$ heritability in a broad sense, Δg, expected genetic progress; FD, fruit diameter; FL, fruit length; CT, cortex thickness; PT, pulp thickness; FC, fruit cavity; SS, soluble solids; PA, pulp acidity; PF, pulp firmness; P, production (kg ha^{-1}).

The wide-ranging heritability estimates had a moderate magnitude (30-60%) for all the evaluated characteristics, except for fruit diameter (FD), fruit cavity (FC) and pulp firmness (PF), which had a low magnitude (<30%), indicating that environmental variance plays an important role in the manifestation of the phenotypic variance of the evaluated characteristics. These estimates differ from those found by Reddy *et al*. (2013b), who reported high heritability values for fruit length (83.92%), average fruit weight (90.35%), length of fruit cavity (89.41%), width of the fruit cavity (66.93%), bark thickness (70.39%) and total soluble solids (86.51%). Rakhi and Rajamony (2005) reported high heritability for fruit length, fruit circumference, average fruit weight, and production per plant, which meant that the variability of these characteristics had a high percentage because of genetic variation with greater correspondence between the phenotypes and the genotype. Reddy *et al*. (2013) found high heritability for the production in melon, which implied that this characteristic can be improved through selection.

The estimated values of genetic progress had a low magnitude (<10%) in all the evaluated characteristics because of the moderate or low heritability. Reddy *et al*. (2013) reported different genetic progress for all the characteristics, with the exception of fruit weight and length. The estimates of genetic advance had a high great magnitude (> 20%) for average fruit weight (57.27%), bark thickness (49.04%), total soluble solids (24.68%), and fruit production (30.50%) and a moderate magnitude (10-20%) for the fruit length (17.21%), fruit diameter (10.02%) and pulp thickness (13.51%).

Although the heritability was moderate for fruit weight (FW), soluble solids (SS) and production (kg ha^{-1}), the genetic advance was significant taking into account the agronomic and economic importance of these characteristics (Rakhi and Rajamony, 2005). In general, selection for melon types with high production and quality should focus on average fruit weight, production per hectare and content of soluble solids.

CONCLUSIONS

Moderate genetic variability was found in all the characteristics evaluated in the 20 half-sib families of the creole melon; therefore, there are possibilities for improving these characteristics through recurrent selection. All the families were superior to the control treatment in 7 of the 10 fruit characteristics. Family 93 stood out for fruit weight and production (kg ha^{-1}), and family 196 did so for total soluble solids. Heritability broadly had a moderate magnitude for all the analyzed characteristics. The genetic progress had a low magnitude for most characteristics. In recurrent selection processes, the characteristics demanded by the market, mainly soluble solids and average fruit weight, must be considered.

Conflict of interests: this manuscript was prepared and reviewed with the participation of all authors, who declare that there exists no conflict of interest that puts at risk the validity of the presented results.

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Socioecological resilience of typical citrus fruit agroecosystems

Resiliencia socioecológica de agroecosistemas citrícolas tipificados

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Valencia orange picking (*Citrus Sinensis* L., Osbeck) in Lejanias (Colombia).

Photo: J.A. Cleves-Leguizamo

ABSTRACT

This paper, as a tool for analysis, considered the capacity of the interaction that open systems have against the occurrence of disturbances so that they can continue to function with minimal losses of energy, called resilience, an emerging characteristic of agroecosystems. To establish a method to measure this characteristic, ten variables were evaluated, including seven cultural variables: level of schooling, land tenure type, saving capacity, social organizational identity, farm infrastructure, weed control and production system; and three ecosystem variables: water resource availability, phytosanitary management and Main Agroecological Structure (MAS) in typical citrus fruit agroecosystems. These were methodologically grouped into six recommended domains: groups of farms and citrus growers with similar ecosystem and cultural conditions that have been characterized and typified in a previous study. In each of these groups, three farm types were selected for a total of 18 production units (department of Meta, Colombia). To determine the difference between the variables, Chi-square tests were applied (using the Pearson and Fisher statistics). Network analysis was applied to determine the relationship between the variables. The resilience was not significantly correlated with level of schooling, farm infrastructure or MAS. The relationship between the resilience and cultural variables presented a high significance, whereas the ecosystem variables showed a low statistical significance.

Additional key words: citriculture; Orinoquía; productive systems; low tropics.

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RESUMEN

En este artículo, como una herramienta de análisis se consideró la capacidad de interacción que tienen los sistemas abiertos frente a la ocurrencia de un disturbio, de tal manera que puedan seguir funcionando con mínimas pérdidas de energía e información, denominada resiliencia y es considerada como una característica emergente de los agroecosistemas. Con el objetivo de establecer un método para medir esta característica se evaluaron diez variables, siete culturales: nivel de escolaridad, tipo de tenencia de la tierra, capacidad de ahorro, pertenencia a alguna organización social, infraestructura de la finca, control de arvenses y sistema de producción; y tres variables ecosistémicas: disponibilidad del recurso hídrico, manejo fitosanitario y Estructura Agroecológica Principal (EAP) en agroecosistemas citrícolas tipificados. Estas variables fueron agrupadas metodológicamente en seis dominios de recomendación, es decir grupos de fincas y de citricultores con condiciones ecosistémicos y a la vez culturales similares, que en trabajo previo habían sido caracterizados y tipificados. En cada uno de estos grupos se seleccionaron tres fincas tipo para un total de 18 unidades productivas (Meta, Colombia). Para determinar diferencia entre las variables se aplicaron pruebas de Chi Cuadrado (empleando los estadísticos de Pearson y Fisher). Para determinar la relación entre las variables se aplicó análisis de redes, pudiéndose determinar que el nivel de escolaridad, infraestructura de la finca y EAP no presentaron relación de significancia con la resiliencia. La relación entre las variables culturales presentaron una alta significancia, mientras que las variables ecosistémicas evidenciaron baja significancia estadística con la resiliencia.

Palabras clave adicionales: citricultura; Orinoquía; sistemas productivos; Trópico bajo.

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INTRODUCTION

In Latin America, climate variability and extreme events have impacted different regions (Altieri *et al*., 2012). In this same region, the intensity and presence of extreme events is increasing, and, in Colombia, located in an area with a direct influence from the warming of the Pacific waters, the impact is much stronger than in any other country in the region (PNUD, 2011).

In our country, the temperature regime is determined by its geographical position and its physiographic characteristics (Pabón and Hurtado, 2002). In the Orinoquía region, the distribution of the average air temperature is very uniform, with values ranging from 24 to 28°C. In contrast, pluviometric variations are significant (Pabón *et al*., 2001).

In previous studies, the wide genetic plasticity of the Orange Valencia (*Citrus sinensis* L., Osbeck) was demonstrated for the environmental conditions of the Colombian Orinoquia (Cleves-Leguizamo, 2018a).

This study aimed to give continuity to the analysis of the incidence of the cultural components associated with the production of citrus fruits in the Department of Meta, a place with adequate soil and climate conditions for citrus production (Orduz and Mateus, 2012).

This situation is more significant if we take into account that, in the last decade, the domestic market has shown signs of a citrus shortage as a result of the lower production generated by extreme weather events and deterioration of crops, which has led to a reduction in production (Aguilar *et al*., 2012). It is necessary to increase the planted area by 15.000 ha, in addition to replacing at least 20,000 ha that have finished their production cycle (Mateus *et al*., 2010).

Citrus fruits are permanent crops, which require long-term decisions aimed at the sustainability of productive systems (Cleves-Leguizamo *et al*., 2012). This makes it necessary to understand the effects of human interventions on nature, the transformation of the ecosystem and the impact that such alterations have on communities (Maya, 2003; Nicholls and Altieri, 2012).

The concept of resilience was initially a contribution from the field of Ecology, defined as a system's capacity to persist in the face of disturbances, while keeping its original structure and function stable. This is achieved through learning, adaptation and self-organization processes considered critical characteristics for recovering equilibrium and system control (Holling, 2001).

To assess resilience in socio-ecological systems, it is necessary to understand and evaluate the dynamic relationship established between human beings and the environment, taking into account ecological and cultural dimensions, including institutions and social capital, as well as leadership ability and community organization (Jiggins and Rolling, 2000).

In this document, resilience is understood as the ability of a system to absorb disturbances, adapt and reorganize. This is done by fulfilling essential productive functions such as food, fiber and ecosystem services, while preserving the system's structure, identity and interactions with the environment (Cleves-Leguizamo, 2018a).

According to Folke (2006) and Walker *et al*. (2004), resilience has four components: i) *Resistance:* ease or difficulty of systemic change; ii) *Latitude:* maximum point of resistance at which a system can respond before losing its resilience; iii) *Precariousness*: proximity to the system's critical threshold and *iv*) *Panarchy:* derived from the interactions between the previous components.

Given that the agroecosystem is both an ecosystem and cultural complex, resilience also extends to the social context. It is therefore understood as the ability of human groups to cope with not only environmental changes but those generated by social, political, economic and commercial factors (Adger, 2000). This socio-ecosystem resilience is made explicit in communities that depend on ecological and environmental resources for their livelihood, as is the case with agroecosystems (Farhad, 2012).

The importance of studying cultural and ecological variables associated with the resilience of citrus agroecosystems implies considering some basic concepts such as: i) *Biotope:* the physicochemical characteristics of water, soil and atmospheres; ii) *Biocenosis:* the set of organisms that are related to each other and that collectively depend on the environment (Toledo, 1990); iii) *Ecosystem:* the complex and dynamic relationship established between organisms and physical elements of a place, giving rise to a functional unit (MEA, 2005), with energy flows and nutrient cycling that make interdisciplinary approaches necessary for describing its structure and function (Hart,

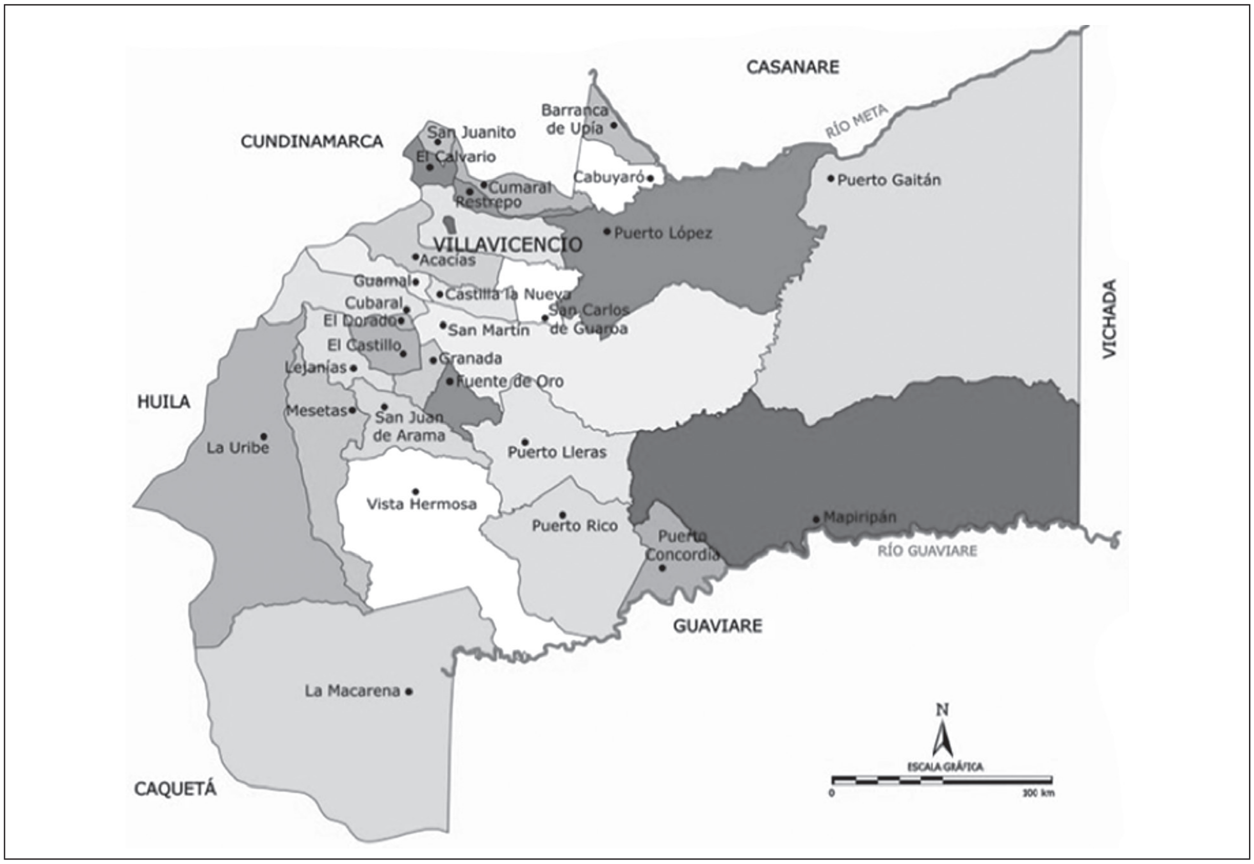

Figure 1. Location of study area municipalities. Source: IGAC (2004).

1985); and iv) *Agroecosystem:* multiple relationships and interactions between cultural and ecosystem elements such as soil, plants and organisms at different trophic levels, with diffuse limits that transcend the geographical scope of the crop or farm. The latter (agroecosystems) are considered by some authors as the object of studies with the science of agroecology, which demands a systemic analytical approach (León and Altieri 2009; León, 2010; León, 2012).

This paper aimed to analyze the relationship between resilience and an array of ecosystem and cultural attributes, with resilience defined as the emerging capacity of non-equilibrium systems to respond to varied disturbances. For this specific case, this study was undertaken in citrus production systems located in the Department of Meta, Colombia.

MATERIALS AND METHODS

The present study was carried out in the municipalities of Villavicencio, Granada, Guamal and Lejanías, where the Department of Meta (Colombia) concentrates 89% of its planted area and 95% of its citrus production (SDA, 2016) (Fig. 1).

A survey was designed with the collaboration of community members, unions, technicians and farmers. The primary data were used to structure an Excel database and to conduct a multivariate statistical analysis, resulting in six "domains of recommendation" for the dendrogram, i.e. six farmer groups with similar internal attributes and external heterogeneity (Cleves-Leguizamo and Jarma-Orozco, 2014) (Fig. 2).

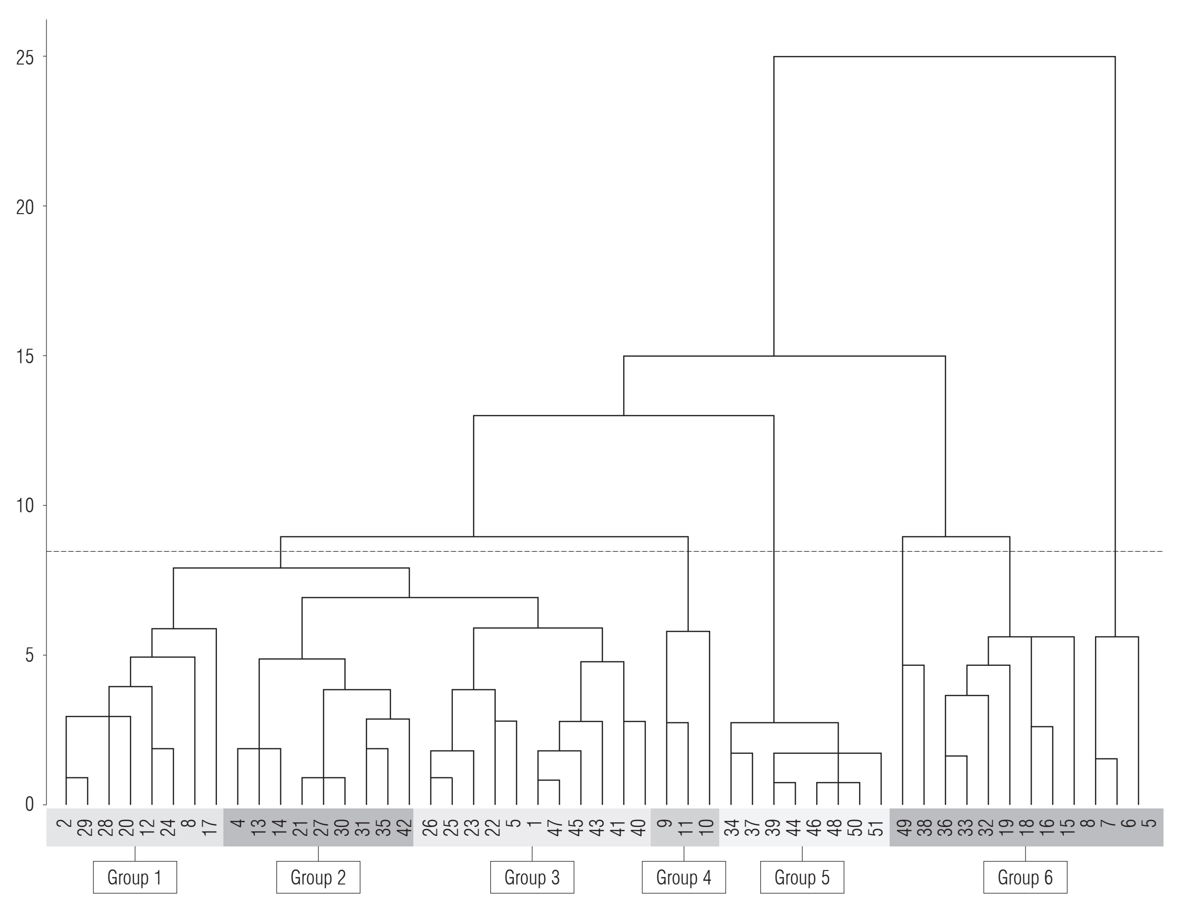

Figure 2. Dendrogram, six recommendation domains. Source: Cleves-Leguizamo and Jarma-Orozco (2014).

Table 1. General characteristics of recommendation domains.

Group	Area (ha)	Characteristics
1	6.33	Farms with phytosanitary deficiency, low level of schooling and associativity; no technical assistance; limited income; and exclusive use of family labor
2	2.3	Farms with phytosanitary limitations; medium level of schooling; medium level of infrastructure; reports of incidences of severe effects associated with climatic variability; and no technical assistance
3	9.6	High level of infrastructure; renewal of cultivars of oranges of the Valencia variety (*Citrus sinensis* L. Osbeck) and tangerines (white *Citrus reliculata*) by *Tangelo minneola* (*Citrus reticulata* x *Citrus paradisi*), technified using the Fly Dragon dwarfing pattern, which allows increasing planting density; 60% of citrus growers are linked to some type of association; they have particular technical assistance; extensive experience in citrus management; 50% have savings capacity; 40% have credit; Although they have not received training in climate information management, they relate temperature to preventive phytosanitary management techniques
4	117.33	Highly technical farms with solid logistical, administrative, technical, and financial infrastructure; linked to specialized markets, and able to process climatological information and incorporate it into phytosanitary management; contain gallery forests that promote the connectivity of minor and major agroecosystems; no phytosanitary limitations; with high productivity; able to perform batch rotation integrated with livestock species; in the process of developing quality certification with a view to offering their products in specialized markets
5	4.25	Renewed crops, young (5 years old); low level of schooling and infrastructure; high experience in crop management; medium productivity, organization, savings and credit availability; access to climatological information
6	6.79	Farms mainly engaged in agrotourism work, without cultivation of critical crops, phytosanitary management is limited to weed control, very low productivity; Plantations with a high age (16 years) without renewal

Source: Cleves-Leguizamo and Jarma-Orozco (2014).

The most important attributes were established by Cleves-Leguizamo and Jarma-Orozco (2014); the most relevant ecosystem and cultural attributes of the six groups of citrus growers are presented below (Tab. 1).

To define the variables used to determine resilience, experts in citrus cultivation in the Orinoquia region were consulted. The prioritized variables were the following: 1) education level, 2) type of land tenure, 3) savings capacity, 4) belonging to a social organization, 5) farm infrastructure, 6) water resource availability, 7) phytosanitary crop assessment, 8) weed control, 9) type of production system and 10) Main Agroecological Structure (MAS). Three farms were chosen from each of these groups because of the minimum number of repetitions in the experiment design. The farms were then numbered randomly from 1 to 18 (Tab. 2).

In order to determine the relationship between the variables and resilience from this sampling population, statistical tests including Chi Square, Pearson and Fisher were carried out. Next, a network of interactions was constructed between the analyzed attributes.

Table 2. Groups and associated farms.

Group	Number of associated farms
1	1, 2 and 3
2	4, 5 and 6
3	7, 8 and 9
4	10, 11 and 12
5	13, 14 and 15
6	16, 17 and 18

An estimate for resilience was made applying the methodology proposed by the Ibero-American Network of Agroecology for the Development of Agricultural Systems Resilient to Change, REGRADES (Henao, 2013), using the indicated variables (Tab. 3).

Data analysis

The results of the 10 variables prioritized in the expert consultation established a measurement of resilience. These measurements were analyzed by group (treatment) and at the farm level (treatment repetitions) to determine which variables explained the variation in the resilience to a greater extent. The following procedures were followed:

Table 3. Variables used in the measurement of socio-ecological resilience.

Group	Variables	Categories	Value
1	Education level (NIDES)	Primary	1
		Secondary	3
		University	5
2	Type of land tenure (TEDLT)	Landowner	5
		Renter	1
3	Savings capacity (CAPDA)	Yes	5
		No	1
4	Belonging to a social organization (TIDOR)	Yes	5
		No	1
5	Farm infrastructure (INDLF)*	High	5
		Medium	4
		Low	3
		Poor	1
6	Water resource availability (FDAPR)	Yes	5
		No	1
7	Phytosanitary crop assessment (EVSDC)	Excellent	5
		Good	4
		Fair	3
		Poor	1
8	Weed control (CODAR)	Excellent	5
		Good	4
		Fair	3
		Poor	1
9	Type of production system (SIDPR)	Monoculture	1
		Associated	5
10	Main Agroecological Structure (MAS) **	High	5
		Medium	4
		Low	3
		Nonexistent	1

* Related to planting activities. ** Main Agroecological Structure (MAS), is a methodological instrument for the evaluation of agricultural systems. It has ten components and was originally proposed by León (2012) and later evaluated by Cleves-Leguizamo *et al.* (2017) as a planning instrument for land use in agroecosystems. Its relevance for permanent crops has also been evaluated by León-Sicard *et al*. (2018) as well as Cleves-Leguizamo *et al.* (2018b).

Characterization of the sample using relative frequencies of each of the responses given by the producers

Use of the REDAGRES methodology to aggregate resilience variables using simple, weight-free averages, which corresponded with survey responses that included environmental and cultural aspects associated with citric agroecosystems. Each variable was assigned a grade based on its condition (optimum, average and low), determining adjustment for low-graded variables, improvement for average-level variables and conservation for optimally graded variables (Altieri and Nicholls, 2013).

1) Development of contingency tables: resilience per group and resilience per 10 variables.
2) Chi-squared and Fisher's exact test to analyze the relationships between the 10 variables.
3) Summary of the 72 chi-squared and Fisher's exact test results to analyze the relationships between the 10 variables.
4) Construction and analysis of the adjacency matrix of the 10 variables, utilizing the software UCINET (Borgatti *et al*., 2002).
5) Elaboration of a network diagram using NETDRAW (Borgatti *et al*., 2002).

RESULTS AND DISCUSSION

With the purpose of establishing whether the methodology proposed for the estimation of socio-ecological resilience was affected by the general characteristics that were used in the classification (group of farms) and by the type of management implemented at the level of the productive unit (farm), the resilience value was established by farm and by group. For the interpretation of the result, a resilience scale was defined with five categories (Tab. 4).

This proposal of analysis and interpretation established that the methodology used for the calculation of resilience is adequate for the production conditions of citrus fruits in the Department of Meta from the point of view of experiment design, where the groups of farms act as "treatments" and farms are "repetitions" of these treatments.

Table 4. Ranges proposed for the characterization of socio-ecological resilience values.

Range	Interpretation of resilience
4.1 – 5.0	Very high
3.1 – 4.0	High
2.1 – 3.0	Medium
1.1 – 2.0	Low
0.0 – 1.0	Very low

The values were also used to determine the relationship between the variables and socio-ecological resilience in order to establish which of them contribute most to explaining the variation in resilience. Below are the general results of the sample (section 3.1.), the results by group (3.2.) and by farm (3.3.), the qualitative analysis of the variables (3.4.), the contribution of these variables to the total value of resilience (3.5.) and, finally, the relationship between the variables and resilience (3.6.).

Characterization of the sample

The sample was characterized by determining relative frequencies of responses (Tab. 5) for surveys conducted on eighteen farms.

In general terms, the farmers have a low to medium educational level, at least from the point of view of formal education. In relation to income, 66% of the population has savings capacity. With respect to organizations, 60% do not belong to any type of organization that facilitates social articulation in any way (Aguilar *et al*., 2010).

70% of the properties are managed directly by their owners, have a medium to high level farm infrastructure, and have established a monoculture production system; 83% have access to irrigation water, perform good pest management through phytosanitary evaluation and most control weeds with mixed media (mechanical and chemical). None report adopting hedges for this purpose. Finally, the main agroecological structure of the farms included in the study is between medium and low (Cleves-Leguizamo, 2018a).

The conjugation of previous environmental and cultural characteristics determined the socio-ecological resilience (Reay, 2019).

Resilience / group determination

To determine the resilience of the six analyzed groups, average values were calculated for each of the three farms in each group (Fig. 4).

Table 5. Relative frequencies of survey responses.

Variable	Consolidated survey results
Education level	50% possess a low level of education (primary), 27.78% medium level and 22.22% possess a high level of education
Type of land tenure	16.67% rent land, 83.33% are landowners
Savings capacity	66.67% possess savings capacity, 33.33% do not have excess income and are unable to save
Belonging to a social organization	55.56% do not belong to any association or organization, 44.44% belong to an organization
Farm infrastructure	22.22% have deficient farm structure, 27.78% have a low level of structure, 11.11% medium level and 38.89% high level
Water resource availability	83.33% have a water source for irrigation, 16.67% do not have a water source
Phytosanitary crop assessment	11.11% of farms have an excellent level of crop assessment; 38.89% good; 27.78% fair and 22.22% poor
Weed control	5.56% perform mechanical weed control, 27.78% perform chemical control and 66.67% perform mixed weed control
Type of production system	33.33% have an associated system of production, 66.67% are monoculture
Main Agroecological structure	16.67% have a high main agroecological structure; 33.33% medium; 33.33% low; 16.67% nonexistent

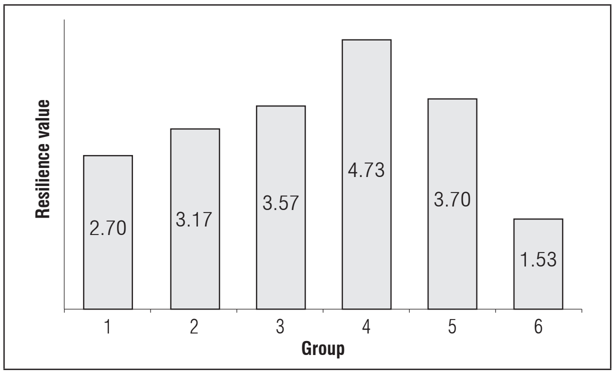

Figure 4. Average values of socioecological resilience for farm groups.

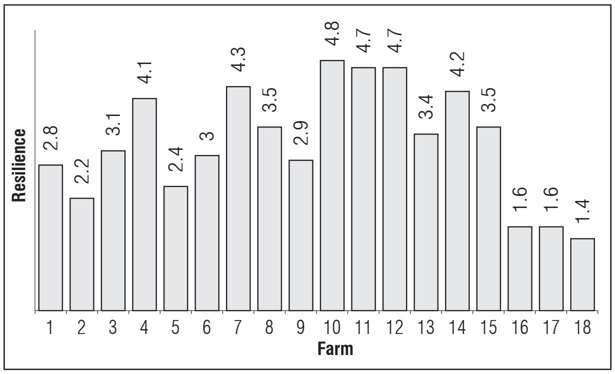

Figure 5. Resilience values obtained per farm.

As indicated above, the farms with an agro-industrial productive approach (group 4) have the highest resilience (4.7) since ecosystem and cultural variables offer the best conditions to face externalities; they have a greater capacity to respond to negative events and a greater capacity to capitalize on positive events (Adger, 2000).

Group 6 (resilience of 1.5), on the other hand, had the lowest value and, therefore, will not be able to recover in the face of negative socio-ecosystem externalities and will not be able to take advantage of positive externalities. Group 1 had an equally low resilience value (2.7). Two groups were placed at an intermediate level of resilience (3.6 for group 3, and 3.7 for group 5), which had a medium capacity to face adverse externalities.

These results showed that the general characteristics that were taken as references for the typification of the groups (Tab. 1) significantly affected the socioecological resilience (Cleves-Leguizamo and Jarma-Orozco, 2014). The results of the statistical tests confirmed this statement (Tab. 7).

Resilience by farm

Resilience was determined based on the average of the values of each of the selected variables and associated with each of the eighteen (18) citrus agroecosystems values (Fig. 5).

The highest values of resilience were observed on farms 10, 11 and 12. These three farms made up the group linked to agribusiness, integrating plant and animal species (major and minor), with gallery forests articulated to biological corridors (León-Sicard, 2018). Administratively, these farms are developing quality certification processes to offer their products in specialized markets. Farms 16, 17 and 18 had the lowest resilience values and belonged to group number 6, which had plantations that have not been renewed for more than 25 years and only carry out weed control by the owners. These farms have developed tasks linked to agritourism (Aguilar *et al*., 2012).

On the other hand, farms 1, 2, 3, 5 and 9 had resilience values slightly higher than the previous group and were monocultures, which do not have technical assistance with phytosanitary control that is low efficiency. Finally, farms 4, 6, 7, 8, 13, 14 and 15 presented resilience values between medium and high, associated with a better phytosanitary crop management and access to technical assistance.

Relationship between the variables and resilience

Although it is pertinent to know the values of the socio-ecosystem resilience of farms and groups, it is necessary to know the relationships between the variables that constitute it. For this reason, to determine the relationship between the variables and the category of resilience, a combination of Pearson's Chi Squared and Fisher's exact tests were performed with a 95% confidence level in order to avoid possible biases as a result of the sample size. Fisher's exact test was used when the samples were small and the assumption of a minimum expected value equal to 5 of the Pearson Chi-square tests was not met.

In all cases, the null hypothesis established no relationship between the variables. Therefore, values below 0.05, in asymptotic and/or exact significance, established that there were statistically significant relationships between the variables (Tab. 6).

Table 6. Results obtained for the statistical tests used to establish the relationship between the variables and the resilience of the farm.

Test	Pearson's Chi-squared test			Fisher's exact test		
Variables	Value	Asymptotic significance	Is the relationship significant?	Value	Exact significance	Is the relationship significant?
Education level	10.575	0.102	No	9.316	0.087	No
Type of land tenure	18	0	Yes	10.822	0.001	Si
Savings capacity	8.025	0.045	Yes	7.472	0.037	Si
Belonging to a social organization	11.723	0.008	Yes	11.068	0.004	Si
Farm infrastructure	16.723	0.053	No	12.101	0.095	No
Availability of water resources	7.44	0.059	No	5.406	0.051	No
Phytosanitary management	20.537	0.015	Yes	13.722	0.024	Si
Weed control	15.48	0.017	Yes	13.151	0.004	Si
Production system	18	0	Yes	15.661	0	Si
MAS	15.55	0.077	No	11.16	0.177	No
Group relations	31.8	0.007	Yes	19.647	0.012	Si

Qualitative analysis of variables

The *Education level* variable did not present a significant relationship with resilience, mainly because of the vast experience that farmers have in crop management (Aguilar *et al*., 2010). The *Type of land tenure* presented a significant relationship with resilience. This indicates that the owners who have developed rooting processes and who know their environment better have a greater sense of belonging that is evidenced in the management of the farm (Mateus *et al*., 2010).

Savings capacity is related to resilience, which means that people with a greater availability of economic resources have a greater capacity to respond. *Belonging to a social organization* is also significantly related to resilience. It is easier to face all kinds of externalities of the agroecosystem if you belong to an organization that can offer support in such circumstances (Aguilar *et al*., 2012).

Farm infrastructure did not show a significant relationship with resilience. It should be noted that the farms had an average infrastructure between medium and high, at 62% (Mateus *et al*., 2012).

Water resource availability showed no significant relationship with resilience, possibly because precipitation supplies the crop's water requirement. However, having a source of water for irrigation increases the resilience of the agroecosystem (Cleves-Leguizamo *et al*., 2016; Reay, 2019).

Cultural variables such as *Phytosanitary Management* and *Weed control* are related to resilience. Both contribute to better crop management in the face of adverse externalities fundamentally associated with disturbances linked to change and climatic variability (Quintero-Pertuz and Carbonó-Delahoz, 2015; Reay, 2019). Such changes, although resulting from internal natural processes (radiative forcing) as well as anthropogenic activities that affect the composition of the atmosphere, are fundamentally changes in land use and soil vocation (Pabón and Hurtado, 2002; IPCC, 2001; IPCC, 2007; IPCC, 2013).

This situation is relevant in Colombia because of its geodetic position, which makes it vulnerable to the occurrence of meteorological phenomena more than any other country in the region. Temperature and precipitation variations affect crop yield with a greater intensity in long-cycle crops such as citrus (Naciones Unidas, 1992; Boshell *et al*., 2011; Cleves-Leguizamo *et al*., 2016).

The *Production System* was significantly related to resilience since the biodiversity of the productive system contributes differentially to the resilience of the agroecosystem, in accordance with the results obtained by Altieri and Nicholls (2013). The authors reported that biodiversity agroecosystems are more resilient.

The *Main Agroecological Structure* did not present a statistically significant relationship with resilience because this index does not describe the complexity of productive systems (León-Sicard *et al*., 2018).

Belonging to a social organization and resilience showed a statistically significant relationship. This result showed that the characterization and typing prior to the study allowed an accurate grouping of the farms (Cleves-Leguizamo and Jarma-Orozco, 2014).

The value of farm resilience was related to group resilience, highlighting the consistency of the resilience results for each of the farms and groups.

Analysis of resilience between groups

To determine differences between the groups, an ANOVA was performed. This test showed that there were significant statistical differences between the groups, with a 95% confidence level (Tab. 7). Once it was determined that there was a statistical difference, multiple comparison tests (Duncan, Tukey and Dunnet) were carried out to establish clusters and differences between the groups. The results are shown below (Tab. 8 and 9).

Table 7. Analysis of variance of resilience by farm groups.

	Grades	Sum of squares	Quadratic mean	F	Pr > F
Model	5	17.273	3.454	12.54	0.0002
Error	12	3.306	0.275		
Total	17	20.580			

The Duncan test was used to compare all pairs of means. This does not require a previous F test, as does DMS, so it can be carried out without the F test. On the other hand, the Tukey test uses a single value with which all possible pairs of means are compared. Finally, in the Dunnet test, the means are compared against the control group. The results of the Duncan and Tukey test are in Tab. 8, and the results of the Dunnet test are in Tab. 9.

The test results confirmed that group 4 had the highest level of resilience; group 6 had the lowest; groups 2, 3 and 5 had a medium level of resilience; and there were no significant differences between group 1 and group 6.

Table 8. Duncan and Tukey test results for resilience by farm/group.

Group	Half Static	Duncan's Grouping	Tukey Grouping	
4	4.73	A	A	
5	3.70	B	A	B
3	3.56	C	A	B
2	3.16	D		B
1	2.70	E	C	B
6	1.53	F	C	

Table 9. Dunnet test results for resilience by farm group.

Comparison group		Difference between means	Confidence limits		
4	6	3.20	1.95	4.44	***
5	6	2.16	0.92	3.41	***
3	6	2.03	0.78	3.27	***
2	6	1.63	0.38	2.87	***
1	6	1.16	-0.07	2.41	

*** indicates comparisons were significant at the $P \leq 0.05$ level.

The differences found with the various means comparison techniques between the groups were consistent with the initial categories determined at the start of the study, indicating that the typing was correct. Likewise, the socio-ecological variables characterized the agroecosystems, which correctly determined the resilience at the farm and group level (Folke, 2006).

Variable correlation matrix

To determine the degree of relationship between the variables, 78 Pearson's Chi-squared and Fisher's exact tests were performed in the SPSS statistical program. Fisher's test was used to determine relationships between variables since it is used for small samples. In all cases, a 95% confidence level was used along with a null hypothesis of independence between the variables (Tab. 10).

From this matrix, a network diagram was developed using the NetDraw program, where the size of the nodes represented the number of relationships between the variables used to calculate resilience (Fig. 6).

The resilience of a farm was related to the variables *Savings capacity, Production system, Belonging to a social* *organization, Phytosanitary management, Weed control, Type of land tenure,* and *Group resilience.* While the *Education level, Availability of water resources, Farm infrastructure* and *Main agroecological structure* were not related to resilience at the farm level.

These results show that the practices and characteristics of the productive unit are determining factors in socio-ecological resilience; the statistical analysis that supports this statement will be presented later (León-Sicard *et al*., 2018) (Tab. 10).

Table 10. Summary of Chi-squared test results for variables used in the evaluation of socio-ecological resilience.

	NIDES	TEDLT	CAPDA	TIDOR	INDLF	FDAPR	EVSDC	CODAR	SIDPR	MAS	RESFI	RESGR	GRUPO
NIDES	1.000	1.000	0.819	0.032	0.769	0.272	0.520	0.415	0.071	0.056	0.087	0.029	0.046
TEDLT		1.000	1.000	0.216	0.010	0.056	0.010	0.025	0.515	0.113	0.001	0.004	0.007
CAPDA			1.000	0.638	0.566	1.000	0.759	0.387	0.054	0.775	0.037	0.878	0.961
TIDOR				1.000	0.904	0.216	0.030	0.018	0.002	0.131	0.004	0.026	0.134
INDLF					1.000	0.357	0.095	0.012	0.257	0.129	0.095	0.031	0.006
FDAPR						1.000	0.010	0.326	0.515	0.382	0.051	0.070	0.338
EVSDC							1.000	0.024	0.106	0.358	0.024	0.031	0.117
CODAR								1.000	0.150	0.143	0.004	0.037	0.103
SIDPR									1.000	0.055	0.000	0.044	0.175
MAS										1.000	0.177	0.000	0.005
RESFI											1.000	0.003	0.012
RESGR												1.000	0.000
GRUPO													1.000

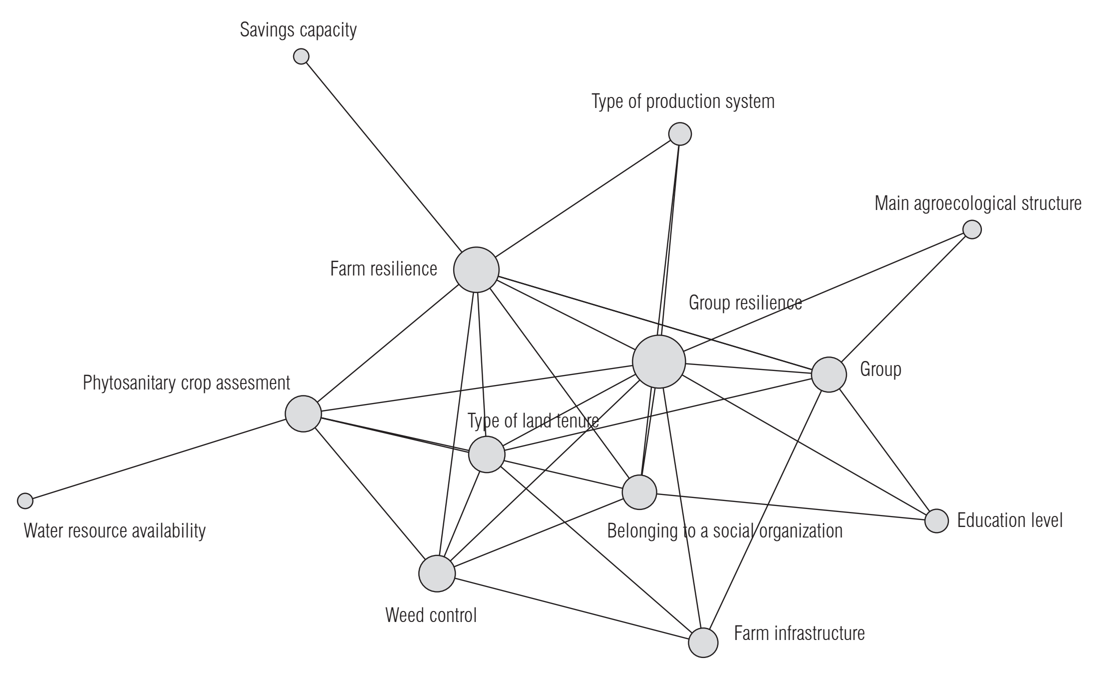

Figure 6. Network of interactions between variables used for the evaluation of socio-ecological resilience.

These results corresponded to the Fisher's and Pearson tests, and indicated that the socio-ecological resilience of citrus producers in the Department of Meta is influenced by the variables that directly affect the productivity of the crop, as well as by the relationships and economic capacity of the producer (Mateus *et al*., 2010), which allow them to make up for the deficiencies they may have individually, for example in terms of education, training, availability of infrastructure and technical assistance (Aguilar *et al*., 2012).

The variable *Farm infrastructure* was related to *Type of land tenure, Weed control, Group* and *Group level resilience. Farm infrastructure was* related to farm resilience in the network chart although it was not statistically significant according to the Fisher's and Pearson's tests. For group resilience, physical capital was important to better assimilating externalities derived from climate change and its variability.

The *MAS* variable was only related to resilience at the group level. As mentioned, it was strongly marked by ecological attributes related to agroecosystem arrangements, rather than with cultural variables (Cleves-Leguizamo, 2018b).

The *Availability of water resources* was only related to *Phytosanitary management,* and *Savings capacity* was only related to farm resilience. These variables only had a relationship in the interaction network (Reay, 2019).

This analysis allows us to infer that variables such as: *Phytosanitary management, Weed control* and *Production system* are useful tools for the implementation of union actions that contribute to the technical improvement of citrus crops in the region (Orduz and Mateus, 2012).

Finally, variables such as: *Phytosanitary management, Weed control and Production system*, which presented a significant relationship with resilience, are useful tools for the implementation of union actions that contribute to the technical improvement of citrus crops in the region (Cleves-Leguizamo *et al*., 2012; Orduz, and Mateus, 2012).

The variables *Type of land tenure, Savings capacity* and *Belonging to an organization* can also be key aspects for the implementation of sociocultural actions that can benefit citrus producers in the Department of Meta.

It is important to note that these relationships are represented in terms of the values of statistical significance, which is calculated from the sample data, so, for larger samples, established relationships could eventually vary (Cleves-Leguizamo, 2018a).

CONCLUSIONS

Cultural variables, in other words those determined by human actions, present the highest statistical significance in their relationship with resilience.

The variables *Education level, Farm infrastructure, Availability of water resources* and *MAS* did not show a significant relationship with resilience.

The behavior of the groups characterized and typified in this study was consistent with respect to resilience, confirming the differences between them and validating the characteristics of the recommendation domains.

Since the internal consistency of the groups has been confirmed, strategies can be proposed by virtue of the internal homogeneity of the groups and, in turn, the heterogeneity between them.

The variables included in this study presented between one (1) and ten (10) interactions, confirming the systemic nature of the developed analysis.

The *MAS* did not present a statistically significant relationship with resilience because this index, despite analyzing five ecosystem attributes and five cultural attributes, failed to describe the complexity of productive systems reliably, mainly because it did not evaluate fundamental attributes such as availability of water resources and edaphic aptitude.

Conflict of interest: this manuscript was prepared and reviewed with the participation of all authors, who declare that there exists no conflict of interest that that puts at risk the validity of the presented results.

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Physical analysis of peduncles of dwarf cashew clones for consumption, processed or natural

Análisis físico de pedúnculos en clones de anacardio enano para consumo en fresco y procesado

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Pacajus cashew clone (CCP 76).

Photo: F.C. Vidal Neto

ABSTRACT

The physical characteristics of cashew-dwarf peduncles were evaluated to later indicate the best clones for natural consumption and/or processing, considering that consumers appreciate products for their visual attractiveness. The objective of this study was to analyze peduncles of dwarf cashew clones obtained from the Genetic Improvement Program of Embrapa Agroindústria Tropical, Cruz-CE experimental area, in order to make inferences about the physical characteristics and indicate the best clones for natural consumption and/or processing. The analyzed variables were: total mass of the cashew (MT), chestnut mass (MC), peduncle mass (MP), apical diameter (DA), basal diameter (DB), length and firmness. The experiment design was a randomized complete block design with 25 treatments (clones) with 3 replications and up to 12 cashews per plot (four plants in total). The analysis of variance and the comparison of the means by the Scott and Knott test was realized. When analyzing the MT values, it was found that 100% of the clones were classified as types 4 (approximately 150 g), 5 (approximately 120 g) and 6 (approximately 80 g) cashews/tray, except 149-1. As for MC, there was a variation from 8.18 to 15.08 g. General averages of 95.16, 50.07 and 41.38 mm were found for the variables length, DB and DA of peduncle, respectively. It is concluded that, in general, all clones presented good characteristics; however, clone 108-6 is the preference for most consumers since it has red staining, as did the control (CCP 76). Therefore, all clones presented desirable characteristics for natural consumption and/or processing.

Additional key words: *Anacardium occidentale* L.; post-harvest quality; clones; production.

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RESUMEN

Se evaluaron las características físicas de los anacardos enanos, considerando que los consumidores primero aprecian los productos por su atractivo visual. Este trabajo tuvo como objetivo analizar pedúnculos de clones de anacardos enanos obtenidos del Programa de Mejoramiento Genético de la Agroindustria Embrapa, realizado en el área experimental de Cruz-CE, con el propósito de hacer inferencias sobre sus características físicas, para luego indicar los mejores clones destinados al consumo fresco y / o procesamiento. Las variables analizadas fueron: masa total de anacardo (MT), masa de nuez (MN), masa del pedúnculo (MP), diámetro apical (DA), diámetro basal (DB), longitud y firmeza. El diseño experimental fue en bloques al azar con 25 tratamientos (clones) con 3 repeticiones, y se cosecharon hasta 12 anacardos por parcela (la parcela tenía un total de cuatro plantas). Se realizó un análisis de varianza y comparación de medias con la prueba de Scott y Knott. Al analizar los valores de MT, se encontró que el 100% de los clones se clasificaron como tipo 4 (aproximadamente 150 g), 5 (aproximadamente 120 g) y 6 (aproximadamente 80 g) anacardos/bandeja, excepto 149-1. En cuanto a la MN hubo una variación de 8,18 a 15,08 g. Promedios generales de 95,16; 50,07 y 41,38 mm, para las variables longitud, DB y DA del pedúnculo, respectivamente. Se concluye que, en general, todos los clones mostraron buenas características, sin embargo, el clon 108-6 es de mayor preferencia para los consumidores, ya que presenta color rojo como el control (CCP 76). Por lo tanto, todos los clones mostraron características deseables para el consumo y/o procesamiento fresco.

Palabras clave adicionales: *Anacardium occidentale* L.; calidad poscosecha; clones; producción.

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INTRODUCTION

Physical variables such as appearance, size, color, shape, firmness are of great importance since they are the main characteristics that attract consumers. However, one should seek to meet the requirements of the intended market according to their needs, depending on the type of product to be obtained from the selected clone (Almeida *et al*., 2018). Qualitative attributes such as color, size, firmness and shape are more important than quantitative ones in physical analyses because they assist in the establishment of the fruit harvest point (Lopes, 2011).

Moreover, these are of great importance in marketing since they are the main characteristics available to consumers, who prioritize buying fruits that visibly have a more attractive appearance. Fruit quality is genetically established, so there is no human intervention because quality is determined by the capacity of genes. However, for expression to occur at the full genetic potential, plants are subject to several factors, such as nutritional requirements and appropriate soil and climatic conditions (Aular and Natale, 2013).

Nutrients and minerals interfere significantly with fruit quality and productivity, where soil fertility is one of the main factors for the development of the genetic potential of plants. Considering that a chemical analysis of the soil is necessary in order to characterize its capacity to provide nutrients, so that, each type of soil can be suitable for the cultivar to be produced (Wang *et al*., 2015).

In order to meet the main interest in the final product, the desirable characteristics for a clone to present good peduncle production are: low plant size to facilitate manual harvesting and to avoid damage the product; peduncle with color ranging from orange to red, pyriform shape, masses ranging from 100 to 140 g and have good firmness (Moura *et al*., 2015). Therefore, the objective of this study was to evaluate peduncles of different clones of dwarf-cashew in order to infer the physical characteristics for later indication for natural consumption and/or processing.

MATERIAL AND METHODS

The cashews (peduncle and chestnut) from dwarf cashew clones came from an experimental unit that is being evaluated in an experiment on competition of dwarf cashew clones in Cruz - CE, in the microregion of the Coast of Camocim and Acarau mesoregion, do Noroeste Cearense, with the geographical coordinates: Latitude: 02°55'04" S and Longitude: 40°10'18" W, average rainfall: 1,136 mm and average temperature: 23.0°C.

This experiment was conducted under field conditions from September 2017 to September 2018. The experiment design was a randomized block, with 25 treatments (Tab. 1) with 4 plants and three replications. The treatments consisted of different dwarf cashew clones that were previously selected based on the local edaphic and climatic conditions, with CCP 76 as a control. CCP 76 was chosen as a control because its features are considered the industry standard: good palatability, good proportion of soluble solids, titratable acidity, and color that appeals to consumers, etc. Peduncles were harvested at the end of September 2017 in the early hours of the morning and immediately packed in plastic boxes with only one layer of fruits, protecting them from mechanical injuries.

According to the variables: total cashew mass (MT), chestnut mass (MC), peduncle mass (MP), apical diameter (AD), basal diameter (DB), length and firmness, the cashews were characterized, with MT (g) (cashew nuts and peduncle) - measured by weighing each individual fruit on a semianalitic balance; MC (g) - determined after cashew weaning by weighing the cashew nut on a semianalitic balance; MP (g) – obtained with the difference between the total mass and the cashew nut mass; length, BD (near cashew nut) and AD (side opposite side of the cashew nut) of the penducle (mm), measured with a digital caliper; and peduncle firmness (N), determined using a Mc Cormick model FT 011 with 8 mm diameter nozzle on two points on opposite sides of the medial part of the peduncle.

The experiment design was a randomized complete block design (DBC) with 25 treatments (clones) with 3 replications and up to 12 cashews per plot (four plants in total). The results were submitted to the normality and heterogeneity of variance test, and then the F test was performed, which showed significant difference between the evaluated clones. The analysis of variance (ANOVA) was performed using the Genes software, and for the comparison of the means, the Scott and Knott test at 5% probability was used. Differences were considered significant when $P \leq 0.05$ (Banzatto and Kronka, 2013).

Table 1. Identification of treatments and material origin.

Treatment	Plot	Clones	Material origin
1	101/206/312	113-1	Híbr. MG Resinose 2007
2	102/209/315	MG-1	MG 2005
3	103/217/321	MG-17	MG 2005
4	104/212/302	MG-41	MG 2005
5	105/203/316	MG-57	MG 2005
6	106/210/308	MG-65	MG 2005
7	10/207/313	MG-76	MG 2005
8	108/224/311	108/6	Progênies Pacajus 2007
9	109/204/307	146/7	Progênies Pacajus 2007
10	110/218/309	SLC 12-20	Exp. Clones Anão 1998
11	111/201/322	116-2	Progênies Pacajus 2007
12	112/225/324	114/2	Progênies Pacajus 2005
13	113/220/320	114/4	Progênies Pacajus 2005
14	114/202/303	133/1	Progênies Pacajus 2005
15	115/219/306	149/1	Progênies Pacajus 2005
16	116/223/319	105/5	Progênies Pacajus 2007
17	117/215/305	143/7	Progênies Pacajus 2007
18	118/213/310	H-51	Híbr. MG Resinose 2007
19	119/222/317	H-71	Híbr. MG Resinose 2007
20	120/221/325	155/2	Progênies Pacajus 2007
21	121/205/323	END II 6-9	Clone
22	122/211/318	PRO 805/4	Clone
23	123/216/314	BRS 189	Cultivar
24	124/214/304	CCP 76	Cultivar
25	125/208/301	BRS 226	Cultivar

RESULTS AND DISCUSSION

The statistical analysis revealed a significant effect from the treatments (clones) on all evaluated characteristics. Because of the large number of clones, it was convenient to separate them into groups to facilitate an understanding of the results. The criterion of separation in groups was the significant difference between them, which followed a decreasing order of the means, that is, group 1 will always present clones with higher averages for the variables. Each group consisted of clones that did not differ from each other. Depending on the analyzed variable, the number of groups changed according to the need to group clones with the same importance. The clones have always been compared to the control since it has the characteristics desired for natural consumption and processing markets.

The total mass (MT) of cashew varied from 66.45 to 143.36 g, with a 76.91 g amplitude and a general average of 105.53 g (Fig. 1). However, the classification of the cashews was based on the number of fruits per tray, which usually ranged from 4 to 9, corresponding to an average total mass of 600 g (Moura *et al*., 2015).

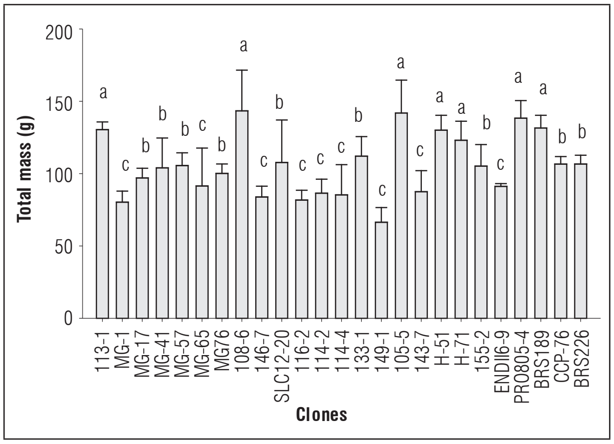

Figure 1. Total mass of cashews of dwarf cashew clones from Cruz, CE. 2017. Averages followed by the same letter do not differ from each other according to the Scott and Knott test ($P \leq 0.05$) ($n = 3$) ± standard error. Values between 113.27 and 150.82 g are considered ideal.

In the present study, the clones 108-6, 105-5, PRO805-4, BRS189, 113-1, H-51 and H-71, of this group had values between 102 and 143.36 g and were classified as type 4 cashews / tray. Group 2 (the control CCP-76 (106.57 g), 133-1, SLC12-20, BRS226, MG-57, 155-2, MG41, MG17 and MG76) had TM values that were 97,07 to 112.07 g and clones classified as type 5 cashews / tray. Group 3 (MG-65, ENDII6-9, 143-7, 114-2, 114-4, 146-7, 116-2, MG-1 and 149-1) had TM values between 66.45 and 91.49 g and clones classified as type 6.

In general, the present study showed that the objectives of genetic improvement of dwarf cashew clones are being reached since the values of type 4, 5 and 6 (4, 5 and 6 cashews per tray.) are considered the market standard because they were obtained by evaluating the variable TM.

Thus, Moura e*t al*. (2001), working on the physical characterization of peduncles of cashew clones for *natural* commercialization, obtained average masses for the cashews in a study with nine clones that ranged from 88.5 g to 155.4 g; the maximum value of BRS 189 clone was lower to that found in this study.

For the CCP76 control in the aforementioned studies, they found values of 150.8 and 155 g (respectively) for TM; this clone is the mass standard for natural commercialization. However, they were superior to those found in this study for this same clone, possibly because first experiment was irrigated and the second work was carried out in 2006, which presented annual rainfall (1,151.2 mm), the annual average of the year of accomplishment of this work, 2017 (190.2 mm) (Moura *et al*., 2001).

Lopes *et al*. (2011), working with the physical characterization of peduncles of dwarf cashew clones at different maturation stages, obtained average masses for cashews that varied from 80.28 g (BRS 189) to 83.72 g (CCP 09). The value of the BRS 189 clone was lower than that found in this study. On the other hand, when evaluating the clones CCP 76 and BRS 265, which had the highest and the lowest mean of total mass, 113.27 and 54.36 g, respectively, CCP76 presented an average value higher than that found in this study, probably because of the local climatic conditions of the studied areas.

The present study showed that a variation of 55.01 to 130.64 g, with an amplitude of 75.63 g and an overall mean of 95.16 g (Fig. 2), was observed for the peduncle mass (PM). Group 1 (108-6, 105-5, PRO805-4, BRS189, 113-1, H-51 and H-71) was composed of clones with a higher PM, and the values of this group were between 112.43 and 130.64 g. It is important to highlight that they obtained higher values than the control. Group 2 (the CCP-76 control (98.39 g)) next to clones 133-1, BRS226, MG-57, MG-41, MG-17, SLC12-20, 155-2 and MG76). They presented variations for averages between 87.77 and 101.33 g, inferior to group 1 and superior to the other groups. Group 3 (114-2, MG-65, ENDII6-9, 143-7, 146-7, 114-4, 116-2, MG-1 and 149-1) had PM averages between 55.01 and 82.16 g. The CCP 76 clone was studied by other authors. Comparing the two studies, it is possible to see that, in the first case, the clone obtained values of 92.7 g, lower than the one found in this study (Pereira *et al*., 2005).

On the other hand, it was higher than the one obtained in this study, with an average of 172.5 g (Lopes *et al*., 2011), which can be explained by the high rainfall

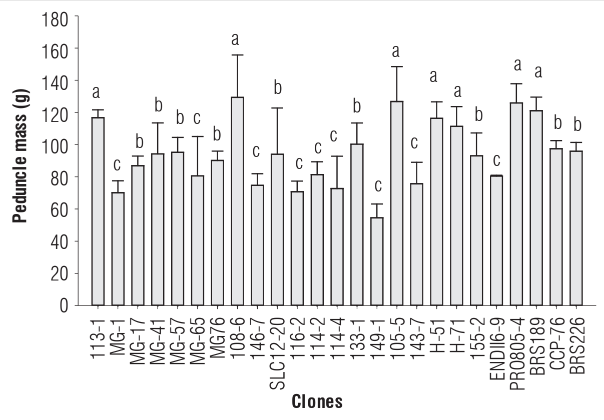

Figure 2. **Peduncle mass of cashews of dwarf cashew clones from Cruz, CE. 2017. Averages followed by the same letter do not differ from each other according to the Scott and Knott test ($P \leq 0.05$) ($n=3$) ± standard error. Values between 101.03 and 141.80 g are considered ideal.**

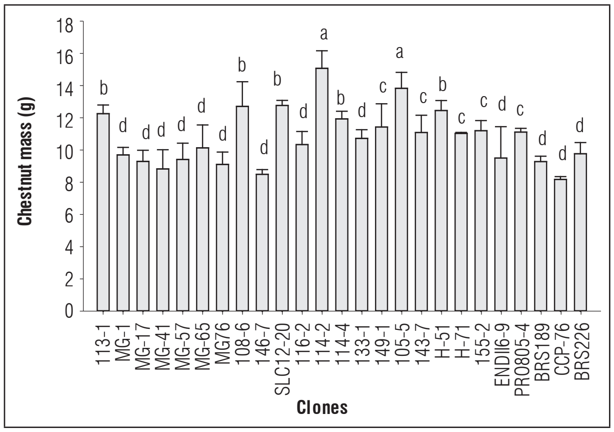

Figure 3. **Chestnut mass of cashews of dwarf cashew clones from Cruz, CE. 2017. Averages followed by the same letter do not differ from each other according to the Scott and Knott test ($P \leq 0.05$) ($n=3$) ± standard error. Values between 9.02 and 11.47 g are considered ideal.**

of 1,925.2 mm during the course of the experiment, about 10 times more than the rainy season during the present study. It is worth mentioning that the area of the experiment was also different, so possibly other factors may also have been involved in these differences.

Abreu (2007), when analyzing clones CCP 76 and BRS 226, obtained PM values of 145.80 and 144.52 g, respectively, values higher than those found in this study because of the difference in the amount of water received by the plants in the rainy season since it was also done under dry conditions. In order to provide greater gains in the processing of the peduncle, cashews with higher MP values are much more attractive since processing industries look for a greater yield of pulp.

The differentiation of the peduncles between clones is important so that there is separation and standardization during the process of directing for export and supplying the various markets. Standardization makes the economic exploitation of this product more attractive because of the ease in choosing the type of peduncle that meets consumer need.

According to the present study, there was a variation of 8.18 to 15.08 g in the mass of the cashew nuts (CM) of the different dwarf cashew clones, with an amplitude of 6.9 g and a general average of 10.79 g (Fig. 3). Group 1 (114-2 and 105-5) was composed of the clones that reached the highest CM values, which were 13.84 to 15.08 g, higher than the control. Group 2 was composed of clones (SLC12-20, 108-6, H-51, 113-1, and 114-4), which presented values between 11.94 and 12.77 g and also stood out when compared to the value of the control. Group 3 (149-1, 155-2, PRO805-4, 143-7 and H-71) had clones with CM values that were between 11.04 and 11.43 g, standing out from the control. The control CCP 76 was used as the control group (133-1, 116-2, MG-65, BRS226, MG-1, ENDII6-9, MG-57, MG17, BRS189, MG76, MG 41 and 146-7), which presented MC values between 8.18 and 10.73 g; the control had the lowest. Among the groups mentioned, 1, 2 and 3 can be highlighted because of large-sized almonds, which are appreciated by the chestnut processing industries because of the excellent mass, which may provide type SLW almonds (Special Large Whole) and corresponds to 180 almonds/453.59 g (the minimum yield of almond for commercial production is 20% of the mass of the chestnut).

In a study evaluating the physical attributes of peduncles from different clones at different stages of maturation, it was observed that CCP 76, at stage 7 (commercial maturation), obtained results superior to the other clones with 11.21 g, higher than that found in this study (Lopes *et al*., 2011). Analyzing a comparative ecology of two clones grafted under irrigation conditions found for CCP 76 nuts at the commercial maturation stage produced values varying from 6.9

to 8.4 g, similar values to that found for the same clone in the present study. These differences in MC were due to the difference in the amount of cashews that were produced from the same inflorescence (Lopes *et al*., 2011).

However, in the present study, cultural practices were not applied, such as pruning and thinning, in order to control the size and mass of the fruits, with development of more fruits on the same branch. These fruits tended to be smaller and, consequently, had a lower mass, which may explain these differences.

Among the studied variables, the size of the peduncle was directly related to three measurements: basal diameter (near the chestnut), apical diameter and length. It was observed that there was a variation of 42.21 to 58.38 mm for basal diameter (BD) for the different dwarf cashew clones, with an amplitude of 16.17 mm and an overall average of 50.07 mm (Fig. 4).

Group 1 (PRO805-4, 108-6, H-71, 105-5 and 133-1) had the best BD values, in a range between 54.47 and 58.38 mm, superior to the control. Group 2 (clones BRS226, 155-2 and MG-41 together with CCP 76 (54.10 mm) + ENDII6-9) had BD values between 51.72 and 53.03 mm, lower than group 1 and higher than groups 3 and 4. Group 3 (H-51, 143-7, 113-1, SLC12-20, MG-57, 114-2, MG-17 and MG76) was composed of clones expressing BD values between 47.91 and 50.17 mm, lower than the control and superior to group 4. Group 4 (116-2, BRS 189, 149-1, 114-4, MG-1, 146-7 and MG-65) had clones with lower BD values, ranging from 42.21 to 45.90 mm.

Studying physical characteristics of different cashew clones, Gomes e*t al*. (2006) found the highest values for clones CCP 76, BRS 189, Embrapa 50 and Embrapa 51, 58.1; 57.6; 54.7 and 54.7 mm, respectively. Values higher than those found in this study.

Abreu (2007), studying the quality and total antioxidant activity of peduncles of commercial clones of dwarf cashews, obtained a general average of 57.68 mm, whose variation was from 50.63 to 61.97 mm. Therefore, the values were higher than those found in this study. On the other hand, Lopes e*t al*. (2011) found even higher values for CCP 76, 65.87 mm.

For the variable apical diameter (AD), a variation of 30.97 to 49.46 mm was found, with an amplitude of 18.49 mm and an overall mean of 41.38 mm (Fig. 5). The clones (BRS189, H-71, 155-2, 114-2, PRO805-4, 105-5, 133-1, BRS226, ENDII6-9, + clone 143-7 and SLC12-20) presented the highest AD values, between 42.15 and 49.46, standing out from group 2 (Tab. 1).

Group 2 (MG-41, H-51, 114-4, 149-1, 108-6, MG-57, 116-2, MG76, MG-1, 146-7, MG-65, MG-17 and 113 -1) presented AD values between 30.97 mm and 40.99 mm, which presented lower values than group 1.

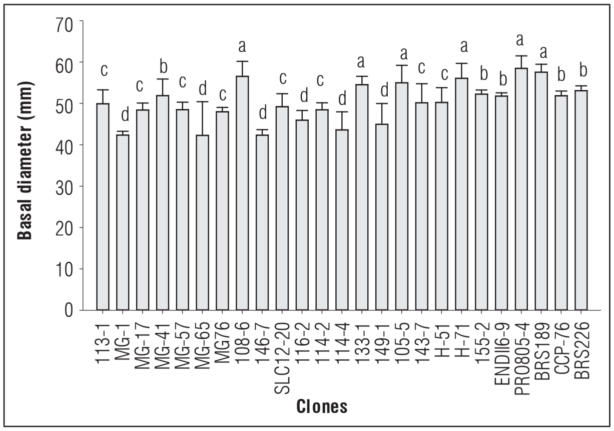

Figure 4. Basal diameter of peduncles of dwarf cashew clones from Cruz, CE. 2017. Averages followed by the same letter do not differ from each other according to the Scott and Knott test ($P \leq 0.05$) ($n = 3$) ± standard error. Values between 53.73 and 61.82 g were considered ideal.

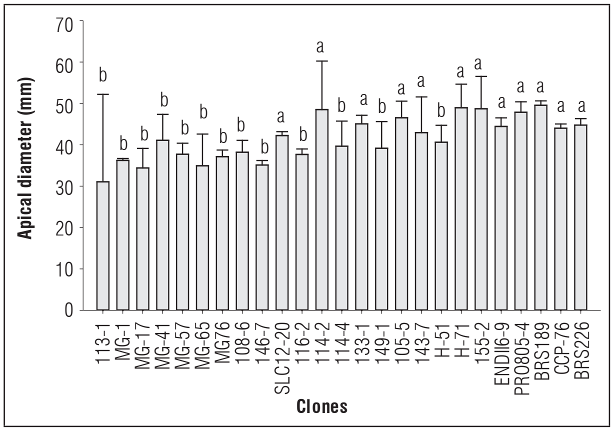

Figure 5. Apical diameter of peduncles of dwarf cashew clones from Cruz, CE. 2017. Averages followed by the same letter do not differ from each other according to the Scott and Knott test ($P \leq 0.05$) ($n = 3$) ± standard error. Values between 40.81 and 50.27 mm are considered ideal.

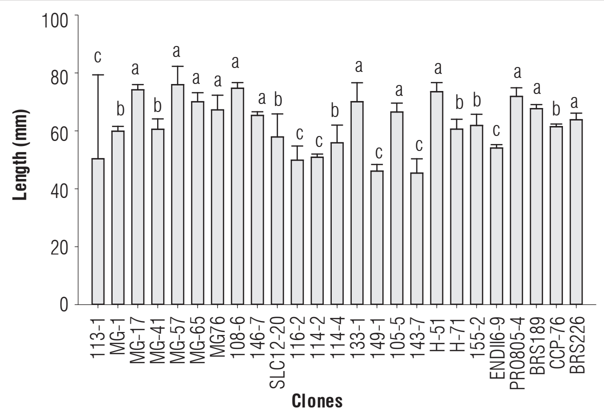

Figure 6. Length of peduncles of dwarf cashew clones from Cruz, CE. 2017. Averages followed by the same letter do not differ from each other according to the Scott and Knott test ($P\leq0.05$) ($n=3$)±standard error. Values between 67.24 and 76.44 mm are considered ideal.

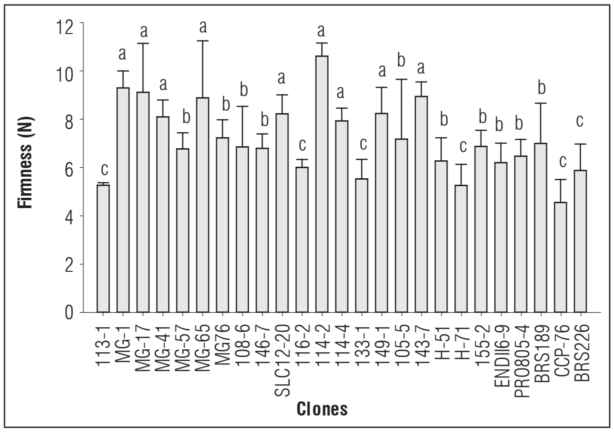

Figure 7. Average firmness of peduncles of dwarf cashew clones from Cruz, CE. 2017. Averages followed by the same letter do not differ from each other according to the Scott and Knott test ($P\leq0.05$) ($n=3$)±standard error. Values between 5.83 and 16.19 N are considered ideal.

Working with CCP clone 76, Pereira *et al*. (2005), in the Northern Region of Minas Gerais, obtained an AD value higher than 44.40 mm. In the present study, the AD obtained for the same clone was 43.93 mm. It was observed that, for the commercial stage, there was an average variation of 33.99 to 48.87 mm (Lopes *et al*., 2011), lower than the variation found in this study. When analyzing the peduncle length, a variation of 45.54 to 76.11 mm (Fig. 6) was observed, with an amplitude of 30.57 mm and an overall mean of 62.37 mm.

Group 1 was composed of clones (MG57, 108-6, MG-17, H-51, PRO805-4, 133-1, MG-65, BRS189, MG76, 105-5 and 146-7) and presented the highest medium lengths, with values varying from 63.96 to 76.11 mm, standing out from the other clones, including the CCP 76 control. Group 2 (155-2 + the CCP-76 control (61.56) next to clones H-71, MG-41, MG-1, SLC12-20 and 114-4) showed values ranging from 55.97 to 61.99 mm, inferior to group 1. Group 3 (ENDII6-9, 114-2, 113-1, 116-2, 149-1 and 143-7) presented averages ranging from 45.54 to 54.19 mm, less than the CCP 76 control.

In a study carried out by Lopes *et al*. (2011) on the physical attributes of peduncles of four different clones at different stages of maturation, it was generally observed that there was an increase in length during development for all clones. The mature CCP 76 had the highest mean, with 67.24 mm, which was higher than the length found for this same clone in the present study (61.56 mm).

As for firmness of the peduncles of the different clones of dwarf cashew, there was a variation of 4.57 to 10.67 N, with an amplitude of 6.1 N and a general average of 7.22 N (Fig. 7). Group 1 (114-2, 114-4, MG-1, MG-17, 143-7, MG-65, 149-1, SLC12-20 and MG-41) was composed of clones that achieved the best firmness averages, with values between 7.97 and 10.67 N, superior to the other groups and presenting greater resistance to mechanical damage. Group 2 (MG76, 105-5, BRS189, 155-2, 108-6, 146-7, MG-57, PRO805-4 H-516ENDII6-9) obtained firmness values ranging from 6.23 to 7.26 N, higher than the control. Group 3 (116-2, BRS226, 133-1, 113-1, H-71 near the control (CCP-76)) showed the lowest firmness values, varying between 4.57 and 6.03 N.

Pereira *et al*. (2005) found, for clone CCP 76, a firmness value in the central part of the peduncle of 16.95 N, which, according to Almeida *et al*. (2011), was higher than any other study carried out with this clone in the commercial maturity stage. On the other hand, Lopes *et al*. (2011), studying the physical characterization of peduncles of dwarf cashew clones in different stages of maturation, observed a value of 7.78 N for CCP 76 in stage 7, superior to the one found in this study for the same clone.

Moura *et al*. (2001) assessed the physical characteristics of cashew peduncles for natural commercialization, which presented a value of 5.83 N for CCP76, superior to that found in the present study (4.57). Thus, studies have reported that peduncles with a greater firmness value have a longer post-harvest life, which was also sought in this study since it is one of the main objectives of researchers in this line of research.

CONCLUSIONS

For the variables total mass, peduncle mass, basal diameter and length, the best evaluated clone was 108-6 because it presented the highest average; however, when analyzing the nut mass and firmness, it was observed that they were better represented by clone 114-2. Clone 116-2 stood out for apical diameter. Overall, all clones showed good characteristics; however, clone 108-6 was better when evaluating total mass, peduncle mass, basal diameter and length, which stood out from the control (CCP 76).

In general, all clones presented good characteristics; however, clone 108-6 is preferred by most consumers since it has red staining, as does the control (CCP 76). Therefore, all clones presented desirable characteristics for natural consumption and/or processing.

Conflict of interest: this manuscript was prepared and reviewed with the participation of all authors, who declare that there exists no conflict of interest that that puts at risk the validity of the presented results.

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Molecular characterization using SSR markers in 50 shrub pea genotypes (*Pisum sativum* L.) from the GRICAND Collection, Colombia

Caracterización molecular con marcadores SSR para 50 genotipos de arveja arbustiva (*Pisum sativum* L.) de la Colección GRICAND, Colombia

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Shrubby pea crop (*Pisum sativum* L.) with Afila gene in Nariño (Colombia).

Photo: D. Herrera, GRICAND

ABSTRACT

The pea (*Pisum sativum* L.) is one of the more important legume crops produced globally. We studied the structure and genetic diversity in a collection of 50 pea accessions with 16 simple sequence repeat (SSR) markers, whose average polymorphic information content (PIC) was 0.62. The SSR markers amplified a total of 28 alleles with an average of 4 alleles per locus, with locus AB71 and D21 amplifying the largest number of alleles (6). The observed heterozygosity (Ho) was 0.09±0.08 and the expected heterozygosity (He) was 0.42, indicating an elevated level of inbreeding (F_{is} = 0.60). The genetic relationships were inferred with a similarity index (DICE) and a bayesian analysis (STRUCTURE), detecting 2 clusters for the genotypes, with a high similarity of the morphological characteristics of each genotype. The results of this study will be useful for the creation of future breeding programs.

Additional key words: genetic diversity; genetic structure; pre-breeding; SSR markers; grain legumes; plant habit.

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RESUMEN

La arveja (*Pisum sativum* L.) es uno de los cultivos de leguminosas más importantes producido a nivel mundial. Estudiamos la estructura y diversidad genética en una colección de 50 accesiones de arveja con 16 marcadores de Secuencias simples repetidas (SSR), cuyo promedio del contenido de información polimórfica (PIC) fue de 0,62. Los marcadores SSR amplificaron un total de 28 alelos con un promedio de 4 alelos por locus, siendo el locus AB71 y D21 los que amplificaron el mayor número de alelos (6). La heterocigosidad observada (Ho) fue de 0,09 y la esperada (He) de 0,42, indicando un alto nivel de endogamia (F_{is} = 0,60). Se infirieron las relaciones genéticas por medio de un análisis de similitud (DICE) y un análisis bayesiano (STRUCTURE) detectando 2 agrupaciones para los genotipos de arveja analizados, con una alta similitud con las características agromorfológicas de cada genotipo. Los resultados del presente estudio serán útiles para la creación de futuros programas de fitomejoramiento en arveja.

Palabras clave adicionales: diversidad genética; estructura genética; marcadores SSR; pre-mejoramiento; leguminosas de grano; hábito de crecimiento.

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INTRODUCTION

Pisum sativum, known as pea or sweet pea, is a self-pollinated legume, with a genome made up of seven chromosomes (2*n* = 14) (Smýkal *et al*., 2008). It is one of the staple foods of the basic market basket of the Colombian family because of its high content of protein and micronutrients, such as zinc and iron (Amarakoon *et al*., 2012; Peralta *et al*., 2013).

It is possible to find shrub-type peas, semi shrub-type peas and snowy-peas within *P. sativum*. Shrub peas are low-bearing, and their growth ends in flower, semi-shrubs are medium-sized peas that end in leaflets and snowy peas are high, require a tutor and end in leaflets. Two types of planting are used: with tutor and crawling planting without tutor or broadcast planting. Planting with a tutor generates high yields and a higher fresh pod quality; however, one of the limiting factors in this production system is the cost of the tutoring because the structure of the plants does not have enough tendrils to be left on the tutors without the plants falling on the ground; a high usage of polypropylene threads is necessary for their mooring (Checa-Coral and Rodriguez, 2015).

In Colombia, the pea is the second most important legume, after the bean, with a cultivated area of 35,211 ha and a green pod yield of 4.1 t ha^{-1}. Its production is concentrated in six departments: Nariño, Cundinamarca, Boyaca, Tolima, Huila, and Nariño, the latter being the main producer with 15,816 ha (Checa-Coral and Rodriguez, 2015). This department has suitable areas for producing shrub peas, a suitable alternative for diversification in the cereal zone of Nariño because of its capacity for adaptation, high yield potential and possibility of harvesting green pods or dry-grained sheaths, depending on market conditions. However, in Nariño, the varieties of peas include snowy peas, which require tutoring systems to help the plant stay off the ground, allowing an increase in potential yield. According to Checa-Coral *et al*. (2017), employing a tutoring system represents 52% of total crop costs, which has led farmers with limited finances to not use tutored systems for the cultivation of peas, significantly increasing the incidence of foliar diseases such as mildew (*Erysiphe polygoni*) and Ascochyta (*Ascochyta pisi*), reducing yield and deteriorating quality of the final product. Because of technification difficulties in snowy pea tutoring systems, studies on shrub peas are vital, within which genotypes with the *Afila* gene (a*F*) are found, which, in the homozygote condition, transforms leaves into tendrils (Mike, 2008; Smýkal *et al*., 2013), reducing tipping and preventing the sheaths from coming into contact with the ground, avoiding pod rotting under high relative humidity conditions (Checa-Coral *et al*., 2017). This type of pea with a shrub growth habit tends to obtain low-bearing and early genotypes, with sheath qualities for fresh consumption and for the agro-industry (Checa-Coral *et al*., 2017). This material is adapted to the cereal zone and, with an adequate harvest time, could be an alternative to partially cover the national demand for dry peas and for producers to obtain good profitability because of the low cost of implementation of this crop.

Given the importance of shrub peas as a crop in the Department of Nariño-Colombia, it is necessary to initiate genetic improvement programs for genotypes with this growth habit. It is advisable to carry out molecular characterization of collections that allow breeders to identify genes of interest to associate them with agronomic characteristics and thus generate results that express genetic diversity. Knowledge on genetic diversity fosters the efficient use of germplasm, identifying and eliminating duplicates, and helps the establishment of nucleus collections (Ghafoor *et al*., 2005). Genetic diversity is the main input for the genetic improvement of a cultivated species. Local and wild varieties are kept in germplasm banks as genetic resources; however, their use in genetic performance improvement has been limited (Ali *et al*., 2007).

One of the most used molecular methods for characterization and genetic diversity studies are molecular markers. Microsatellites have become the most used markers because of their high polymorphism and easy handling in the laboratory; these markers have the ability to differentiate homozygous and heterozygote individuals. These markers consist of DNA fragments with a few nucleotides in length, between 2 and 6 base pairs, which are randomly repeated several times (Vieira *et al*., 2016). In 2005, a research group established sets of specific SSR microsatellites for *P. sativum* (Loridon *et al*., 2005), which have been used in several studies to observe the genetic diversity of the pea; for example, 21 SSR markers were used in a collection of more than 1,000 introductions. Zong *et al*. (2009) found that the genetic diversity of the Chinese collection has several differences that were detected in the global gene pool, such as the presence of rare alleles. These markers have been used in various studies over the years thanks to their high polymorphic information content, codominance and reproducibility (Smýkal *et al*., 2008; Cieslarová *et al*., 2011; Rana *et al*., 2017). SSRs have also been used for studies of disease resistance; for instance, it has been found that resistance to *Aphanomyces euteiches* (cause of rotten root) is moderately heritable (Hamon *et al*., 2011).

Having specific pea genotypes of determined growth or shrubs adapted to the region, for which an adequate cultivation technology is established, improves the income of farming families in municipalities that used to be wheat producers in Nariño and allows farmers to return to agricultural activities by generating new work opportunities (Checa-Coral *et al*., 2017).

The aim of this work was to carry out the molecular characterization of 50 shrub pea genotypes from the collection of the GRICAND Research Group of the Universidad de Nariño, using SSR microsatellite molecular markers to serve as a starting point for the establishment of breeding programs for the species.

MATERIALS AND METHODS

Plant material

The study population was formed by 50 genotypes of *P. sativum* with a shrub-like growth habit, which are part of the *Pisum sativum* L. collection of the GRICAN Research Group on Andean Crops of the Universidad de Nariño. Sowing and germination of the plant material was carried out during the last three weeks of September, 2018 in the greenhouse of the Universidad Nacional de Colombia, Palmira Campus. Three plants of each genetic material were sown, and the sowing was carried out in germinators used for seedlings. When the plants were approximately 10 cm high, young leaflets were extracted, collecting approximately 5 g of foliar tissue which was stored in ultra-freezers at 80°C. Simultaneously and in order to renew the seed in the *Servicio Nacional de Aprendizaje* of Colombia - SENA, 50 pea genotypes were multiplied in the field, using furrows 2 m in length with a distance between furrows of 60 cm, depositing one seed per site. The lot was used to register the characteristics of leaf type and grain form based on UPOV descriptors (UPOV, 2009) (Tab. 1).

Table 1. Morphological characteristics of leaf type and grain form for 50 genotypes of *Pisum sativum* L.

Genotype	Leaf type	Grain form
ARB001	3	2
ARB002	3	2
ARB003	3	2
ARB004	3	4
ARB005	3	2
ARB006	3	4
ARB007	3	4
ARB008	3	4
ARB009	3	4
ARB010	3	4
ARB011	3	4
ARB012	3	4
ARB013	3	3
ARB014	3	4
ARB015	3	4
ARB016	3	3
ARB017	3	4
ARB018	3	4
ARB019	3	4
ARB020	3	4
ARB021	3	4
ARB022	3	4
ARB023	3	4
ARB024	3	4
ARB025	1	1
ARB026	1	1
ARB027	1	1
ARB028	1	1
ARB029	1	4
ARB030	1	1
ARB031	1	1
ARB032	1	4
ARB033	1	4
ARB034	1	4
ARB035	1	4
ARB036	1	4
ARB037	1	4
ARB038	1	4
ARB039	1	1
ARB040	1	2
ARB041	1	4
ARB042	1	1
ARB043	1	2
ARB044	1	4
ARB045	1	2
ARB046	1	4
ARB047	1	1
ARB048	1	4
ARB049	1	2
ARB050	1	2

Leaf type: 1 = normal or terminated in tendril, 3 = without leaves, tendril only. Grain form: 1 = round, 2 = angular round, 3 = elongated oval, 4 = several rectangular shapes.

DNA extraction

The SPIN Plant Mini Kit® (STRATEC Biomedical AG, Birkenfeld, Germany) with its respective procedure was used for the DNA extraction. The quantification of the genetic material was carried out with a Colibri Spectrophotometer® (Titertek-Berthold, Pforzheim, Germany) to obtain more precise measurements. The DNA dilutions were carried out for the normalization and homogenization of DNA concentration, with values of 30 ng μL^{-1}, for the subsequent molecular evaluations with PCR reactions.

Genotyping

The genotyping used 16 molecular microsatellite fluorescent markers (SSR), described by Loridon *et al*. (2005) (Tab. 2). These markers were subjected to a process of standardization to determine what was the best hybridization temperature of each one. Subsequently, the amplification of the samples was done with the PCR technique.

Once products of amplification were obtained, the markers were mixed in plates with 96 wells where

PCR products were combined; each well contained a maximum of three markers, which could not be the same color (PET, VIC, FAM, NED) and had to have distant molecular weights. These plates were sent to the laboratories of Macrogen (Seoul) for the analysis of the fragments of the SSR microsatellites.

SSR microsatellites statistical analysis

The size of the alleles in base pairs, was estimated using standard lane size ROX-500 (Applied Biosystems, Foster City, CA) with GENEIOUS Software® v 11.1 (http://www.geneious.com) (Kearse *et al*., 2012). The number of polymorphic alleles observed, expected heterozygosity, and genetic diversity of Nei (1973), were observed using Arlequin Program v 3.5.2.2 (Excoffier and Lischer, 2010). Likewise, the Polymorphic Information Content was calculated (Botstein *et al*., 1980).

For a distance or similarity analysis, two methodologies may be used: a Principal Coordinates Analysis (PCoA) or a dendrogram (tree diagrams). A PCoA generates a scattering graph that can have two or three dimensions, where the distances between the samples shown in the graph reflect the genetic distances between them. On the other hand, a dendrogram performs a grouping of the samples that are genetically similar (Nisar *et al*., 2017). The PCoA was carried out using GenAlex v 6.5 program (Peakall and Smouse, 2012). On the other hand, the dendrogram was performed considering the created matrix and the similarity method of UPGMA/DICE using the Simqual programs of the NTSYS-PC package (Rohlf, 2006).

To assess the genetic structure of the genotypes, Structure v 2.3.4 was used (Falush *et al*., 2007). The criteria recommended by Porras-Hurtado *et al*. (2013) were used: the number of subpopulations (K-value) was 1-10, with 10 runs, for a *Burn-in* period of 100,000 steps and 200,000 interactions of the Monte Carlo Markov Chain (MCMC). To determine the best K, the method of Evanno *et al*. (2005) was considered. The Structure Selector Program was used (http://lmme.qdio.ac.cn/StructureSelector/). These steps were repeated the necessary times until the structural behavior of the samples was clearly defined. In order to determine a significant genetic differentiation between the possible groups created in Structure, an analysis of molecular variance was carried out (AMOVA), along with determination of using Arlequin v 3.5.2.2 (Excoffier and Lischer, 2010).

Table 2. SSR markers employed in research with their sequence forward and backward, hybridization temperature and molecular weight.

Marker	Forward sequence 5′-3´	Reverse sequence 5´-3´	Tm^a	Molecular size
AA5	TGCCAATCCTGAGGTATTAACACC	CATTTTTGCAGTTGCAATTTCGT	61	235
AA355	AGAAAAATTCTAGCATGATACTG	GGAAATATAACCTCAATAACACA	51	180
AB53	CGTCGTTGTTGCCGGTAG	AAACACGTCATCTCGACCTGC	51	120
AD147	AGCCCAAGTTTCTTCTGAATCC	AAATTCGCAGAGCGTTTGTTAC	61	330
D21	TATTCTCCTCCAAAATTTCCTT	GTCAAAATTAGCCAAATTCCTC	51	200
AA122	GGGTCTGCATAAGTAGAAGCCA	AAGGTGTTTCCCCTAGACATCA	61	190
AA135	CCGTTACACATCATTAAGATG	TCCATATCCAGATTAGTCAGA	51	291
AD146	TGCTCAAGTCAATATATGAAGA	CAAGCAAATAGTTGTTTTGTTA	51	390
AD148	GAAACATCATTGTGTCTTCTTG	TTCCATCACTTGATTGATAAAC	54	190
AA238	TATCATCAAGGTCCAATTTAGT	AGCTAAATCGTACCTAATCTGT	51	190
D23	ATGGTTGTCCCAGGATAGATAA	GAAAACATTGGAGAGTGGAGTA	51	170
AB71	CCAACCATTTGTGAGTTCCCTT	TTCGTCGAACCACGAGAATAGA	61	145
AD60	CTGAAGCACTTTTGACAACTAC	ATCATATAGCGACGAATACACC	51	216
AC58	TCCGCAATTTGGTAACACTG	CGTCCATTTCTTTTATGCTGAG	61	205
AD175	CTTGTGCAGAAGCATTTGATTA	AGAGACAATGGATGCTCATAGT	55	405

Tm^a: hybridization temperature.

RESULTS AND DISCUSSION

Analysis of SSR microsatellites

Seven out of the 16 markers showed polymorphisms, and 27 alleles were found within the 50 genotypes (Tab. 3). Loci with a greater allelic richness included AB71 and AD21, with 6 alleles each. The average number of alleles was 4, with a range of 2 to 6 alleles per locus (Tab. 3); this result is greater than that reported by Ahmad *et al*. (2012) but similar in other studies on the *Pisum* genus (Cupic *et al*., 2009; Ponnaiah *et al*., 2011) and less than that reported by Hagenblad *et al*. (2014), where a range of 5 to 12 alleles was found, as was recently reported by Rana *et al*. (2017). The size of the alleles had a range of 50-493 base pairs. The polymorphic information content (PIC) had an average of 0.62; according to Botstein *et al*. (1980), this value indicated that the markers were highly informative since they were above 0.50 and indicated that there could be an allelic variation within the samples. Similar values were reported in other pea research (Nasiri *et al*., 2009; Ahmad *et al*., 2012). Studies that have evaluated a larger number of Pisum accessions have observed a larger allele, indicating that some markers may become more polymorphic depending on the number of accessions that have been implemented (Ahmad *et al*., 2012). The inbreeding coefficient (F_{is}) was 0.60 which indicated that there was a level of inbreeding, which explained that the observed heterozygosity (Ho) was less than the expected heterozygosity (He) (0.09 and 0.42, respectively). This indicated that there was a high homozygosity normal behavior in the *P. sativum* individuals because of its pollinated nature (self-fertilization). The low observed heterozygosity has been reported in other research on *P. sativum*: Handerson *et al*. (2014) obtained a Ho of 0.031, Smýkal *et al*. (2008) obtained a Ho of 0.069 with a collection from the Czech Republic and Teshome *et al*. (2015) used a collection of *P. sativum* from Ethiopia, finding a Ho of 0.05, which confirms that *P. sativum* is a species that tends towards homozygosis (Teshome *et al*., 2015). On the other hand, according to Vallejo and Estrada (2002), in autogamous plants (such as *Pisum sativum*), inbreeding is expected to give a value equal or very close to one (1). Likewise, loci AB71 and AA135 had Ho values of 0.20 and 0.16, respectively. The inbreeding values and Ho values could be explained by the fact that the seeds were obtained through open field sowing, which increased the probability that pollinating insects visited the flowers. Some research has suggested that the main pollinators of *Pisum* belong to the order of diptera, hymenopteros and lepidoptera (Naeem *et al*., 2018). In addition, in some cases, *Pisum* pistil may remain in a suitable state after anthesis (Kosterin and Bogdanova, 2014). These factors could suggest that the inbreeding and Ho resulted from the existence of some cases of allogamy or cross-pollination in the collection.

Genetic diversity

In the dendrogram (Fig. 1A), two main groups were observed with a similarity coefficient (DICE) of approximately 0.74 (Group I and Group II). With an approximate coefficient of similarity of 0.93, most of the sub-groups (15 subgroupings) were formed, which were highly related to the groups formed with the PcoA (Fig. 1B). In Group 1 of the dendrogram, sub-groups of genotypes with a high coefficient

Table 3. SSR markers with annealing temperature (Ta), number of alleles (Na), effective number of alleles (ENA), allele size range, polymorphic information content (PIC), observed heterozygosity (Ho) and expected heterozygosity (He), inbreeding coefficient (F_{is}).

Marker	TA (ºC)	Na	ENA	Allele size BP range	PIC	Ho	He	F_{is}
AA122	62	5.0	2.12	155-195	0.70	0.05	0.53	0.91
AA5	59	4.0	3.07	229-241	0.69	0.07	0.67	0.89
D21	51	6.0	3.46	185-278	0.74	0.16	0.71	0.77
AB71	59	6.0	4.65	304-334	0.81	0.20	0.79	0.75
AA135	54	2.0	1.17	114-119	0.40	0.16	0.15	-0.09
AD60	52	2.0	1.08	50-58	0.50	0.00	0.08	1.00
AA355	56	2.0	1.02	483-493	0.50	0.02	0.02	-0.01
Average		4.0	2.00	-	0.62	0.09	0.42	0.60

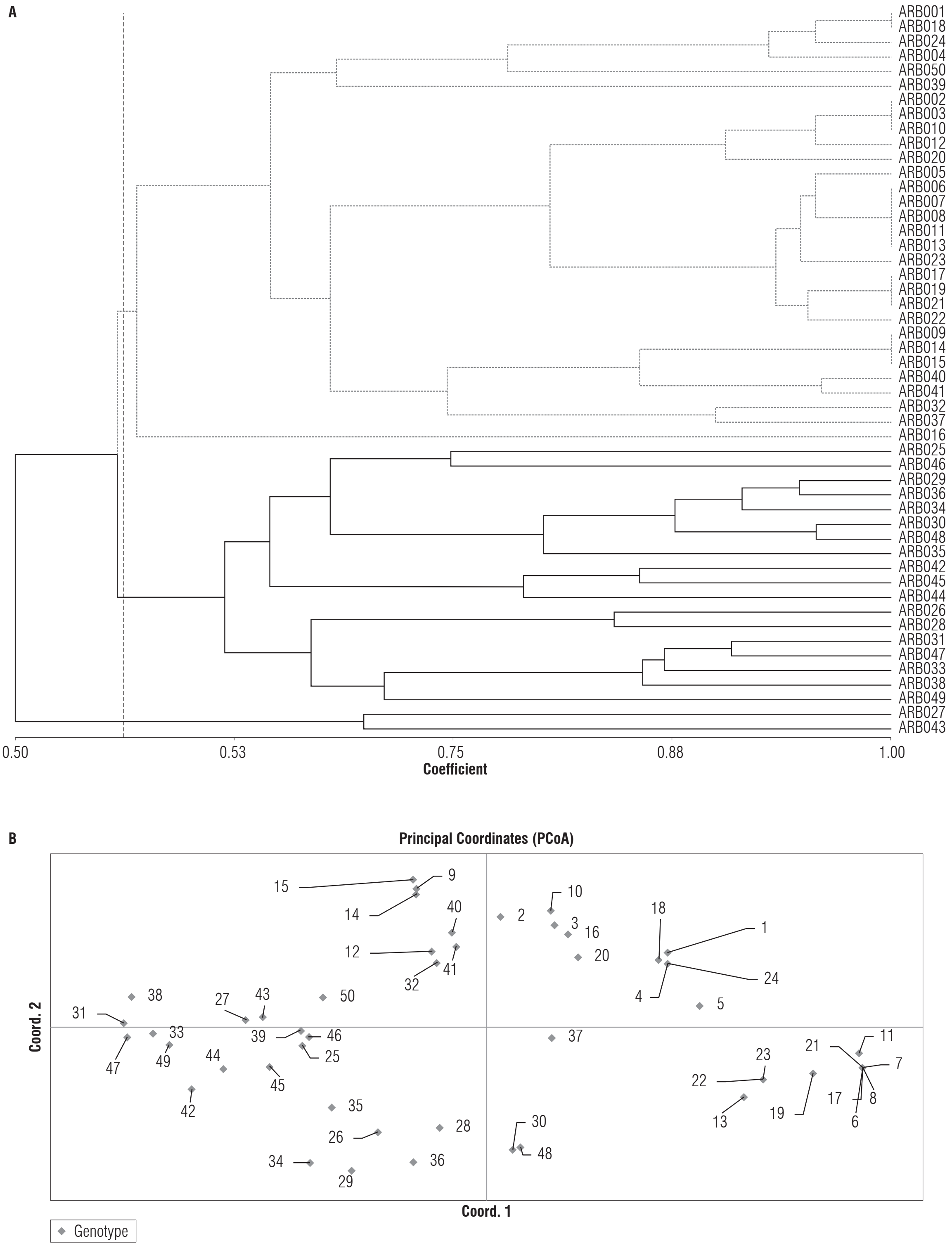

Figure 1. Similarity analysis for 50 genotypes of *P. sativum* with SSR markers: A) Dendrogram using the DICE/UPGMA method. B) Analysis of main coordinates (PCoA).

of similarity (<0.98) are observed, such as ARB 004 with 024; ARB 006 with 007, 008, 017 and 021; and ARB 022 with 023, among others. These high similarities could have been the product of the genotypes used in this study, with a very small population, a shrub growth and a particular area: Department of Nariño, along with sharing the same morphological characteristics.

The graph of main coordinates analysis (Fig. 1B) was carried out with the first two coordinates, which explained 51.20% of the total variation within the samples. Coordinate one explained 29.32%, and coordinate two explained 21.88%. By observing the groupings formed in the dendrogram (Fig. 1A) and in the PCoA analysis (Fig. 1B), it was observed that they were highly associated.

The analysis of the structure of the 50 *P. sativum* accessions showed a K = 2 with the higher hierarchical level of the genetic structure, which was supported by the Bayesian analysis (Fig. 2A). These two groupings (Group I and Group II) formed by the STRUCTURE analysis were highly associated with the groups found in the dendrogram. The accessions separated in two groups by STRUCTURE, in the same way as groups I and II of the dendrogram. On the other hand, the AMOVA analysis showed a FST value of 21.92% ($P<0.00001$), indicating that there was significant genetic differentiation between the two large groupings. Because the structure analysis was performed for each of the two groups separately, each group threw a hierarchy level K = 2, which indicated that each group has two subgroups (Fig. 2B and 2C).

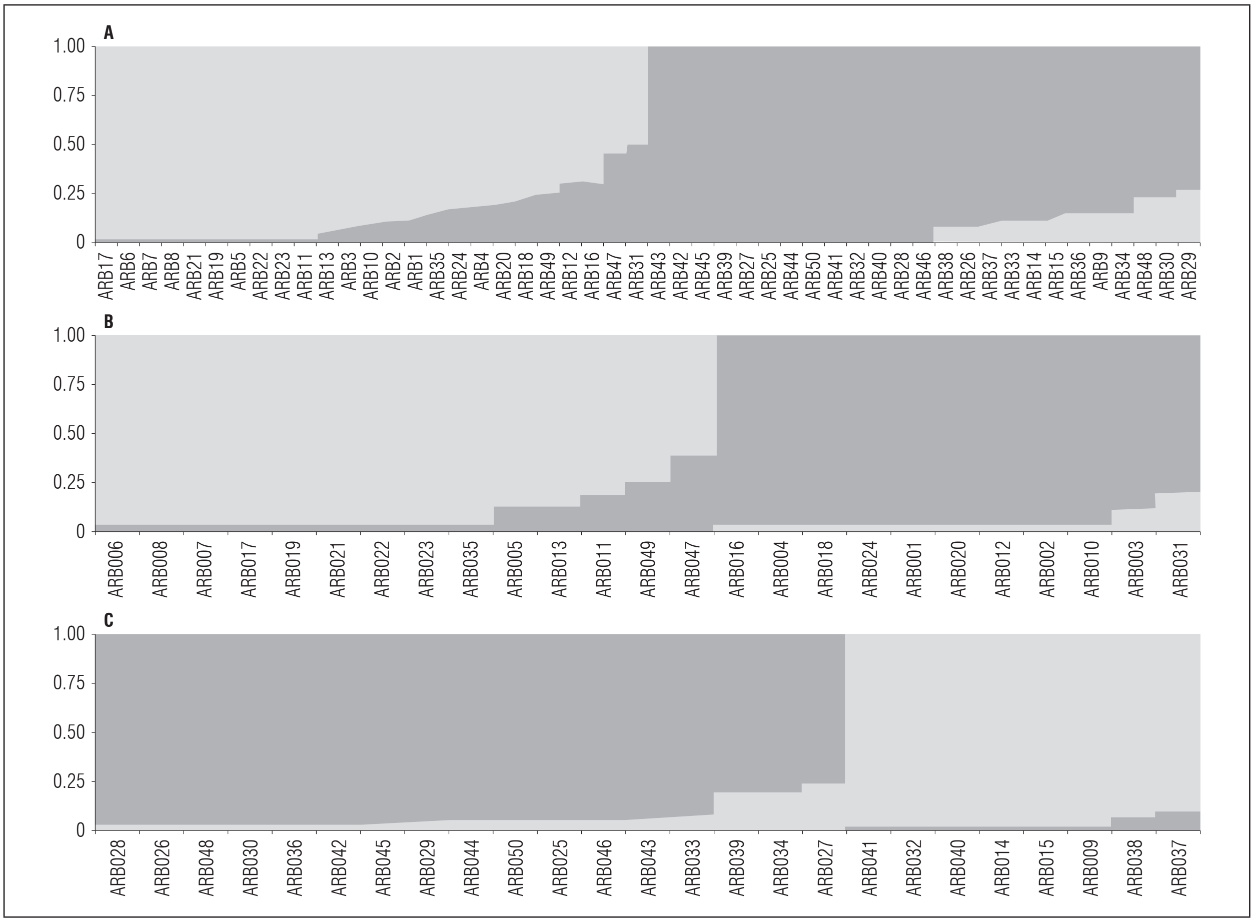

Figure 2. A) Bayesian analysis of the genetic structure (K = 2) in the 50 shrub pea genotypes based on seven microsatellite loci. B) Bayesian analysis of the genetic structure (K = 2) in group I (Group I) of the shrub pea genotypes. C) Bayesian analysis of the genetic structure (K = 2) in group II (Group II) of the shrub pea genotypes. Each bar equals an individual genotype.

These K values have been demonstrated by Nasiri *et al*. (2009) and Ahmad *et al*. (2012), where a K = 2 were obtained. Other authors have reported some not very distant groups with K = 3 (Jing *et al*., 2012; Rana *et al*., 2017). The high similarity between the groups found in both the dendrogram, PCoA and STRUCTURE may have been due to the fact that the genotypes in the same group may share similar morphological features. According to the morphological traits (Tab. 1), it was observed that most genotypes belonging to group I had a sharp blade form (without leaves), with tendrils only and a rectangular grain form in several forms. It was observed that the genotypes of grouping II had a normal leaf form with lateral leaflets that finished in tendrils and, like Group I, a rectangular grain form in various forms. Morphological level differences were not found for the subgroups.

Finally, this is the first study that employed SSR microsatellite markers for the study of genetic diversity in the *Pisum sativum* Collection of the Department of Nariño (Colombia). Understanding the collection of *P. sativum* with the *Afila* gene is of great importance for the horticultural sector since it prevents the deterioration of product quality by reducing foliar diseases.

Although the use of SSR can be an effective tool for characterizing germplasm, for future research, it is recommended to have information at the geographic level on the origin of the genotypes, which will allow a much more complete system for the analysis of diversity and the possible development of new growths.

CONCLUSION

This study demonstrated the usefulness of molecular markers for molecularly characterizing *P. sativum* pea accessions. Understanding the richness of alleles is a priority and a significant help in different breeding and replanting programs since they are a pillar for better development of this crop. The morphological traits used in this study, along with the molecular points, explained the differences found within and between the accessions. The results of the diversity and genetic structure are useful for identifying contrasting groups to guide the selection of parents. The genetic variability between the accessions was sufficiently high, suggesting that the genetic diversity of the pea is sufficient for the possible combination of favorable genes.

Using these results as a first step, a self-pollination process of several generations shall follow in order to obtain pure lines. Hence, it is necessary to integrate genetic, morphological, disease resistance and production results to establish a possible relationship with the data obtained and, thus, be able to propose improvement programs. Research is currently underway to adjust planting distances, densities and economic analyses to exploit the productive potential of these genotypes.

In the future, the release of the first volatile variety with the *Afila* gene for the country is expected. These new varieties reduce the costs of tutoring (52%) by half or more.

Conflict of interests: The manuscript was prepared and reviewed with the participation of the authors, who declare that there exists no conflict of interest that puts at risk the validity of the presented results.

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Molybdenum dosage and application timing in sweet corn

Dosis y épocas de aplicación de molibdeno en maíz dulce

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Sweet corn production in the experimental area of Jaboticabal, Sao Paulo (Brazil).

Photo: J.W. Mendoza-Cortez

ABSTRACT

Nowadays, there is a greater offer of sweet corn hybrids with a high productive potential, shorter cycle and more intense metabolism than common corn, resulting in this crop being very demanding in terms of nutrients. The present study aimed to evaluate the effect of three levels of molybdenum (Mo) (150, 300 and 450 g ha^{-1}), via foliar spraying, with different application timing (E_1: 100% at 15 days after emergence - DDE; E_2: 100% at 30 DDE; E_3: 100% at 45 DDE; E_4: 50% at 15 and 30 DDE, and E_5: 33.3% at 15, 30 and 45 DDE) on the agronomic performance of sweet corn 'SVN 9298'. There was a significant effect from the interaction of the factors on the foliar concentration of Mo, obtaining the highest value with 450 g ha^{-1} of Mo, applied at 45 DDE, with an increase of 1.2% in the foliar content from that observed in the plants without a Mo application. Also, the foliar concentration of nitrogen (N) was higher with the highest Mo level. The highest number of commercial ears (49,583), total productivity of ears (20,942 kg ha^{-1}) and productivity of commercial ears (13,211 kg ha^{-1}) were obtained with estimated levels of 316, 450 and 311 g ha^{-1} of Mo, respectively. Likewise, the dose that maximized grain productivity (5,055 kg ha^{-1}), which is the most important component that defines the agronomic performance of sweet corn, was the estimated dose of 334 g ha^{-1} of Mo.

Additional key words: *Zea mays* var. *saccharata*; plant nutrition; micronutrient fertilizers; foliar application; crop performance.

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RESUMEN

Actualmente, debido a la mayor oferta de híbridos de maíz dulce con alto potencial productivo, ciclo más corto y un metabolismo más intenso que el maíz común, hacen que este cultivo sea muy exigente en nutrientes. En ese sentido, el presente trabajo tuvo como objetivo evaluar el efecto de tres dosis de molibdeno (Mo) (150, 300 y 450 g ha^{-1}), vía foliar, en diferentes épocas de aplicación (E_1: 100% a los 15 días después de la emergencia - DDE; E_2: 100% a los 30 DDE; E_3: 100% a los 45 DDE; E_4: 50% a los 15 y 30 DDE, y E_5: 33,3% a los 15, 30 y 45 DDE), sobre el desempeño agronómico del maíz dulce 'SVN 9298'. Se presentó interacción significativa de los factores sobre la concentración foliar de Mo, obteniéndose el mayor valor con 450 g ha^{-1} de Mo aplicado a los 45 DDE, siendo equivalente al incremento de 1,2% con relación al contenido foliar observado en las plantas sin aplicación de Mo. También, la concentración foliar del nitrógeno (N) fue mayor con la dosis más alta de Mo utilizada. Los mejores resultados para el número de espigas comerciales (49.477), productividad total de espigas (20.942 kg ha^{-1}) y productividad de espigas comerciales (13.211 kg ha^{-1}) fueron obtenidos con las dosis estimadas de 316, 450 y 311g ha^{-1} de Mo, respectivamente. Asimismo, la dosis que maximizó la productividad de granos (5.055 kg ha^{-1}), que es el componente más importante que define el desempeño agronómico del maíz dulce, fue obtenida con la cantidad estimada de 334 g ha^{-1} de Mo.

Palabras clave adicionales: *Zea mays* var. *saccharata*; nutrición vegetal; fertilizantes de micronutrientes; aplicación foliar; desempeño de cultivos.

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INTRODUCTION

In Brazil, sweet corn (*Zea mays* var. *saccharata*) is grown in approximately 30,000 ha, with 90% of that area located in the State of Goiás (Luz *et al.*, 2014). In the State of São Paulo, sweet corn is in great demand in the industrial sector (because of the sweet taste of the grains and the effect of different mutant alleles that control the conversion of starch into sugar), especially for the manufacture of canned food (Mendoza-Cortez *et al.*, 2016).

Mineral nutrition and crop fertilization are among the factors that influence high productivity. Although it is necessary in only small amounts (0.1 to 0.2 mg kg^{-1} of dry matter) (Maynard and Hochmuth, 2007), Mo is a micronutrient of great importance because it is a nitrate reductase enzyme (Hamlin, 2007; Fageria, 2009), indispensable in the metabolism of N and, therefore, having an indirect effect on the growth and productivity of crops.

Most soils contain enough amounts of this micronutrient; however, its absorption is affected under conditions of low temperatures, high N fertility (Hamlin, 2007), high rainfall and, mainly, acidic soils, where it is strongly adsorbed at the surface of iron and aluminum oxides, becoming unavailable to plants (Fageria, 2009).

A deficiency of this micronutrient is more evident during the reproductive phase than in the vegetative phase (Hamlin, 2007). Mo-deficient corn plants are more likely to have premature sprouting of the grains, which is accentuated when there is a greater availability of N in the soil (Tanner, 1978). In addition, there is a reduction in the size of tassels, male flowers and anthers; there is anthesis suppression and delay , decrease in the activity of some enzymes and increase of others, which affect the viability of pollen during development (Agarwala *et al.*, 1979). On the other hand, Mo toxicity in plants is rare, even when there is a high absorption rate of this micronutrient, especially under field conditions (Leite *et al.*, 2007).

Methods to prevent Mo deficiencies include in-band or field-wide applications, foliar applications and seed treatment. According to Hamlin (2007), foliar applications are much more efficient than soil applications, especially in acidic soils or under dry weather conditions.

The small number of studies carried out on Mo applications, via foliar or associated with N applied to the soil, are for conventional corn and corn used for popcorn (Valentini *et al.*, 2005; Teixeira, 2006; Araújo *et al.*, 2010; Pereira, 2010; Santos *et al.*, 2010; Santos *et al.*, 2012; Gaspareto *et al.*, 2014; Caioni, 2015). There is a shortage of current information on sweet corn under Brazilian conditions.

In this context, the objective of the present study was to evaluate the agronomic performance of sweet corn as a function of molybdenum dose, supplied via foliar applications, with different application timings.

MATERIALS AND METHODS

This experiment was conducted in the municipality of Jaboticabal, State of Sao Paulo, Brazil, between August and November of 2015.

The soil in the experimental area was classified as Latossolo Vermelho (EMBRAPA, 2006). Before the installation of the experiment, the soil was sampled at a depth of 0 to 20 cm, and, according to the results of the chemical analysis, the soil had: pH of 5.7; 22 g dm^{-3} of organic matter; 40 and 11 mg dm^{-3} of P (resin) and S, respectively; 44; 14; 3.3 and 30 mmol$_c$ dm^{-3} of Ca, Mg, K and H+Al, respectively; and 67% soil base saturation. Liming was not performed because the percentage of saturation by soil bases was higher than recommended for the cultivation of sweet corn (≥60%). Also, according to the granulometric analysis, the soil had 592, 161 and 247 g kg^{-1} of clay, silt and sand, respectively.

The experiment was installed in a randomized complete block design, in a 3x5+1 factorial scheme, with four replications. The treatments corresponded to three doses of molybdenum (150, 300 and 450 g ha^{-1}) and five application timings (E$_1$: 100% at 15 days after emergence - DDE; E$_2$: 100% at 30 DDE; E$_3$: 100% at 45 DDE; E$_4$: 50% at 15 and 30 DDE and E$_5$: 33.3% at 15, 30 and 45 DDE). The control treatment (C) corresponded to the non-application of molybdenum.

The experimental unit consisted of five rows of plants, 7.5 m long. The variables were evaluated in the plants located in the central five meters of the three central rows of each experimental unit.

The sweet corn 'SVN 9298', from Monsanto, was sown on August 24, 2015, with 0.50 m between rows. After 7 DDE, clearing was done in order to adjust the distance between plants to 0.33 m, which allowed an estimated population of 60,606 plants/ha.

Based on the recommendation of Cantarella *et al*. (1997), 60 kg ha^{-1} of P_2O_5 (simple superphosphate) and 50 kg ha^{-1} of K_2O (potassium chloride) were applied at planting. In coverage, 40 kg ha^{-1} K_2O were applied only once, in stage V$_4$. In the case of N (urea), 170 kg ha^{-1} were applied, fractionated three times in equal amounts (at 3 DDE; at 20 d after the first application, which coincided with the beginning of the definition of the productive potential, in stage V$_4$; and 20 d after the second application, when the number of rows of grain was defined in the ear, in stage V$_6$).

Ammonium molybdate was used as the source of Mo, via foliar spraying. Tween® adhesive dispersant was used, at 10 mL per 100 L of water. The volumes of the solution (ammonium molybdate plus dispersant) in the foliar applications at 15, 30 and 45 DDE were 270, 400 and 600 L ha^{-1}, respectively.

The evaluated characteristics were: nitrogen content (g kg^{-1}) and molybdenum (mg kg^{-1}) in the diagnostic leaf, total number of ears (ears/ha), number of commercial ears (commercial ears/ha), total ear productivity (kg ha^{-1}), commercial ear productivity (kg ha^{-1}), ear length (cm), ear diameter (cm) and grain productivity (kg ha^{-1}).

The data were subjected to analysis of variance (F-Test), and, when there was a significant effect, regression analysis was performed for the Mo doses and the Tukey test (5%) was used for the Mo application timing, using AgroEstat (Barbosa and Maldonado Junior, 2015).

RESULTS AND DISCUSSION

There was an effect from the interaction of the evaluated factors on the Mo concentration in the sweet corn leaves (Tab. 1). Adjustments were only obtained for Mo concentrations when the application was made at 45 DDE (E$_3$) and when it was fractionated at 15, 30 and 45 DDE (E$_5$) (Fig. 1).

In the splitting of the factor interactions, it was verified that, in E$_3$, the minimum concentration of foliar Mo (3.0 mg kg^{-1}) was obtained with the dose of 240 g ha^{-1}. However, with the increase in Mo doses, there was an increase in the micronutrient content, reaching a maximum of 6.6 mg kg^{-1} with the highest Mo dose. In E$_5$, the lowest (2.85 mg kg^{-1}) and highest (4.0 mg kg^{-1}) concentration of foliar Mo was obtained with 150 and 450 g ha^{-1} of Mo, respectively (Fig. 1).

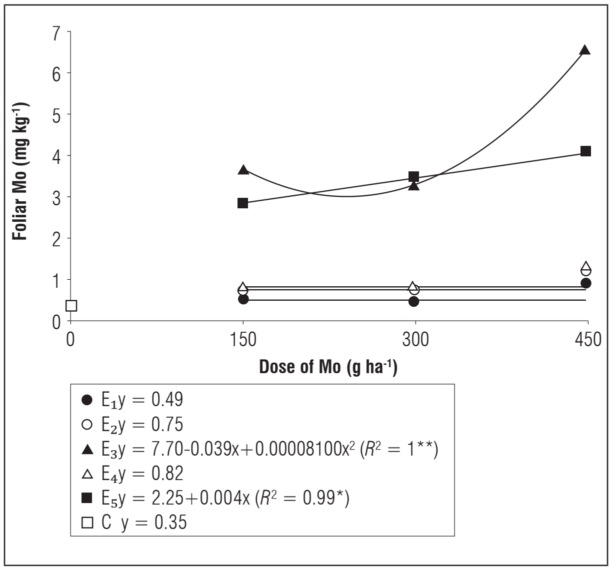

Figure 1. Foliar concentration of Mo as a function of doses and timing of Mo foliar applications in sweet corn 'SVN9298' (C = control, without application of Mo).

The highest concentration of Mo in E_3 can be attributed to the greater foliar area that the corn plants had between the evaluated periods, according to the observation made by Kraemer *et al.* (2009), who found a greater foliar absorption of nutrients when plants had a greater foliar area. Also, the fact that the spraying in E_3 was done without dose fractioning may have contributed to the improvement of the efficiency of foliar fertilization.

Plants not fertilized with Mo had 0.35 mg kg^{-1} of Mo, differing from the average content (2 mg kg^{-1}) observed in the plants that received this element via foliar spraying (Tab. 1).

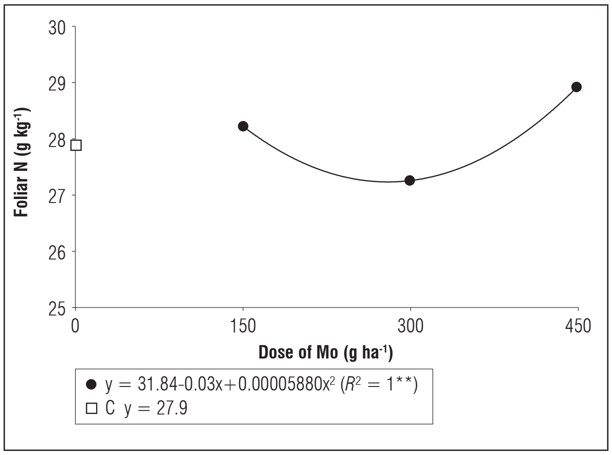

Figure 2. Foliar concentration of N and control treatment (C) as a function of the Mo foliar application in sweet corn 'SVN 9298'.

Although the levels of molybdenum in the soil were not determined, the foliar analysis on the diagnostic leaf allowed for a correcting follow-up foliar fertilization with Mo. The foliar levels of Mo estimated in the fertilized and unfertilized plants were above the critical level considered suitable for this crop, which is 0.1 to 0.2 mg kg^{-1}, according to Cantarella *et al.* (1997).

Despite the divergence in the results of the foliar application of Mo on the foliar concentration of Mo, positive in corn used for grain (Araújo *et al.*, 2010; Caioni, 2015) and popcorn (Teixeira, 2006) and negative in conventional corn (Teixeira, 2006; Pereira, 2010). Bodi *et al.* (2015), Kovács *et al.* (2015) and Taiz and Zeiger (2009) highlighted the importance of providing this micronutrient for increasing its concentration in corn because of its role in N metabolism,

Table 1. F valor for analysis of variance of molybdenum (CMo) and nitrogen (CN) foliar concentrations, total number of ears (NTE), number of commercial ears (NEC), ear length (COM), ear diameter (DIAM), total productivity of ears with husk (PTEP), commercial productivity of ears without husk (PECSP) and grain productivity (PG) of sweet corn 'SVN 9298', as a function of the doses and timing of Mo foliar applications.

Sources of variation	CMo	CN	NTE	NEC	COM	DIAM	PTEP	PECSP	PG
Doses of molybdenum (D)	16.95**	4.17*	<0.00^{NS}	14.52**	0.96^{NS}	0.36^{NS}	2.23^{NS}	2.89^{NS}	3.08^{NS}
Application timing (E)	90.24**	1.27^{NS}	0.28^{NS}	2.53^{NS}	0.79^{NS}	1.53^{NS}	1.87^{NS}	1.77^{NS}	0.68^{NS}
D × E	4.25**	0.92^{NS}	0.05^{NS}	1.98^{NS}	0.68^{NS}	1.75^{NS}	0.91^{NS}	0.95^{NS}	1.72^{NS}
(D × E) × C	22.65**	0.08^{NS}	15.02**	16.2^{NS}	6.81*	29.43**	0.60^{NS}	2.33^{NS}	5.47*
CV (%)	35.21	6.53	1.40	6.25	3.37	2.33	6.57	9.54	6.58

^{NS}, * and **: non-significant and significant at 5% and 1% probability levels by F-test, respectively.

C: control treatment, without application of Mo.

directly affecting the growth and development of plants.

The effect of Mo doses on the N content was observed, with a quadratic adjustment of this variable (Fig. 2). The N concentrations were within the appropriate range for corn (27 to 35 g kg^{-1}), according to Cantarella *et al*. (1997).

The N content in the diagnostic leaf for the nutritional status of sweet corn sprayed with Mo (28.1 g kg^{-1}) was similar to that obtained in the control plants, which did not receive the micronutrient (27.9 g kg^{-1}).

It was verified that the lowest concentration of N (27.2 g kg^{-1}) was obtained with 280.35 g ha^{-1} of Mo. Starting at this dose, there were increases in the foliar content of N, reaching 29.3 g kg^{-1} with the highest dose of Mo (450 g ha^{-1}) (Fig. 2). According to various authors, the importance of Mo lies in the role it plays in the assimilation of N since it is a component of the enzyme nitrate reductase, which is responsible for catalyzing the biological reduction of NO_3^- to NO_2^-, which is subsequently reduced to NH_4^+ (Hamlin , 2007; Fageria, 2009; Tejada-Jiménez *et al.*, 2013; Kóvacs *et al.*, 2015).

The total number of ears (NTE) was not influenced by the doses, the timing of the foliar applications of Mo, or the interaction of these factors (Tab. 1). Meanwhile, there was a significant difference between the means of the control treatment (58,503 ears/ha) and the factorial treatments (60,191 ears/ha), with an increase of 2.9% in the NTE in the sweet corn. According to Araújo *et al.* (2010), this variable can be altered when some nutritional imbalance occurs, a fact that was not found in this study, either as the result of a deficiency or excess, or even as the result of foliar concentrations of Mo and N. Therefore, the higher NTE resulted from the higher foliar contents of Mo and N, as a consequence of the molybdenum fertilization (Fig. 1 and 2). Although significant, the difference between the NTE of the fertilized and unfertilized plants with Mo was small (1,688 ears/ha). Ferreira *et al.* (2001) and Teixeira (2006), evaluating different Mo doses in corn used for popcorn and conventional corn, did not observe the effect of micronutrient applications on NTE. According to these authors, the lack of response to molybdenum fertilization was because the soil had a satisfactory amount of Mo or because the Mo concentration in the seeds was adequate (Weir and Hudson, 1966).

The number of commercial ears (NEC) was influenced by the foliar application of Mo doses (Tab. 1); this variable was adjusted to the quadratic equation (Fig. 3). The increase in Mo doses benefited the NEC, obtaining the maximum value (49,583 commercial ears/ha) with 316 g ha^{-1} of Mo, approximately 10% higher than the NEC verified in the control treatment, which was 44,803 commercial ears/ha.

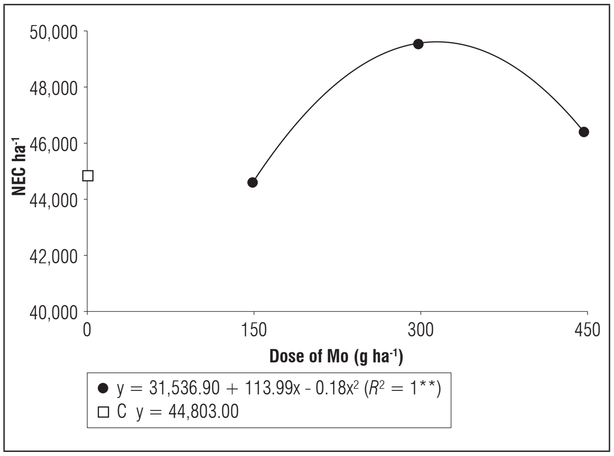

Figure 3. Total number of commercial ears (NEC) and control treatment (C) as a function of the Mo foliar applications in sweet corn 'SVN 9298'.

Molybdenum doses, application timing and the interaction between these factors had no significant effect on the length and diameter of the sweet corn ears. However, there were significant differences between the means of the control treatment and the factorial treatments for these two characteristics (Tab. 1), verifying an increase of 5.3% in length (from 17.6 to 18.5 cm) and 7% in ear diameter (4.7 to 5.1 cm). The values obtained for the length and diameter of the ears were higher than that required for commercial sweet corn (>15 cm in length and > 3 cm in diameter) (Albuquerque *et al.*, 2008). The results differed from those of Caioni (2015), who found no effect from foliar applications of Mo on these variables. According to various authors, the length and diameter of corn ears are greatly influenced by genotype and depend less on the environment and fertilization (Fernandes *et al.*, 2005; Ohland *et al.*, 2005; Goes *et al.*, 2012).

There was no significant effect of Mo doses, application timing, or the interaction of these factors on the total production of ears with husk (PTEP), the total production of commercial ears without husk (PECSP) or the grain production (PG) (Tab. 1).

There was adjustment of the increasing linear equation for the PTEP (Fig. 4), obtaining the maximum value (20,942 kg ha^{-1}) with the dose of 450 g ha^{-1} of Mo, via foliar spraying. Productivity similar to the present study was also recorded by Cardoso *et al.* (2010) and Luz *et al.* (2015) in green maize 'HTMV1' (21,374 kg ha^{-1}) and sweet corn 'SWC01' (21,790 kg ha^{-1}), respectively. The effect of molybdenum fertilization on PTEP was probably due to the fact that Mo promoted a better use of absorbed N, as verified by various authors (Valentini *et al.*, 2005; Caioni, 2015; Kovács *et al.*, 2015). Because of the multiple functions that it fulfills in the metabolism of plants (acting mainly in the synthesis of proteins and as a component of chlorophyll), N stimulates growth and development (increasing the green mass index and photosynthetic performance), altering various yield components and positively influencing the productivity of ears and grains (Gaspareto *et al.*, 2014), a fact that was found in this study for the productivity of sweet corn.

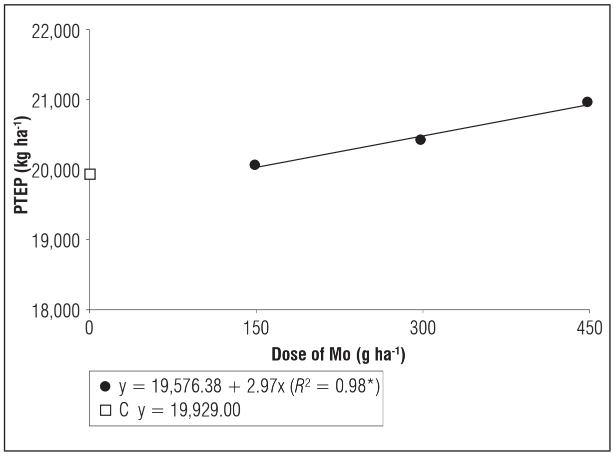

Figure 4. Total productivity of ears with husk (PTEP) and control treatment (C) as a function of the Mo foliar applications in sweet corn 'SVN 9298'.

The productivity of commercial ears without husk (PECSP) was adjusted to the quadratic equation as a function of Mo doses (Fig. 5). With the estimated dose of 311.3 g ha^{-1} of Mo, applied via foliar spraying, the highest PECSP (13,211 kg ha^{-1}) was obtained, lower than that found by Cruz *et al.* (2015), who verified a productivity of 9,919 kg ha^{-1} of PECSP with the hybrid 'GSS 41243' when evaluating different N doses.

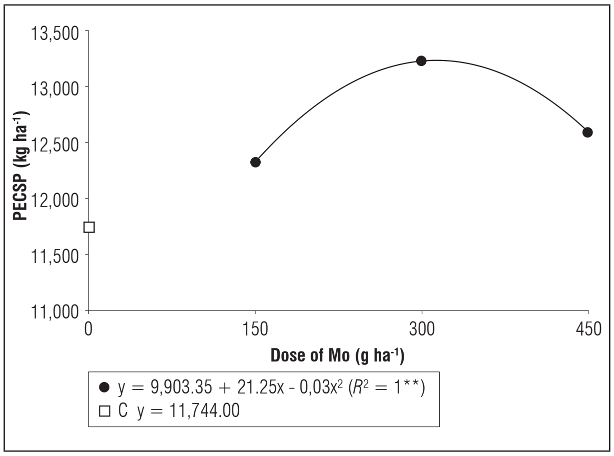

Figure 5. Productivity of commercial ears without husk (PECSP) and control treatment (C) as a function of the Mo foliar applications in sweet corn 'SVN 9298'.

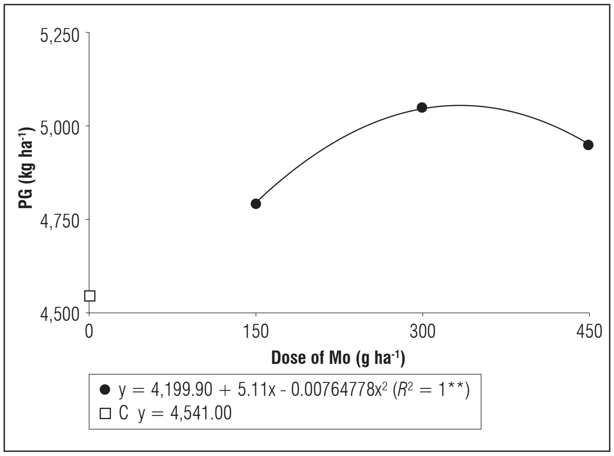

Figure 6. Grain productivity (PG) and control treatment (C) as a function of the Mo foliar applications in sweet corn 'SVN 9298'.

As stated for the PECSP, grain productivity (PG) was adjusted to the quadratic model (Fig. 6). The highest PG (5,055 kg ha^{-1}) was obtained with a dose of 334.4 g ha^{-1} of Mo, via foliar spraying, with an increase of 8.6% in relation to the control treatment (4,541 kg ha^{-1}). The same positive effect of the foliar Mo applications was observed by Caioni (2015), reaching a productivity of 9,028 kg ha^{-1} of grains. Araújo *et al.* (2010) obtained productivity of 4,251 kg ha^{-1} of grains in corn used for popcorn, with doses of up to 1,600 g ha^{-1} of Mo. An increase in PG was also noted by Valentini *et al.* (2005) in conventional corn. The maximum PG, close to that observed in this study, was verified by Pereira *et al.* (1999) with the application of 111 g ha^{-1} of foliar Mo. The authors obtained a 6.7%

increase in PG in relation to treatments without the application of micronutrients. On the other hand, Silva *et al.* (2018) did not verify the effect of different doses of Mo (0, 25, 105, 210, 315 and 420 g ha^{-1}) on grain productivity because the Mo concentrations in the soil and in the seed were sufficient to meet the demand of the corn plants.

Positive results for foliar Mo applications on grain productivity in conventional corn were verified by Santos *et al.* (2010) and Heidarzade *et al.* (2016). Caioni *et al.* (2017) observed effects of nitrogen and molybdenum fertilization on the productivity of grains in corn, with increases in productivity as Mo doses increased, concluding that this effect was probably due to the greater accumulation of proteins in grains as a result of the greater availability of N in the soil solution. According to Araújo *et al.* (2010), the addition of Mo, via foliar spraying, can increase the activity of the enzyme nitrate reductase, which participates directly in the metabolism of N. In addition, N acts on pollen formation and on the reduction of abortion of ovules, consequently affecting grain formation and crop productivity (Carvalho and Nakagawa, 2000).

No toxicity symptoms were observed when the highest Mo dose was applied, indicating that the sweet-corn hybrid ‘SVN 9298’ can tolerate high doses of this micronutrient without impairing grain productivity. Teixeira (2006), applying different doses of Mo (between 0 to 1600 g ha^{-1}) via foliar spraying, did not observe symptoms of toxicity in corn plants, concluding that this crop has a high response capacity at high doses of this micronutrient. Vieira *et al.* (2005), evaluating high Mo doses (between 0 and 1440 g ha^{-1}) via foliar spraying in a bean crop, verified that the application of high doses of this micronutrient did not present a toxic effect for the crop. According to some authors, the use of high doses of molybdenum should promote the accumulation of this nutrient in toxic amounts in plants; however, this does not occur because plants, in general, have reasonable tolerance to the excessive accumulation of this micronutrient (Leite *et al.*, 2007).

CONCLUSIONS

Under the conditions of this experiment, the application timing and the molybdenum doses influenced the foliar concentration of the micronutrient in sweet corn ‘SVN 9298’. Independent of the application timing, the molybdenum foliar spraying influenced the concentration of nitrogen and the agronomic performance of sweet corn ‘SVN 9298’.

The total number of commercial ears, the highest total productivity of ears with husk, the highest productivity of commercial ears without husk and the higher grain productivity of sweet corn ‘SVN 9298’ were obtained with 316; 450; 311 and 334 g ha^{-1} of molybdenum, via foliar spraying, respectively.

Conflict of interests: The manuscript was prepared and reviewed with the participation of the authors, who declare that there exists no conflict of interest that puts at risk the validity of the presented results.

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Weed control in organic maize crop with direct sowing

El control de malezas en cultivos de maíz orgánico en la siembra directa

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Experimental corn field.

Photo: A.L. Giraldeli

ABSTRACT

One of the main difficulties in the organic, no-tillage system is weed management. The aim of this study was to evaluate the effect of the association between mechanical and cultural control methods (reduction of spacing and consortium with *Canavalia ensiformis* DC) on weed phytosociology and grain yield in maize with the organic, no-tillage system. The experiment design was a randomized complete block design with four replications, in a 2×3×2 factorial scheme, where the factors were, first factor: presence and absence of *C. ensiformis*; second factor: three weed management methods (without control, mowing and weeding), and third factor: two maize spacings (0.4 and 0.8 m). We evaluated the phytosociology of the weed community in maize stages V4 and V8, maize yield and dry matter of *C. ensiformis*. In the V4 stage, 21 weed species were identified, which were reduced to 16 species in V8. *Cyperus rotundus* presented a higher IR (Importance Value Index) in V4, and *Panicum maximum* had the highest IR in V8, independent of management. The *C. ensiformis* consortium with maize with 0.8 m spacing and weeding provided a higher grain yield.

Additional key words: *Canavalia ensiformis* DC; weeding; cultural management; mechanical management; mowing.

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RESUMEN

Una de las principales dificultades en el sistema orgánico de siembra directa es el manejo de malezas. El objetivo de este estudio fue evaluar el efecto de la asociación de métodos mecánicos y de control de cultivos (reducción de espaciamiento y cultivos intercalados con *Canavalia ensiformis* DC) en la fitosociología de malezas y el rendimiento de granos de maíz bajo un sistema de siembra directa orgánico. El diseño experimental fue de bloques al azar con cuatro repeticiones, en un esquema factorial 2×3×2, con el primer factor: presencia y ausencia de *C. ensiformis*; segundo factor: tres métodos de manejo de malezas (no controlado, corte y deshierbe), tercer factor: espacio entre dos hileras (0,4 y 0,8 m). Se evaluó la fitosociología de la comunidad de malezas en las etapas V4 y V8 del maíz, el rendimiento de grano del maíz y la materia seca del *C. ensiformis*. En la etapa V4 se identificaron 21 especies de malezas, reduciéndose a 16 especies en V8. *Cyperus rotundus* tiene la mayor IR (importancia relativa) en V4 y *Panicum maximum* la mayor IR en V8, independientemente del manejo. *C. ensiformis* en consorcio con maíz en el espacio de 0,8 m con desmalezado proporcionan un mayor rendimiento de grano.

Palabras clave adicionales: *Canavalia ensiformis* DC; deshierbe; fitosociología; gestión cultural; gestión mecánica; siega.

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INTRODUCTION

Weed control is one of the main technical difficulties in no-till, organic maize production, which, according to Brazilian law, excludes the use of herbicides (MAPA, 2011).

Weeds adversely interfere with crops because of their direct competition for water, sunlight, nutrients and space. In addition, some species produce allelochemicals that are detrimental to the development of other plants (Concenço *et al*., 2014). A 56.55% decrease in maize yield occurred when weeds were controlled 28 days after emergence (DAE), reducing kernel yield from 10,605.8 to 4,608.15 kg ha^{-1} (Balbinot *et al*., 2016).

In the organic system, the predominant weed management wass hand hoeing or ripping out in the tillage system (Spagnolo *et al*., 2017) and mowing in the no-till system (Gomes and Christoffoleti, 2008). These management systems can make organic production unfeasible on medium- or large-size properties because they increase production costs and are not always effective. Vaz de Melo *et al*. (2007) found a higher total weed biomass production in the organic system when compared to the no-till system with use of herbicides, especially because of the high re-sprouting ability of some weed species. Proposals to improve efficiency of weed management in no-till organic maize cultivation include increasing the speed of maize crop establishment and reducing weed infestation. In this regard, it is vital to integrate cultural and mechanical methods of control (Oliveira and Brighenti, 2018).

With respect to the cultural methods, reducing spacing between rows in maize crops from 0.90 to 0.45 m can ensure higher yields at densities of 60,000, 75,000 and 90,000 plants/ha (Stacciarini *et al*., 2015) and suppress weed biomass accumulation (Nunes *et al*., 2010). Takuso *et al*. (2014) observed that, with 0.45 m spacing between rows, there was an increase in plant height, ear insertion height, number of kernels per row and kernels per ear, 100-grain weight and yield. Reduced spacings favored increased sunlight interception by the canopy of cultivated plants; therefore, the crop occupied space more rapidly, reducing the availability of resources for weed growth and development (Knezevic *et al*., 2003).

An alternative is to grow maize intercropped with green manuring species. Similar to what occurs with reduced spacing, an intercropped species occupies space and competes for resources with weeds, reducing infestation (Oliveira and Brighenti, 2018). Correa *et al*. (2014) evaluated the effect of intercropping maize and jack bean (*Canavalia ensiformis* DC.) on weed dynamics during four consecutive growing seasons of maize under the organic, no-till system and found a reduction in the relative importance of *Artemisia verlotorum* Lamotte, *Bidens pilosa* L. and *Digitaria* sp. species, which are often found with greater infestation in the organic, no-till system because they are not efficiently controlled with mowing (Vaz de Melo *et al*., 2007).

Thus, this study aimed to assess the effects of associating mechanical control methods (mowing and hoeing) with cultural methods (spacing reduction and intercropping with jack bean) on the phytosociology, weed dry matter accumulation and maize kernels yield under the organic, no-till system.

MATERIAL AND METHODS

This experiment was carried out during the 2009/10 growing season in an experimental area located at the Federal University of São Carlos - UFSCar, *campus* of the municipality of Araras, SP (Brazil), latitude 22º30’78” S and longitude 47º38’59” W, 646 m altitude. According to Köppen, the weather in the region is Cwa, humid tropical, characterized by hot, humid summers and dry winters. The meteorological data during the experiment were obtained from the Automatic Weather Station (AWS) at UFSCar, Araras, SP, Brazil (Fig. 1).

The soil was classified as clayed dystrophic Red Latosol (NVdf) (Embrapa, 2013). The soil chemical characteristics were determined with a soil analysis before the lime application (Tab. 1).

The experiment design consisted of randomized blocks with four replicates and a 2×3×2 factorial arrangement. The first factor consisted of the presence or absence of jack bean (*C. ensiformis*) in the maize rows; the second factor corresponded to two different spacings between the maize rows (0.4 and 0.8 m), and the third factor consisted of three weed control methods (hoeing, mowing, and no weed control). The size of the experimental plot was 20 m² (5×4 m),

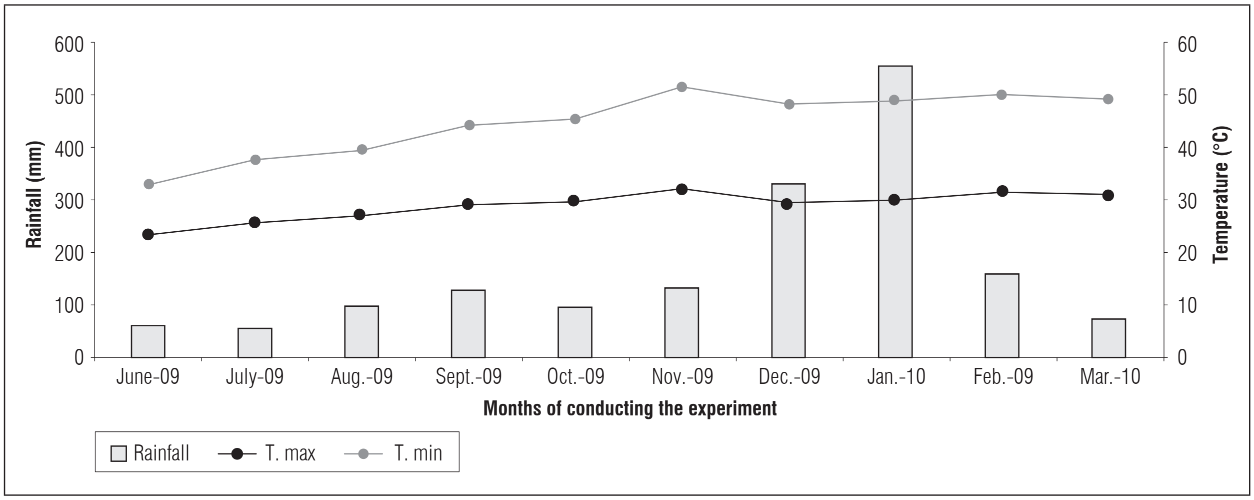

Figure 1. **Representation of rainfall, minimum average temperature, and maximum average temperature for the experiment period. Araras, São Paulo, Brazil, 2009/10.**

Table 1. **Results of soil chemical and physical analysis of the experiment area at a depth of 0 to 20 cm. Araras, São Paulo, Brazil, 2009/10.**

P Resin	MO	pH	K	Ca	Mg	H + Al	Al	SB	CTC	V	S	B	Cu	Fe	Mn	Zn
mg dm^{-3}	g dm^{-3}	($CaCl_2$)	mmol$_c$ dm^{-3}							%	mg dm^{-3}					
2	38	4.9	1.8	25	11	47	2.6	37.8	84.8	45	9	1.25	1.1	19	17.2	1.4

and the usable area (net area used for assessments) was 8 m^2 (4×2 m).

In June, 2009, lime was applied to the soil to raise the base saturation to 70%, and 30 d after liming, broadcast sowing of black oat (*Avena strigosa* Schreb.) was carried out at a density of 80 kg ha^{-1} of seeds. In late October, 2009, black oat plants were cut using a backpack brush cutter, and the straw was left on the ground for approximately 5 d for natural desiccation. Then, direct sowing was done using a hand seeder, and maize variety AL 25 was sown in the rows, spaced at 0.4 and 0.8 m apart, according to treatment, for a population of 55,000 plants/ha in both treatments. In the plots with jack bean, eight Fabaceae seeds per meter were sown, concomitantly with maize, using a hand seeder. After emergence of the jack bean plants, they were thinned, keeping six plants/m.

The planting fertilization was defined for an expected maize grain yield of 6.0 to 8.0 t ha^{-1} (Coelho, 2006). A commercial organic fertilizer (Ciafertil®) was used at a rate of 14.12 t ha^{-1} (dry compost weight), approximately 40 m^3 ha^{-1}, to provide the nitrogen required by the maize, i.e., 120 kg ha^{-1} (Raij *et al*., 1996). Accordingly, 68 and 41 kg ha^{-1} of P_2O_5 and K_2O, respectively, were applied according to the chemical characteristics of the compost: 0.85% N, 0.48% P_2O_5, 0.29% K_2O, 3.6% CaO, 0.29% MgO and 0.48% SO_4 on dry basis.

Weed control in the plots using a hoe was done whenever needed to allow the maize plants to grow freely in total absence of infesting species. In the plots using a mower, the weeds were cut close to the ground using a backpack mower at two different times: at stages V4 and V8, when the maize leaves were fully developed.

The weed species, except for the treatment using hoe, were assessed at two different times: when the maize plants were at stage V4, prior to the first weed cutting, and the second assessment when the maize plants were at stage V8, prior the second cutting.

Weeds were collected by throwing a 0.25 m quadrat frame randomly in three samplings per plot. Weed specimens were cut close to the ground, separated per species and dried in a forced-air oven for 72 h at 70°C for determination of dry matter per species and total dry matter per plot. Subsequently, a descriptive analysis was conducted using phytosociological parameters represented by relative frequency (RF), relative density (RD), relative dominance (RDo) and relative importance (RI) of the species present in the weed community at each treatment (Concenço *et al*., 2013).

The grain yield was determined after manual harvesting of ears from each usable plot area, which were then threshed, and the kernels were weighed by correcting the weight to 13% moisture. The jack bean dry matter was determined before harvesting the maize ears by collecting the plants present in 1 m of the planting row, which were dried in an air-circulation oven at a temperature of 65°C to constant weight. The values were described as ton ha^{-1}.

The data relating to maize grain yield and jack bean dry matter were subjected to analysis of variance with the F-test, and the mean values of the treatments were compared with the Tukey test ($P \leq 0.05$) (Pimentel-Gomes and Garcia, 2002). The phytosociological data were analyzed with descriptive statistics.

RESULTS AND DISCUSSION

In the first phytosociology assessment, at stage V4 of the maize plants, prior to weed cutting, 21 weed species were found in the experiment area: *Cyperus rotundus* L., *B. subalternans* DC., *P. maximum* (Jacq.) B.K. Simon & S.W.L. Jacobs., *Acanthospermum hispidum* DC., *Amaranthus viridis* L., *Raphanus raphanistrum* L., *Stachys arvensis* L., *Sonchus oleraceus* L., *Galinsoga parviflora* Cav., *G. quadriradiata* Ruiz & Pav., *Ricinus communis* L., *Parthenium hysterophorus* L., *Chamaesyce hirta* Millsp., *Ageratum conyzoides* L., *Commelina benghalensis* L., *Ipomoea purpurea* (L.) Roth., *Euphorbia heterophylla* L., *Leonotis nepetifolia* (L.) R. Br., *Oxalis latifolia* Kunth., *Phyllanthus tenellus* Roxb. and *Eclipta prostrata* (L.) L. The species *C. rotundus* exhibited the highest percent values of RF, RD, RDo and RI, irrespective of the management systems (Fig. 2).

The species *C. rotundus* is commonly found in soil-revolving agricultural systems because the farming implements used to move soil favor the division of tubers and break apical dominance of this plant (Jakelaitis *et al*., 2003). The experiment area was at the second year after implementation of the no-till system, which can explain the predominance of this species in the area. The plots with exclusive maize cultivation at a spacing of 0.8 m had a greater number of species than the maize intercropped with jack bean with the same spacing arrangement (Fig. 2).

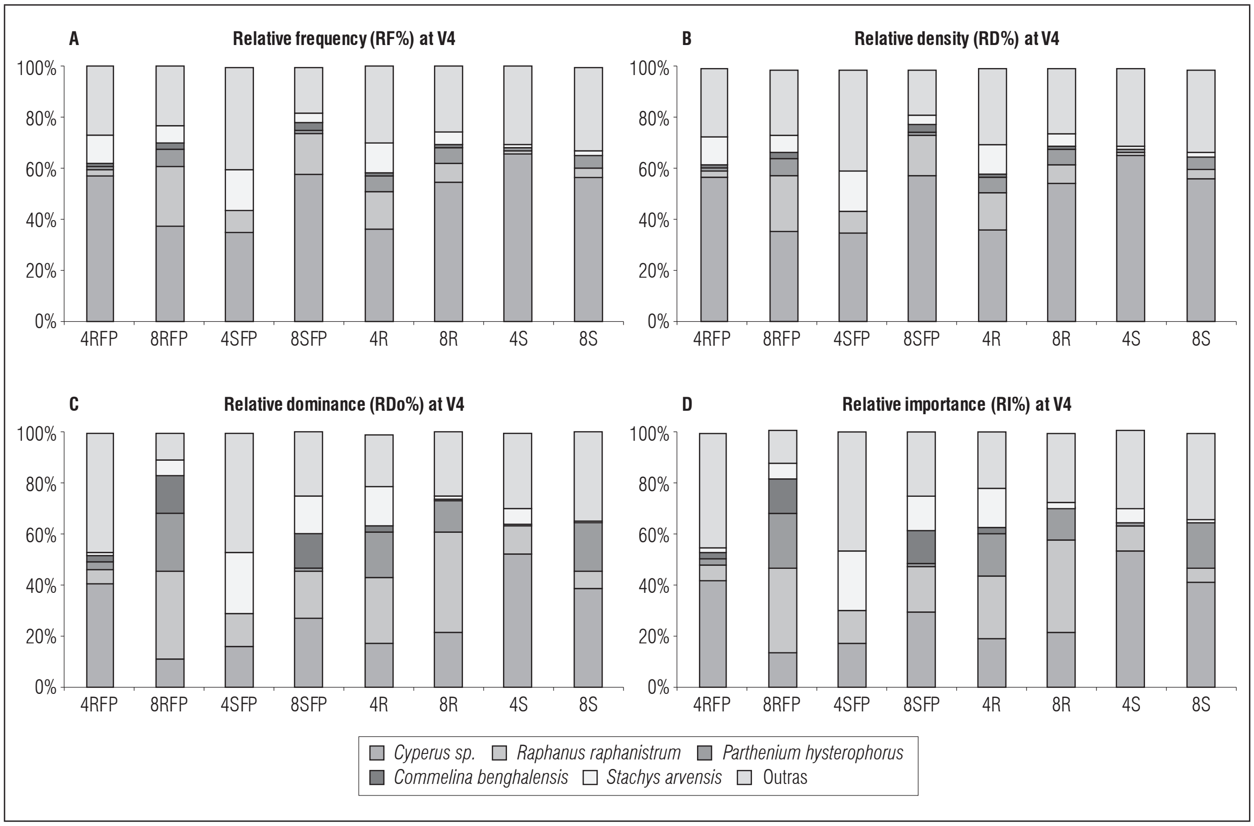

Figure 2. **Effects of weed management systems (R: mowing; S: without control), maize crop spacing (4: 0.4 m; 8: 0.8 m) and presence (FP) and absence of *C. ensiformis* on weed frequency (A), density (B), dominance (C) and relative importance (D) in the maize crop, evaluated in the fourth fully developed maize leaf. Araras, São Paulo, Brazil, 2009/10.**

In the second phytosociology assessment, stage V8, before the second mowing, 16 weed species were found in the experiment area: *P. maximum*, *S. arvensis*, *G. parviflora*, *G. quadriradiata*, *P. tenellus*, *A. verlotorum*, *A. hispidum*, *C. benghalensis*, *A. deflexus* L., *C. iria* L., *Leonurus sibiricus* L., *L. nepetifolia*, *A. viridis*, *I. purpurea*, *A. conyzoides* and *R. raphanistrum* (Fig. 3).

There was a decrease in species richness, down from 21 species in the first assessment to 16 species in the second assessment. The species *B. pilosa*, *S. oleraceus*, *C. hirta* and *O. latifolia*, identified in the first assessment, on the 60th day, after mowing, were not found. In turn, the species began to be part of the weed community in the experiment area.

The species *A. deflexus* and *L. sibiricus* were identified in the plots grown with maize intercropped with jack bean, without weed control, irrespective of spacing. The microclimate, shade and moisture provided in this system, along with the greater competition promoted by jack bean, may have contributed to the establishment of some species to the detriment of others.

The species *P. maximum* exhibited the highest RD, RDo and RI, irrespective of the management systems (Fig. 3), different from the values observed in the first assessment, in which the species with the highest RDo was *C. rotundus*. This fact corroborated the premise of a change in the infesting weed population in the area as a result of the greater competition and shading in the maize rows. In this case, the species *P. maximum* may have been favored because, according to Andrade *et al*. (2004), it has a high production capacity, even under 70% shade.

The species *P. maximum* exhibited an equivalent RI% in the 0.4 and 0.8 m spacings in the maize rows, both in the treatment without control and in the treatment with mowing (Fig. 3). This result suggests that reduced spacing and mowing were not effective in reducing growth and establishment of this weed species.

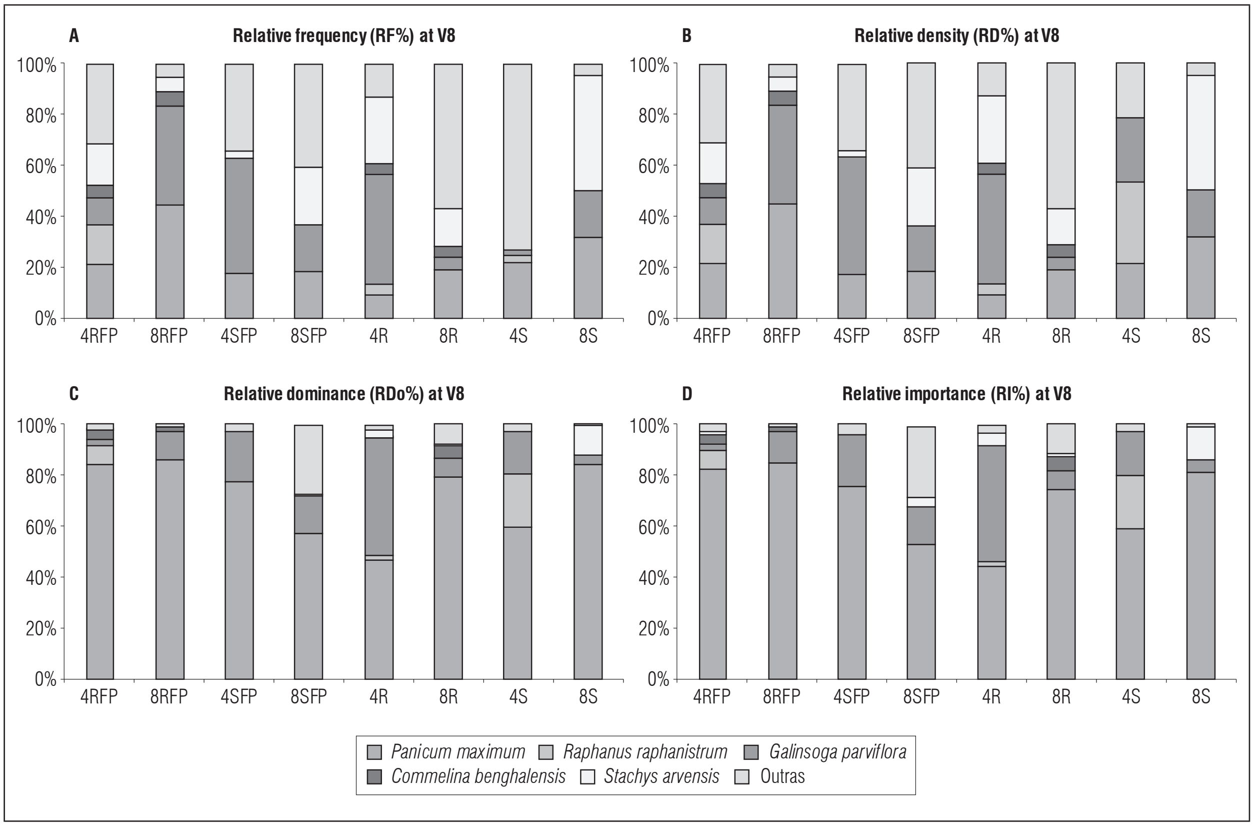

Figure 2. Effects of weed management systems (R: mowing; S: without control), maize crop spacing (4: 0.4 m; 8: 0.8 m) and presence (FP) and absence of *C. ensiformis* on weed frequency (A), density (B), dominance (C) and relative importance (D) in the maize crop, evaluated in the fourth fully developed maize leaf. Araras, São Paulo, Brazil, 2009/10.

Mowing removes shoots, which consequently causes plant stress. The percentage of leaf and stem losses represents a reduction of sunlight interception as well as lower rates of canopy net photosynthesis and a decrease in roots activity (Davidson and Milthorpe, 1966). However, when a species exhibits stem reserves and easy dissemination, mechanical control (mowing) is not quite efficient.

Effective weed control with mowing depends on the species present in the infesting community. For *B. pilosa*, this method proved to be viable when performed 14 and 25 d after maize emergence because it managed to inhibit seed production. But for *C. benghalensis*, this method was not effective, considering the high rate of leaf area, irrespective of when mowing was conducted (Lemos *et al*., 2013). However, Chiovato *et al*. (2007) observed that two cuttings conducted at stages V4 and V8 resulted in a lower accumulation of leaf and stem dry matter in maize plants when subjected to interference by *B. pilosa*.

A reduced RI% with *P. maximum* was also not observed in the treatment of maize intercropped with jack bean at a spacing of 0.4 m (Fig. 3). But in the 0.8 m spacing between rows with maize-intercropped with jack bean, the treatment without control reduced the RI% of *P. maximum*, probably because of the higher competition with jack bean plants and other weed species.

The 0.8 m spacing with jack bean was more effective in restricting the RDo and RI of *P. maximum*. In this treatment, jack bean achieved the highest dry matter accumulation (4 t ha^{-1}, see later), that is, the Fabaceae plants established quickly because of the greater penetration of sunlight, occupying the space before the weed species.

Oliveira *et al*. (2014) observed 10 weed species in a maize monoculture, especially *Rottboelia cochinchinensis* (Lour.) Clayton, *Sorghum arundinaceum* (Desv.) Stapf. and *Alternanthera tenella* Colla., but, in maize intercropped with *C. ensiformis*, *Crotalaria juncea* L. or *Mucuna aterrima* (Piper & Tracy) Holland., eight species were found, resulting in a lower accumulation of weed dry matter, especially for monocotyledons.

The 0.4 m spacing between rows favored the dominance and RI of species *S. arvensis*, *Acanthospermum* sp., *A. viridis* and *C. benghalensis* (Fig. 3). The favoring of these weed plants over other species may have been due to the larger shading area. In these treatments, there was also a reduction of RI for *P. maximum*, which may have contributed to the establishment of other species as a result of the lack of competition.

The analysis of variance for the grain yield variable revealed a significant effect from the following interactions: weed plant management × spacing between maize rows and management of weeds × presence and absence of jack bean (Tab. 2).

Table 2. Maize grain yield as a function of weed management and line spacing. Araras, São Paulo, Brazil, 2009/10.

Weed management	Spacing	
	0.4 m	0.8 m
	t ha^{-1}	
Weeding	1.78 aB	3.51 aA
Mowing	1.10 bA	1.35 bA
Without control	1.91 aA	1.08 bB
CV (%)	28.9	

Means with different letters, lowercase in the columns and uppercase in the rows, indicate a significant statistical differences according to Tukey test ($P \leq 0.05$) ($n=4$).

Mowing for both maize spacing arrangements (0.4 and 0.8 m) reduced the kernel yield (Tab. 2), probably because the weed plants were not efficiently controlled. Reduced spacing hindered mowing, which was done until maize stage V4; after this stage, mowing cut the maize plants.

The grain yields may have been affected by the spacing between rows and seed density. The variety AL 25 is recommended for seeding between 0.8 and 1.0 m, which may have contributed to a reduced yield, even maintaining adequate plant density.

The 0.8 m spacing, associated with hoeing, was the treatment that achieved the highest grain yields (Tab. 2). In the maize-jack bean intercropping, irrespective of the spacing between rows, hoeing provided the highest grain yield, without differences from the treatment without jack bean (Tab. 3).

The maize grain yield was similar to the one observed by Pereira *et al*. (2011) in a monoculture, in maize intercropped with *C. juncea* (cut at the 8th expanded maize leaf) and in maize with *C. juncea* without cutting. Jack bean, at a density of 31,250 plants/ha, did not affect ear length, ear diameter, number of kernel rows per ear, 1,000-seed weight or kernel yield (Saldanha *et al*., 2017).

Table 3. Production of maize grains according to weed management and presence and absence of *Canavalia ensiformis*. Araras, São Paulo, 2009/2010.

Weed management	*Canavalia ensiformis*	
	Presence	Absence
	t ha^{-1}	
Weeding	2.80 aA	2.50 aA
Mowing	0.86 bB	1.58 bB
Without control	0.95 bB	2.05 bAB
CV (%)	28.9	

Means with different letters, lowercase in the columns and uppercase in the rows, indicate a significant statistical differences according to Tukey test ($P \leq 0.05$) ($n=4$).

The presence of jack bean provided the same maize kernel yield with mowing and without weed control (Tab. 3). In these treatments, the same production rates of jack bean dry matter were obtained, lower than the dry matter production for the treatment with hoeing (Tab. 4). This suggests that the weeds with mowing and without control competed with both maize plants and jack bean plants, reducing their development and dry matter production.

Table 4. Yield of dry matter of *Canavalia ensiformis* at the end of the maize cycle, as a function of weed management. Araras, São Paulo, Brazil, 2009/10.

Weed management		Line spacing	
Weeding	4.87 a	0.4	1.50 b
Mowing	2.25 ab	0.8	4.00 a
Without control	1.25 b		
CV (%)	35.0		

Means with different letters indicate a significant statistical differences according to Tukey test ($P \leq 0.05$) ($n=4$).

The jack bean dry matter production was lower in the 0.4 m spacing (Tab. 4). Collier *et al*. (2011) also found a reduced dry matter for jack bean intercropped with maize grown with 0.45 m spacing.

Therefore, more studies are necessary to understand the dynamics of the weed community and the alteration of infesting plants in the transition to and establishment of organic, no-till planting. The chosen management method must always favor maize plants to the detriment of weeds, but, to accomplish this, it is necessary to know which control methods can be adopted without favoring certain species.

CONCLUSIONS

The weed community changed during the maize growing cycle as a result of alterations provided by the growth and establishment of maize and jack bean plants, changing the competition patterns.

Jack bean intercropped with maize with spacing at 0.8 m between rows can be a good alternative for producing maize in organic, no-till cultivation but requires at least one hoeing when the fourth leaf of the maize plants is developed.

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Conflict of interests: this manuscript was prepared and reviewed with the participation of all authors, who declare that there exists no conflict of interest that puts at risk the validity of the presented results.

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Growth analysis of green-leaf lettuce under different sources and doses of organic and mineral fertilization

Análisis de crecimiento de lechuga crespa bajo diferentes fuentes y dosis de fertilización orgánica y mineral

Experiment lettuce plant growth.

Photo: R.R. Rejane

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ABSTRACT

The objective of this study was to evaluate the influence of different sources and doses of organic and mineral fertilization on the production of green-leaf lettuce. The experiment design used randomized blocks in a factorial scheme (6×3), with six doses of fertilizers (1 = 0; 2 = 25; 3 = 50; 4 = 100; 5 = 150, and 6 = 200% of the recommended fertilization for green-leaf lettuce crop) and three sources of fertilizers [cattle manure (CaM) and chicken manure (ChM), decomposed, on a wet basis and applied 100% at planting at the doses: CaM – 0, 12.5, 25, 50, 75, 100 Mg ha^{-1}; ChM – 0, 5, 10, 20, 30, 40 Mg ha^{-1}; mineral fertilization (MF) varying the N levels: 0, 37.5, 75, 150, 225, 300 kg ha^{-1} plus 400 kg ha^{-1} of P_2O_5 and 60 kg ha^{-1} of K_2O]. The fertilization with CaM and ChM was more efficient than the MF at increasing the production of green-leaf lettuce, mainly because of the higher residual effects of P in the Oxisol. The ChM provided a higher soil pH, P and K, while the CaM provided a higher soil Mg, organic carbon and organic matter. The dose with 144% of organic fertilization exclusively on a wet basis corresponding to 72 Mg ha^{-1} of CaM and 29 Mg ha^{-1} of ChM resulted in the highest green-leaf lettuce yield.

Additional key words: *Lactuca sativa* L.; phosphorus; nitrogen; cattle manure; chicken manure.

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RESUMEN

El objetivo de este estudio fue evaluar la influencia de diferentes fuentes y dosis de fertilización orgánica y mineral en la producción de lechuga crespa. Se utilizó un diseño de bloques completos al azar, en un esquema factorial (6 × 3), donde se evaluaron seis dosis de fertilizante (1 = 0, 2 = 25; 3 = 50; 4 = 100; 5 = 150 y 6 = 200%) de la fertilización recomendada para la lechuga, tres fuentes de abono fertilizante (estiércol bonino (EB) y aves de corral (CP)), descompuesto, aplicado húmedo 100% en la siembra: 1 = EB - 0; 12,5; 25; 50; 75 y 100 Mg ha^{-1}; 2 = CP 0; 5; 10; 20; 30 y 40 Mg ha^{-1}; 3 = Fertilización mineral varió solo las cantidades de N; 3 = 0; 37,5; 75; 150; 225 y 300 kg ha^{-1}, junto a 400 kg ha^{-1} de P_2O_5 y 60 kg ha^{-1} de K_2O que se mantuvo en las parcelas con fertilización mineral. La fertilización con EB y CP demostró ser más eficiente que la fertilización mineral en la producción de lechuga crespa, principalmente debido a los mayores efectos residuales del P en el suelo. El estiércol CP proporcionó valores más altos de pH, P y K, mientras que el EB de Mg, materia orgánica y carbono orgánico en el suelo. La dosis del 144% de fertilizantes exclusivamente orgánicos en base húmeda correspondiente a estiércol de 72 Mg ha^{-1} EB y 29 Mg ha^{-1} CP, dieron los mejores rendimientos para la lechuga crespa en un Latosol.

Palabras clave adicionales: *Lactuca sativa* L.; fósforo; nitrógeno; estiércol de ganado; estiércol de aves.

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INTRODUCTION

Lettuce (*Lactuca sativa* L.) is the most cultivated leafy vegetable in Brazil. The States of São Paulo and Minas Gerais are the major producers, where green-leaf lettuce enjoys consumer preference, reaching 70% of the market, followed by American lettuce (15%), flat lettuce (10%) and 5% for other commercial types (Sala and Costa, 2012). In recent years, the consumption of processed lettuce has grown significantly, mainly serving fast-food restaurants, industries and hospitals (Mota *et al*., 2012).

As a short cycle plant, lettuce uses high quantities of mineral fertilizers to meet plant development demands (Queiroz *et al*., 2017), mainly for nitrogen (N). This nutrient is the most required macronutrient in horticultural crops; however, this nutrient presents great losses through leaching, volatilization, and immobilization, requiring special care in its management (Carvalho and Zabot, 2012).

The frequent increase in the cost of mineral fertilizers and increasing environmental pollution as a result of inappropriate agricultural activities highlight the use of organic fertilizers as an attractive option. Organic fertilizers are attractive for all farm sizes because of nutrient cycling and organic matter addition, which may modify a soil's physical, chemical and biological attributes, thus improving soil fertility (Pereira *et al*., 2013). These potential effects of organic fertilizers have generated new demands on researchers for assessing the technical and economic feasibility of such fertilizers (Melo *et al*., 2008).

Agricultural activities produce plant and animal (manure) waste in large quantities, which has been used in agriculture for thousands of years but requires more efficient management in the fertilization of agricultural crops, mainly in vegetable crops with a short cycle (Figueiredo *et al*., 2012; Vergel *et al*., 2016). Knowledge on the dynamics of nutrient mineralization must be fully understood to improve nutrient availability in the soil of short-cycle crops, avoiding immobilization or rapid mineralization of nutrients during periods of high or low demand (Peixoto Filho *et al*., 2013; Vaz *et al*., 2019).

Bovine (cattle) manure (CaM) is widely used as an organic fertilizer for the production of vegetables. When this fertilizer is incorporated, it improves soil aeration and water absorption, as well as the chemical, physical and biological properties of the soil, generating a more balanced nutrient availability for plants (Cunha *et al*., 2012). Abreu *et al*. (2010) emphasized that CaM has a water pH of 6.7, 26.9 g kg^{-1} of organic matter, N, P, K, Ca, Mg and S at 11.4, 16.1, 8.3, 62, 13 and 6.7 g kg^{-1}; and B, Cu, Fe, Mn and Zn at 7.24, 67.8, 147, 146 and 119 mg kg^{-1} (dry basis), respectively. However, this composition depends on animal feed because, when done exclusively in pastures, these values are different than where there is supplementation with concentrates (Peixoto Filho *et al*., 2013). In contrast, chicken manure (ChM) from intensive farming (poultry) is richer in nutrients, water pH of 8.4, 31.1 g kg^{-1} of organic matter (OM), N, P, K, Ca, Mg and S at 32.2; 22.6, 30, 171, 8.9 and 6.9 g kg^{-1}; and B, Cu, Fe, Mn and Zn at 36.5, 69.6, 272, 583 and 631 mg kg^{-1} of in dry basis, respectively (Abreu *et al*., 2010).

In addition, manure fertilizers contain high levels of cellulose, which make residue decomposition and nutrient release slowly, generating positive consequences in vegetable crops (Souza, 2007). The contents of N, P and K in ChM are at higher concentrations than in other species of domestic animals since it contains 5 to 15% water, while other manures have 65 to 85% (Tedesco *et al*., 2008). Using ChM as a form of fertilizer, Abreu *et al*. (2010) and Peixoto Filho *et al*. (2013) demonstrated that lettuce presented better root development and improved production of dry mass and yield when compared to other forms of organic fertilization.

The majority of studies assess the productivity of lettuce after the application of different sources of organic fertilization, but results vary according to the region, soil type and form of irrigation; however, only a few studies have assessed the residual effect of organic fertilization on the production of lettuce in the Cerrado biome. The objective of this study was to evaluate the influence of different sources and doses of organic and mineral fertilization on the production of green-leaf lettuce.

MATERIAL AND METHODS

The study was conducted in the experimental area of the horticulture sector of the Federal Institute of Triângulo Mineiro, Campus Uberaba, at the coordinates 19º45’27” S; 47º55’36” W, at an altitude of 764 m a.s.l. The experimental area was previously prepared with two harrowings, followed by a soybean cultivation cycle and two years without crop activities before the experiment was installed in the winter of 2017.

The climate of the region is characterized as tropical rainy, with a cold dry winter and hot humid summer - Aw climate type, according to Beck *et al*. (2018). The average annual precipitation and temperature are 1,600 mm and 22.6ºC, respectively (Inmet, 2018); however, during the evaluation period, rainfall of 20 mm (June/2017) was recorded; the total accumulated in the year was 1,995.3 mm, above the normal for the region (Fig. 1).

The soil of the experimental area was classified as a dystrophic red latosol (Santos *et al*., 2018), medium texture, presenting the following values in the 0-0.2 m layer four months prior to the liming: 210, 680 and 110 g kg^{-1} of clay, sand and silt, respectively, pH ($CaCl_2$) 6.5; 20.9 mg dm^{-3} of P (Mehlich-1); 161 mg dm^{-3} of K^+; 2.9 $cmol_c$ dm^{-3} of Ca^{2+}; 1.5 $cmol_c$ dm^{-3} of Mg^{2+}; 2 $cmol_c$ dm^{-3} of H+Al and 18 g kg^{-1} of organic matter and base saturation (V) of 71%.

The sowing of the green-leaf lettuce (Vanda cultivar, Sakata®) occurred in plastic trays (200 cells), grown under greenhouse conditions prior to field transplant.

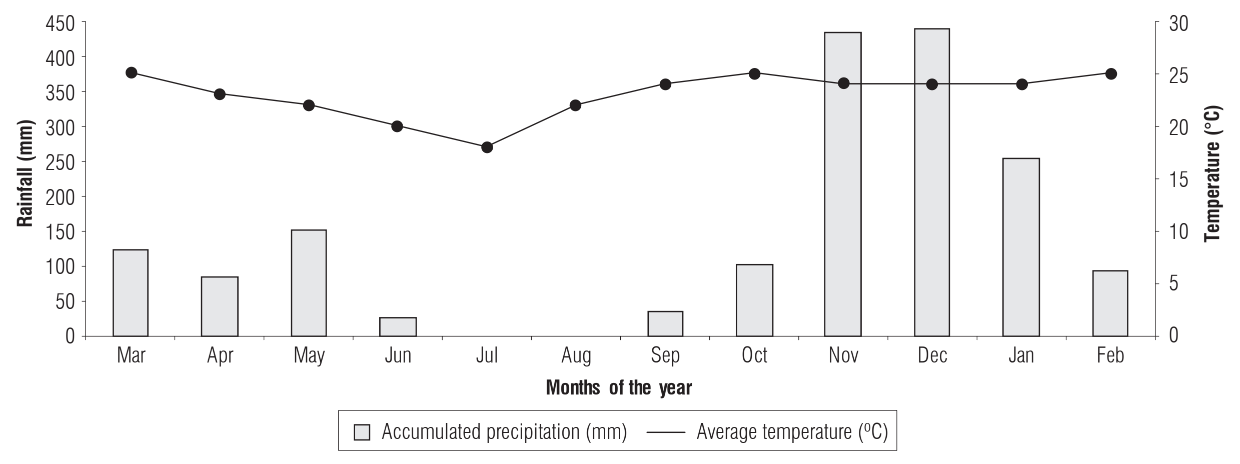

Figure 1. Climate variables obtained from the Meteorological Database for Teaching and Research (Inmet, 2018).

The seedlings were transplanted when they presented 4 to 6 final leaves that were completely expanded (7 to 10 cm height), approximately 25-30 days after sowing (DAS), and spaced 0.25 m between plants and between rows, in 4 planting rows, with 10 plants per row (40 plants per plot) in plots 1 m apart. The experiment plots were mechanically built and were 0.3 m tall, with 2.5×1 m (2.5 m^2). In each plot, only 12 plants from the two central rows were evaluated.

The experiment design used the randomized block design in a 6×3 factorial scheme, with six doses of fertilizers (0, 25, 50, 100, 150 and 200% of the recommended fertilization for lettuce crop) and three fertilizers sources [cattle manure (CaM) and chiken (hens) manure (ChM), decomposed, on a wet basis and applied 100% at planting at the doses: CaM – 0, 12.5, 25, 50, 75, 100 Mg ha^{-1}; ChM – 0, 5, 10, 20, 30, 40 Mg ha^{-1}; mineral fertilization (MF) varying the N levels: 0, 37.5, 75, 150, 225, 300 kg ha^{-1} of N as urea, plus 400 kg ha^{-1} of P_2O_5 (superphosphate, 20% P_2O_5) and 60 kg ha^{-1} of K_2O (KCl, 60% K_2O), as recommended in Ribeiro *et al*. (1999) – urea and KCl were fractioned: 20% at planting, 20, 20 and 40% in successive side-dressing fertilizations; all treatments had 4 replications (*n*=72). The organic fertilizers and minerals were distributed and incorporated in the plots 5 and 3 d before seedling transplant, respectively.

The lettuce plants were irrigated daily (drippers), with a flow rate of 1.8 L h^{-1} spaced every 0.5 m, for approximately 40 min, maintaining the soil close to field capacity. Infestant plants (weeds) were manually controlled.

The harvest occurred 67 DAS and 42 days after transplant (DAT), when the plants presented commercial standards without evidence of flowering and with the maximum vegetative development. The plants were cut just below the basal leaves, close to the soil level, and brought to the laboratory to conduct the evaluations.

After the lettuce harvesting, the number of leaves (NL) was quantified, the plant height (PH) was measured with a graded ruler and the stem diameter (SD) were evaluated with caliper; the shoot fresh mass (SFM), shoot dry mass (SDM) and yield (P) were recorded using a digital scale with a maximum capacity of 20 kg. The harvested plants were dried at 65 °C with a forced air circulation oven for 72 h to determine the dry mass (SDM).

Four days after harvest, soil samples were collected in each plot in the 0-0.2 m soil layer for chemical analysis and determination of the residual effect of the fertilizers. The soil water pH, phosphorus (P) by Mehlich-1, potassium (K^+), calcium (Ca^{2+}), magnesium (Mg^{2+}), potential acidity (H + Al), soil organic matter (SOM), sum of bases (Ca+Mg+K+Na) (SB), effective cation exchange capacity (CEC) (t = SB + Al^{3+}), CEC at pH 7 (T = SB + (H+Al)), and base saturation (V = 100 SB/T) were recorded.

The results were submitted to analysis of variance using the F test for significance. When the results presented significant differences ($P<0.05$), the data were submitted to analysis of regression for the quantitative factors (doses) and Tukey's test to the averages of qualitative factors (sources), both at 5% probability, using the AGROESTAT statistical software.

RESULTS AND DISCUSSION

The lettuce morphological parameters when using the CaM and ChM fertilizers were significantly higher ($P<0.05$) than when mineral fertilizer was applied (urea). For the doses, 150 and 0% were significantly higher and lower than the other doses for all parameters, respectively, but there was no interaction between the sources and doses (Tab. 1).

The use of CaM and ChM improved the soil chemical attributes after soil incorporation and increased all agronomic characteristics of the green-leaf lettuce. These results were due to the low natural fertility of Oxisols in the Cerrado biome. These soils generally present an accelerated rate of plant residue decomposition, low levels of organic matter, Ca, Mg, P and, consequently, a low cationic exchange capacity; thus, the use of organic fertilizers can improve the attributes of such soils.

Cunha *et al*. (2012) and Oliveira *et al*. (2014) showed that the application of organic compounds improves soil aeration and water absorption, as well as the chemical, physical and biological soil attributes, generating a balance in the availability of nutrients to plants as a function of useful microorganisms, macro and micronutrients that are available, natural antibiotics and growth substances, such as the humic fractions of organic matter.

The organic fertilization not only boosted productivity but also produced plants with a better nutritional

Table 1. Agronomic evaluation of green-leaf lettuce under different fertilizers and doses.

Fertilizer	NL	PH	SD	SFM	SDM	Yield
		cm		g/plant		Mg ha^{-1}
	Fertilizers (F)					
Urea	16.4 b	13.9 b	1.1 b	78.5 b	3.4 b	12.6 b
ChM	22.0 a	16.8 a	1.5 a	159.8 a	9.4 a	25.6 a
CaM	23.2 a	17.5 a	1.5 a	172.2 a	9.8 a	27.5 a
F-test	65.87**	18.45**	28.69**	39.09**	34.89**	39.09**
	Doses (D)					
0	16.2 c	12.8 c	1.0 c	62.7 d	3.5b	10.0 d
25	19.8 b	15.1 bc	1.3 b	113.5 c	6.8 ab	18.1 c
50	21.0 ab	16.1 ab	1.4 b	134.2 bc	7.8 a	21.5 bc
100	22.0 ab	17.1 ab	1.5 a	164.7 b	8.7 a	26.3 b
150	23.0 a	18.5 a	1.5 a	185.7 a	9.3 a	29.7 a
200	21.6 ab	17.0 ab	1.4 b	160.3 b	9.3 a	25.6 b
F-test	13.53**	9.71**	10.93**	14.75**	6.57**	14.75*
	Interaction F × D					
F-test	1.16^{ns}	0.69^{ns}	0.86^{ns}	0.96^{ns}	0.92^{ns}	0.96^{ns}
CV (%)	10.69	13.68	14.24	29.14	39.24	29.13

^{ns} = non significant; ** = significant at 5%, by the Tukey test ($P<0.05$).

ChM = chicken manure; CaM = cattle manure; NL = number of leaves; PH = plant height; SD = stem diameter; SFM = shoot fresh mass; SDM = shoot dry mass.

quality (arising from the improvement of soil quality) than the plants grown exclusively with mineral fertilizers (Silva *et al*., 2011). Oliveira *et al*. (2014) emphasized that leafy vegetables respond well to organic fertilization and that the constant use of mineral fertilizers causes reductions in a soil's biological activity, which can affect the productive performance of crop cultures. Vaz *et al*. (2019) emphasized in their study that the commercial productivity of 14.5 Mg ha^{-1} was observed at 157.4 kg of formulated 05-25-15 (NPK) and that doses above 200 kg ha^{-1} reduced the number of total leaves and productivity.

The polynomial regression analysis demonstrated that the best fit to the averages of the analyzed variables was the quadratic model for NL, PH, SD, SFM, SDM and yield; the maximum values were 23.9, 17.4 and 1.6 cm, 182.4 and 11 g/plant and 29.5 Mg ha^{-1}, which corresponded to the doses 229.5, 181.5, 195, 213, 274.5 and 216 kg ha^{-1} of N, which were equivalent to 153, 121, 130, 142, 183 and 144% of the recommended dose, respectively (Fig. 2).

According to Filgueira (2013), increases in the NL and PH favor vegetative development and expand the photosynthetically active area of plants, raising the productive potential. This response was seen in the present study since, in the plots fertilized with CaM, the NL and HP increased by 43.7 and 25.9%, which caused an increase of 36.6% in the SD, 119.4% in the SFM, 188.23% in SDM and 118.25% in the lettuce productivity. In the plots fertilized with ChM, the values did not differ from the plots with CaM ($P>0.05$); the NL and PH increased by 37.5 and 20.9%, which caused an increase of 36.6% in SD, 103.6% in SFM, 176.5% in SDM and 103.2% in the productivity when compared to the plots with mineral fertilization.

The study of the production of lettuce (Vanda cultivar) grown under protected environments and in an open field, with organic and mineral fertilization, using doses of 0, 1 and 2 times the recommendation for organic compost (3.6 kg m^{-2} of NPK), mineral fertilizer [0.02 kg m^{-2} of N, via urea, 0.08 kg m^{-2} of P (simple superphosphate), and 0.008 kg m^{-2} K (KCl)], indicated that the open environment had the highest averages with the highest dose of organic fertilization for PH (15.6 cm), the recommended dose of mineral fertilization for NL (8.6) and SD (1.13 cm) and a greater

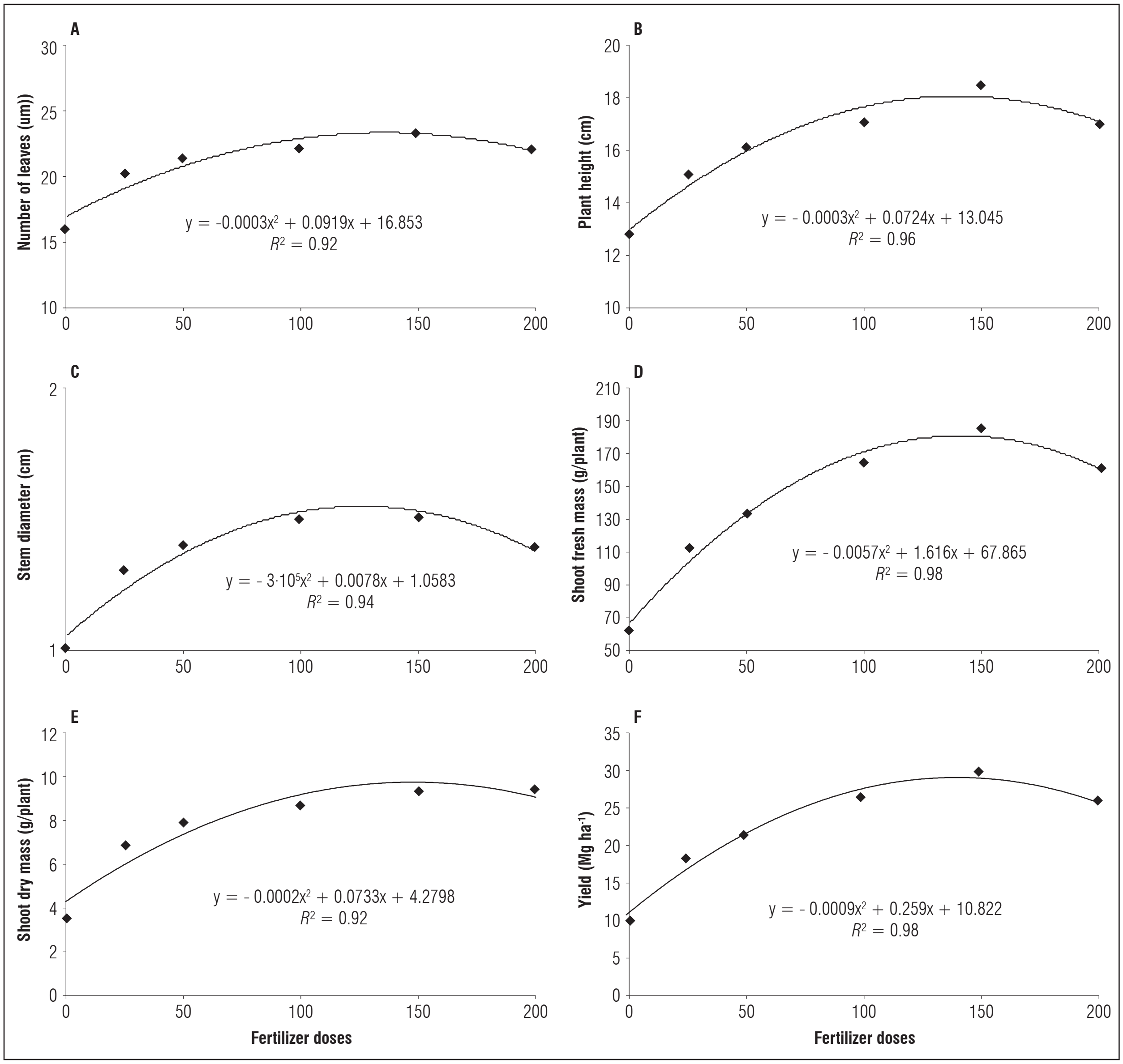

Figure 2. Effect of doses in the lettuce for number of leaves (A), plant height (B), stem diameter (C), shoot fresh mass (D), shoot dry mass (E), and yield (F), corresponding to the doses of 229.5 (153%), 181.5 (121%), 195.0 (130%), 213.0 (142%), 274.5 (183%) and 216.0 (144%) kg ha^{-1} of N.

dose of mineral fertilization for SFM (96.8 g/plant) and productivity (36.0 Mg ha^{-1}) (Cavalheiro *et al*., 2015). These values were lower than those found in the present study for NL (23.9), PH (17.4 cm), SD (1.6 cm), SFM (182.4 g/plant), and SDM (11.0 g/plant), except productivity (29.5 Mg ha^{-1}) (Tab. 2).

However, the greater organic fertilizer doses, which resulted in the greatest SFM, SDM and productivity, were 213 (142%), 274.5 (183%) and 216 (144%) kg ha^{-1} of N, respectively; however, the technical and economic feasibility of such application must be evaluated for each situation (Tab. 2).

The evaluation of the mineral fertilization, ChM, CaM, earthworm humus and organic compost in the production of lettuce (Vera cultivar) indicated a higher production when using ChM (Abreu *et al*., 2010), presenting a SFM and SDM of 542.3 g, significantly higher when compared to the 91.1 g obtained with

Table 2. Regressions of the effects of fertilizer doses on the plant variables.

Variable	Equation	R^2	Value reached	N doses	
				kg ha^{-1}	%
NL	$y = -0.0003x^2 + 0.0919x + 16.853^{**}$	0.92	23.9	229.5	153
PH	$y = -0.0003x^2 + 0.0724x + 13.045^{**}$	0.96	17.4 (cm)	181.5	121
SD	$y = -3E\text{-}05x^2 + 0.0078x + 1.0583^{**}$	0.94	1.6 (cm)	195.0	130
SFM	$y = -0.0057x^2 + 1.616x + 67.865^{**}$	0.98	182.4 (g/plant)	213.0	142
SDM	$y = -0.0002x^2 + 0.0733x + 4.2798^{*}$	0.92	11.0 (g/plant)	274.5	183
Yield	$y = -0.0009x^2 + 0.259x + 10.822^{**}$	0.98	29.5 (Mg ha^{-1})	216.0	144

* = significant at 1% and ** = significant at 5%, by the Tukey test ($P < 0.05$). NL = number of leaves; PH = plant height; SD = stem diameter; SFM = shoot fresh mass; SDM = shoot dry mass.

CaM and the 233.1 g obtained with mineral fertilization. Peixoto Filho *et al*. (2013) assessed the productivity of green-leaf lettuce (Cacheada cultivar) with doses of chicken, cattle and ovine manure in successive cultivations and observed that the ChM provided the best indices for the evaluated parameters: NL (15), SFM (141.4 g), SDM (5.5 g) and P (26.7 Mg ha^{-1}).

For the residual effect on soil chemical attributes for the type of fertilizer used, the CaM showed the highest levels of magnesium (Mg) (1.7 cmol$_c$ dm^{-3}) and organic matter (21 g kg^{-1}). Significant interactions between the fertilizers and doses for soil water pH, P and K were detected (Tab. 3; Fig. 3). In general, it was observed that the ChM resulted in a linear increase in soil pH, P and K, which ranged from 6.5 to 6.8, 19.9 to 86.8 mg dm^{-3} and from 0.2 to 0.5 cmol$_c$ dm^{-3}, respectively; 5, 335 and 104% higher than the control (no fertilizer). The urea fertilizer linearly increased the content of K by 64%, which ranged from 0.4 to 0.6 cmol$_c$ dm^{-3} and linearly decreased the pH value by 7%, which ranged from 6.6 to 6.2.

Table 3. Chemical attributes of Oxisol cultivated with green-leaf lettuce under different fertilizers and doses.

Fertilizer	Soil chemical attributes						
	pH	P	K	Ca	Mg	H+Al	OM
	CaCl$_2$	mg dm^{-3}	cmol$_c$ dm^{-3}				g kg^{-1}
	Fertilizers (F)						
Urea	6.4 b	24.1 c	0.5 a	2.6	1.3 b	1.9	18 b
ChM	6.7 a	49.2 a	0.3 b	2.9	1.5 ab	1.7	19 b
CaM	6.6 a	37.9 b	0.3 b	2.8	1.7 a	1.9	21 a
F-test	3.51*	16.10**	35.98**	2.20^{ns}	5.46**	3.76^{ns}	8.37**
	Doses (D)						
0	6.6	19.6 d	0.3 c	2.4	1.3	2.0	18
25	6.5	28.2 cd	0.4 b	2.7	1.5	1.8	20
50	6.7	33.1 c	0.3 c	2.7	1.5	1.8	19
100	6.5	40.3 b	0.4 b	2.9	1.5	1.9	20
150	6.6	52.0 a	0.4 b	3.0	1.5	1.9	20
200	6.5	49.1 a	0.5 a	3.0	1.6	1.8	19
F-test	0.62^{ns}	7.92**	8.73**	1.92^{ns}	0.81^{ns}	1.25^{ns}	1.84^{ns}
	Interaction F x D						
F-test	2.33*	4.21**	4.87**	1.34^{ns}	0.91^{ns}	1.88^{ns}	1.37^{ns}
CV (%)	4.35	41.46	20.80	20.84	22.87	15.25	10.28

^{ns} = non significant; * = significant at 1% and ** = significant at 5%, by the Tukey test ($P < 0.05$). ChM = chicken manure; CaM = cattle manure.

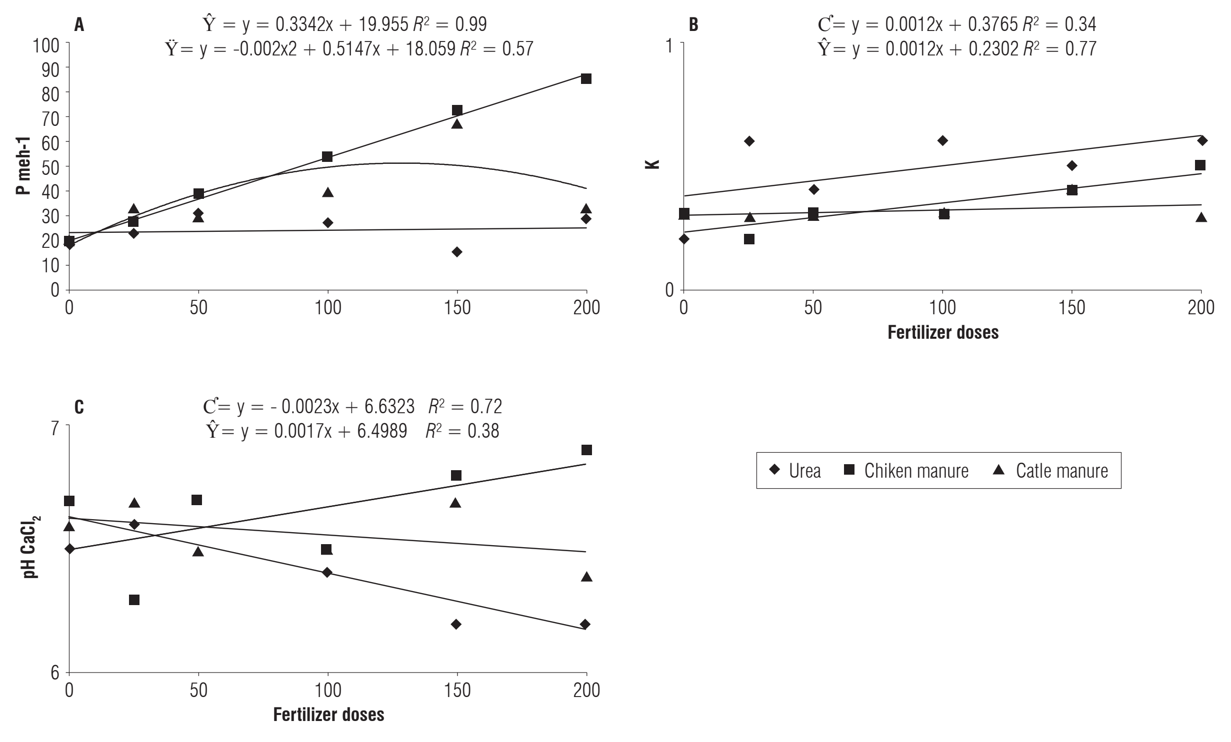

Figure 3. Regressions of the effect of doses of urea, chicken and cattle manure fertilizers on soil chemical attributes.

The increase in P availability, verified with the fertilization with ChM and CaM, was due to the presence of this nutrient in the manure composition, to the soil pH and to the rise in the soil organic matter content. The soil pH near neutrality provided conditions for P availability; in acidic conditions, there is a reaction of $H_2PO_4^-$ with the ionic forms of iron (Fe) and aluminum (Al), forming compounds with low solubility; also, the presence of organic matter blocks the adsorption sites on Fe and Al oxides in the soil, decreasing the adsorption capacity of $H_2PO_4^-$ (Novais and Smyth, 1999).

The study of polynomial regression indicated that the soil pH in the soil fertilized with ChM and urea presented a linear adjustment, reaching values of 6.8 and 6.2, respectively, with the 200% dose. The soil P when fertilized with ChM and CaM presented a linear and quadratic adjustment, respectively, reaching values of 86.8 mg dm^{-3} with the 200% dose and 51.2 mg dm^{-3} with the 129% dose. The soil K in the areas fertilized with urea and ChM presented a linear adjustment, reaching values of 0.6 and 0.5 cmolc dm^{-3} with the 200% fertilized doses, respectively (Tab. 4).

Table 4. Regression equations of the effect of doses of fertilizers on soil chemical attributes.

Soil attribute	Equation	R^2	Fertilizer	Value reached	Dose	Variation
pH	$y = -0.0023x + 6.6323$**	0.72	Urea	6.2	200	-7
	$y = 0.0017x + 6.4989$*	0.38	ChM	6.8	200	5
P (mg dm^{-3})	$y = 0.3342x + 19.955$**	0.99	ChM	86.8	200	335
	$y = -0.002x^2 + 0.5147x + 18.059$*	0.57	CaM	51.2	129	183
K (mg dm^{-3})	$y = 0.0012x + 0.3765$**	0.34	UR	0.6	200	64
	$y = 0.0012x + 0.2302$**	0.77	ChM	0.5	200	104

* = significant at 1%. and ** = significant at 5%, by the Tukey test ($P<0.05$). ChM = chicken manure; CaM = cattle manure; pH = soil water pH; P = phosphorus (Mehlich-1); K = potassium.

Pimentel *et al*. (2009) conducted two experiments on lettuce and carrot [the first experiment was fertilized with organic compound (70% of Napier grass, 10% of remnants of cultures, 20% of cattle manure) at 0, 4.2, 8.4 and 16.8 kg/parcel (dry basis); the second experiment was fertilized with 1/3 of the previous organic compound plus various grasses at 0, 3.4, 6.9 and 13.7 kg parcel^{-1} (dry basis)], and observed that the levels of P and K increased regardless of the organic compound used. The authors reported that the soil pH in both experiments was close to neutrality (6.7 and 6.6), as well as in the present study.

According to Mantovani *et al*. (2005), green-leaf lettuce (cultivar Verônica) fertilized with 0, 30, 60, 90 and 120 Mg ha^{-1} of urban waste compost presented linear increases in soil pH ($CaCl_2$), soil organic matter, P, K, Ca and Mg, with increases of 19, 28, 81, 27, 178 and 100%, respectively, as compared to the control treatment (highest dose of organic fertilizer). This authors also reported that this effect on soil pH can be attributed to the presence of soluble organic anions (R-COO- and R-O-) in organic waste, which can adsorb H^+ from the soil solution with exchange reactions, especially with Ca^{2+} ions.

The correlation of the soil chemical characteristics and biomass production of the lettuce fertilized with organic compounds indicated that the addition of organic compounds increased the production of lettuce dry mass and the content of soil organic matter in the soil (Oliveira *et al*., 2014). These authors also reported that the rise of soil organic matter provided greater P, K and Ca availability, in addition to a reduced soil potential acidity, favoring plant development and increasing productivity, similar to what was observed in the present study.

After harvest, there was still a residual of 49.2 mg dm^{-3} of P in the plots fertilized with ChM (Tab. 5), indicating that there was a greater availability of this nutrient in an Oxisol (high P adsorption), which is a common soil in the Cerrado.

The microbiological quality and productivity of lettuce (Vera cultivar) under mineral and diverse organic fertilization treatments (control without fertilization, chemical fertilization, chicken manure, cattle manure, earthworm humus, organic compost) indicated that the fertilization with chicken manure provided the highest soil pH (7.1), K (0.46 cmol$_c$ dm^{-3}),

Table 5. Soil pH, phosphorus and potassium levels according to fertilizer.

Fertilizer	Doses						Average
	0	25	50	100	150	200	
	pH						
Urea	6.5 aA	6.6 aA	6.7 aA	6.4 aA	6.2 bA	6.2 bA	6.4 b
ChM	6.7 aAB	6.3 aB	6.7 aAB	6.5 aAB	6.8 aAB	6.9 aA	6.7 a
CaM	6.6 aA	6.7 aA	6.5 aA	6.5 aA	6.7 abA	6.4 abA	6.6 ab
Average	6.6 A	6.5 A	6.7 A	6.5 A	6.6 A	6.5 A	--
CV (%)	4.35						
	P (mg dm^{-3})						
Urea	19.2 aA	23.8 aA	30.3 aA	26.5 bA	15.3 bA	29.3 bA	24.1 c
ChM	18.5 aC	27.3 aBC	38.7 aBC	53.9 aAB	72.3 aA	84.5 aA	49.2 a
CaM	20.9 aB	33.6 aB	30.4 aB	40.5 abAB	68.4 aA	33.5 bB	37.9 b
Average	19.6 D	28.2 CD	33.1 BCD	40.3 ABC	52.0 A	49.1 AB	--
CV (%)	41.46						
	K (cmolc dm^{-3})						
Urea	0.2 aC	0.6 aA	0.4 aB	0.6 aAB	0.5 aAB	0.6 aA	0.5 a
ChM	0.3 aBC	0.2 bC	0.3 aBC	0.3 bABC	0.4 aAB	0.5 bA	0.3 b
CaM	0.3 aA	0.3 bA	0.3 aA	0.3 bA	0.4 aA	0.3 cA	0.3 b
Average	0.3 c	0.4 abc	0.3 bc	0.4 ab	0.4 a	0.5 a	--
CV (%)	20.80						

Means followed by different letter indicate significant differences according to Tukey test ($P \leq 0.05$). ChM = chiken manure; CaM = cattle manure.

Ca (6.8 $cmol_c$ dm^{-3}), Mg (1.1 $cmol_c$ dm^{-3}), and CTC (11.2 $cmol_c$ dm^{-3}), while the fertilization with cattle manure showed the highest values for soil organic matter (51.8 g kg^{-1}) and P (16 mg dm^{-3}), results that corroborate an improvement in soil chemical attributes when cultivated with organic fertilizers.

Mantovani *et al*. (2014) found that high doses of P resulted in an increased growth and production of green-leaf lettuce and observed that a dose of 350 mg dm^{-3}, equivalent to 800 kg ha^{-1} of P_2O_5, was the most appropriate dose for clay soils (420 g kg^{-1} of clay). Abreu *et al*. (2010) demonstrated that plants from treatments with organic composts showed a high foliar P and K content, higher than those observed in a mineral fertilization treatment. The authors also pointed out that N and P are the elements that most commonly limit lettuce production as a result of low availability in the soil.

CONCLUSIONS

The fertilization with cattle manure and chicken manure was more efficient than the mineral fertilization for the production of green-leaf lettuce, mainly because of P residual effects in the soil.

The chicken manure provided a higher soil pH, phosphorus and potassium, and the cattle manure provided a higher magnesium, organic carbon and organic matter content.

The 144% dose of organic fertilization (wet basis), corresponding to 72 Mg ha^{-1} of cattle manure and 29 Mg ha^{-1} of chicken manure, resulted in the highest yield for green-leaf lettuce.

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Conflict of interests: The manuscript was prepared and reviewed with the participation of the authors, who declare that there exists no conflict of interest that puts at risk the validity of the presented results.

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How does the physiological activity and growth of tomato plants react to the use of a soil-mineral compound?

¿Cómo reacciona la actividad fisiológica y el crecimiento de las plantas de tomate al uso de un compuesto suelo-mineral?

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Tomato plant experiment.

Photo: I.S. Pereira

ABSTRACT

The tomato crop has a high productive potential that can be depleted by biotic and abiotic stresses. Increased plant resilience to stress conditions has been reported with foliar applications of soil-mineral compounds; however, it is necessary to better understand how plants react to the use of this compound. Thus, this study evaluated the effect of foliar applications of a soil-mineral compound on the physiological and growth attributes of tomato plants. This experiment was carried out in Lagoa Formosa/MG during 2016. Different rates of the soil-mineral compound were used during the crop cycle, forming four distinct managements. The management consisted of different doses of the mineral compound in four stages after transplanting the tomato seedlings. The experiment design used randomized blocks. The following physiological evaluations were performed: total soluble protein, hydrogen peroxide, nitrate reductase enzyme activity, urease, superoxide dismutase (SOD), peroxidase, phenylalanine ammonia lyase, and lipid peroxidation (LP). The growth assessments were plant biomass and yield. Foliar applications of the soil-mineral compound increased the activity of the SOD enzyme by 4.17 and 6.25%. The use of the soil-mineral compound also increased the LP activity and reduced the antioxidant enzyme activity. The foliar application of the soil-mineral compost at doses of 0.5, 0.750, 1.0 and 1.0 kg ha^{-1} at 15, 25, 40 and 60 days after transplanting, respectively, increased the yield of the table tomatoes by 20%.

Additional key words: resistance inducers; oxidative metabolism; productivity; fertilizers.

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RESUMEN

El cultivo de tomate presenta un alto potencial productivo pero puede afectarse debido al estrés biótico y abiótico. Se ha informado un aumento de la resistencia de la planta a las condiciones de estrés con la aplicación foliar de compuestos minerales del suelo, sin embargo, aún es necesario comprender mejor cómo reacciona la planta al uso de este compuesto. Por lo tanto, este estudio evaluó el efecto de la aplicación foliar del compuesto mineral del suelo sobre los atributos fisiológicos y de crecimiento de las plantas de tomate. Este experimento se llevó a cabo en Lagoa Formosa / MG durante 2016. Se usaron diferentes tasas del compuesto mineral del suelo durante el ciclo del cultivo, lo que constituye cuatro manejos distintos. El manejo consistió en diferentes dosis del compuesto mineral en cuatro etapas después del trasplante de las plántulas de tomate. El diseño experimental utilizado fue de bloques al azar. Se realizaron las siguientes evaluaciones fisiológicas: proteína soluble total, peróxido de hidrógeno, actividad de la enzima nitrato reductasa, ureasa, superóxido dismutasa (SOD), peroxidasa, fenilalanina amoniaco liasa y peroxidación lipídica (LP). Las evaluaciones de crecimiento fueron biomasa vegetal y rendimiento. La aplicación foliar del compuesto mineral del suelo aumentó la actividad de la enzima SOD en 4,17 y 6,25%. El uso del compuesto mineral del suelo también aumentó la actividad de LP y redujo la actividad de las enzimas antioxidantes. La aplicación foliar de compost mineral del suelo a dosis de 0,5; 0,750; 1,0 y 1.0 kg ha^{-1} a los 15, 25, 40 y 60 días después del trasplante, respectivamente, aumentó el rendimiento de tomates de mesa al 20%.

Palabras clave adicionales: inductores de resistencia; metabolismo oxidativo; productividad; fertilizantes.

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INTRODUCTION

The tomato (*Solanum lycopersicum* L.) is one of the most consumed vegetables globally, mainly because of its high nutritional value and antioxidant potential (Du *et al*., 2017). Overall tomato production increased from 27.6 million tons in 1960 to 177 million tons in 2016 (FAOSTAT, 2016). In Brazil, table tomato cultivation stands out because of its socioeconomic importance, as it generates jobs, improving the income of rural workers and growers (Silva *et al.,* 2013). Many factors can lead to a reduction in crop productivity, affecting the quality of the final product, which include problems caused by pests and diseases (Zaidi *et al*., 2018).

Disease management has become a challenge in agriculture as it requires pesticides, often with high toxicity (Carrascosa *et al*., 2015). Chemical pesticides, particularly soil fumigants, have been severely restricted in recent decades because of the environmental consequences (Van Bruggen and Finckh, 2016), the residual effects on soil and the long period necessary for degradation. In addition, the use of pesticides in the environment impacts human health, and resistance is acquired by pathogens for the chemical groups. The use of pesticides should be minimized and rationalized to promote more sustainable agriculture (Lamichhane *et al*., 2015).

Phytosanitary management needs to be improved to minimize or mitigate its impact on the environment (Dayan *et al*., 2009). Plants have mechanisms to respond to different types of stress, whether abiotic (Shah *et al*., 2014) or biotic (Shah and Zeier, 2013). These natural defense mechanisms remain inactive or latent until activated after exposure and/or contact with inducing agents (Mandal, 2010).

The defense mechanisms of a plant are genetically controlled, depending on the expression after contact with the host. So, plants can activate the defense mechanism in several ways, including through oxidative activities (Teixeira *et al*., 2017; Xue and Yi, 2017), enzymes involved in the phenylpropanoid route (Sangeetha and Sarada, 2015), and enzymes involved in lipid peroxidation, among other forms. The use of resistance-inducing products for different crops is increasing, for example on vine (Xue and Yi, 2017), wheat (Moya-Elizondo and Jacobsen, 2016), pepper (Siddiqui and Meon, 2009), and peach (Jiao *et al*., 2018), mainly.

When plants recognize the resistance inducers applied, the intracellular signal transduction pathways are activated (Shah *et al*., 2014). Perception occurs when the molecules of the inducing agent bind to receptor molecules that are probably located in the plasma membrane of the plant cell; this reaction triggers the activation of various defense mechanisms, culminating in protection against pathogens (Graham and Myers, 2011). These inductions alter the physiology of the plant and can interfere directly with productivity.

Application of resistance inductors that have Silicon (Si) is a potentially sustainable option to improve biotic and abiotic stresses in several plants (Zhu and Gong, 2014; Liang *et al*., 2015; Cooke and Leishman, 2016; Etesami, 2018).

Despite the importance of the use of these products, there are few studies on resistance inductors based on silicon oxide and aluminum in the tomato to table culture. One source of these nutrients available to growers is through soil-mineral compost. Thus, this study evaluated the effect of foliar applications of a soil-mineral compound on the physiological and growth attributes of tomato plants.

MATERIAL AND METHODS

This experiment was conducted in the 2016 growing season in the commercial area of Lagoa Formosa, Minas Gerais, Brazil (18°30'01.6" S and 46°30'48.2" W) in the first crop of the year. The cultivar "Dominador" was used, which has an indeterminate growth habit, high vigor, average cycle duration of 120 d and fruits with an aptitude for salads. The seedlings were produced in trays and transplanted to the field when they had two leaves, 28 d after sowing.

Cultural management including fungicides, insecticides and herbicides was carried out in all treatments. Each plot was composed of five rows, with a 6 m length, 2 m row spacing, and 0.6 m between plants. The useful area of each plot consisted of the three central lines, discarding 0.5 m at each border. The plants were irrigated with a central pivot and received fertirrigation according to the nutritional needs of the crop.

The experiment design used a randomized block with four treatments and five replications. The treatments consisted of four management types of the soil-mineral compound during the crop cycle: management 1 was the control; management 2 consisted of foliar applications at the dose of 0.75 kg ha^{-1} per application at 15, 25, 40 and 60 days after transplanting (DAT); management 3 consisted of foliar applications of 0.5 kg ha^{-1} at 15 DAT, 0.750 kg ha^{-1} at 25 DAT, 1.0 kg ha^{-1} at 40 DAT, and again at 60 DAT; and management 4 consisted of foliar applications of 1.0 kg ha^{-1} per application at 15, 25, and 40 DAT.

The soil-mineral compound was a fine, balanced powder, prepared by milling, micronization and standardization of special clays that are commercially distributed in Brazil. The soil-mineral compound was a fine powder composed of Al_2O_3 (20.6%), SiO_2 (17.4%), S (9.8%), CaO (1.3%), TiO_2 (0.34%), MgO (0.18%), Fe_2O_3 (0.16%), and P_2O_5 (0.10). The doses were as indicated in the commercial product (Rocksil®).

The foliar applications were done with costal spraying with a CO_2 injection. The bar contained a fan-type nozzle, with a constant pressure of 2.0 bar.

Biochemical evaluations

Leaf samples for the nitrate reductase determinations were performed at 8, 26, 40, 47 and 73 DAT, and, for the other analyses, they were taken only at 73 DAT. Completely expanded leaves were collected from the middle third of the plants.

The activity of the enzymes nitrate reductase, urease, lipid peroxidation, peroxidase enzymes, superoxide dismutase, phenylalanine ammonia-lyase, hydrogen peroxide, and total proteins were determined.

The NR analysis was performed according to the method proposed by Mulder *et al*. (1959). The urease was evaluated throughout extraction, and the analysis of the plant material was done according to the methodology adapted from Hogan *et al*. (1983).

Samples of 200 mg of fresh biomass of leaves were macerated with 4.0 mL of 0.1 mol L^{-1} potassium phosphate buffer pH 6.8. Then, the samples were transferred to Eppendorf flasks and centrifuged at 10,000 rpm for 30 min at 4°C (Kar and Mishra, 1976). Then, the samples were stored at -20°C until determination of the total protein content of the leaf (Bradford, 1976), superoxide dismutase activity (SOD) (Beuchamp and Fridovich, 1971), peroxidase activity (POD) (Teisseire and Guy, 2000), and phenylalanine ammonia-lyase activity (PAL) (Umesha, 2006). With the fresh leaf biomass the hydrogen peroxide content (H_2O_2) (Alexieva *et al*., 2001) and lipid peroxidation (LP) (Heath and Packer, 1968) were also evaluated.

Biomass attributes

One plant per replicate was harvested at 47 and 83 DAT for determination of the root, stem and leaf biomass. The samples were dried in a forced-air oven at 65°C for 48 h before the determination of the dry biomass. Productivity harvests were also performed at 73, 81, 92, 102, 110, 119, 124, 130 DAT.

Statistical analysis

The data were evaluated for normality and homogeneity using Levene and Shapiro-Wilk tests, both at the 5% significance level. A variance analysis was performed, and, when significant, the Tukey test was applied at the 5% level of significance. For some analyses, regression analysis was also performed. The analyses were performed using statistical software Genes (Cruz, 2013).

RESULTS AND DISCUSSION

For the nitrate reductase variable, the variance analysis presented significance only for the management; there was no effect from the harvesting times or interaction. The other attributes of nitrogen metabolism and the activity of antioxidant enzymes had no effect from the management. There was a difference in the productivity attributes.

The nitrate reductase enzyme did not present a significant difference during the evaluation periods although some management showed a tendency for increasing (Fig. 1). The nitrate reductase enzyme acts on nitrogen assimilation in plants and reduces nitrate to nitrite through NADPH (nicotinamide adenine dinucleotide phosphate hydrogen) energy. Thus, the increase in this enzyme activity contributes to the increase of the assimilation of nitrogen (N) and the growth and development of the plant (Taiz *et al*., 2016).

Resistance induction represents an extra energy expenditure for plant defense and reduces nitrate reductase metabolism and consequently plant growth. Thus, we verified that the foliar applications of the soil-mineral compound may not have activated resistance mechanisms related to the nitrogen metabolism enzymes.

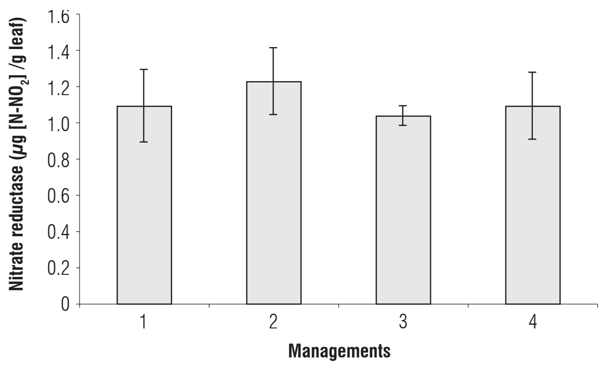

Figure 1. Activity of the enzyme nitrate reductase when submitted to different managements for foliar applications of the soil-mineral compound. Nitrate reductase (µg N-NO$_2$/g leaf). The vertical bars indicate ± standard error.

According to Lawlor (2002), the role of nitrogen in the production of dry phytomass and, consequently, remobilization to reserve organs is directly related to photosynthesis. Photon energy is converted into chemical energy and stored in ATP (Adenosine triphosphate) and secondary metabolites, primarily NADH, which is used in the synthesis of carbon and nitrogen assimilates, particularly amino acids (Lawlor, 2002).

It was found that the activity of nitrate reductase reduces during harvests, more accentuated after flowering. We hypothesize that the fruiting drain competes for the N present in the plant, which explains this reduction in enzyme activity.

The protein content and the activity of the urease enzyme did not differ between the management types; however, it was noted that treatments 2, 3 and 4 increased the total protein content (Fig. 2A).

The quantification of the protein content in the leaf during the reproductive growth evidences the redistribution of amino acids from leaves to reproductive growth. A higher protein content in the leaves may be an indicator of greater assimilation of atmospheric CO_2 because the main enzyme of photosynthesis is composed of N, the ribulose-1.5-bisphosphate carboxylase/oxygenase (Rubisco) (Taiz *et al*., 2016).

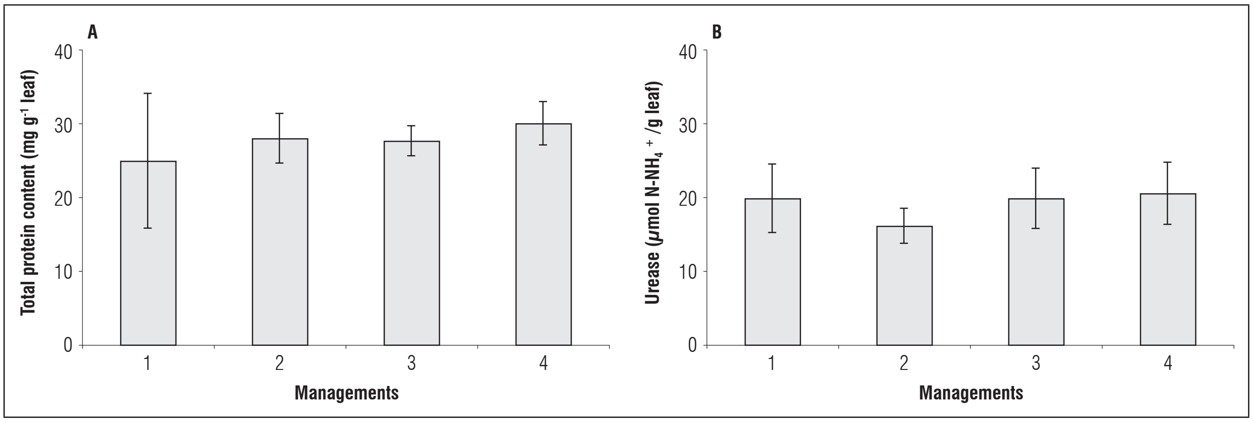

Figure 2. Protein content (A) and urease enzyme activity (B) in tomato leaves at 73 DAT when submitted to different management types for foliar applications of the soil-mineral compound. The vertical bars indicate ± standard error.

Oxidative metabolism

The managements 2, 3 and 4 presented higher levels of hydrogen peroxide than management 1 (control), without significant differences (Fig. 3A). Management 4 presented, on average, values of hydrogen peroxide production that were approximately 56% higher than treatment 1 (control), which demonstrated that the resistance inducer activated the oxidative metabolism in some signal transduction pathways in the plant tissue.

The increase of this free radical indicates that the defense signaling of the plants probably activated genes related to pathogenesis. Thus, the increase in the synthesis of hydrogen peroxide does not always result in negative effects in plants, such as destruction of membranes, because this molecule can function as a signal agent in plants, which includes responses to pathogen elicitors, stomatal closure, acquired systemic resistance, and programmed cell death (Chen and Gallie, 2005).

For the enzyme POD, there was a positive trend in managements 2, 3 and 4, without statistical significance (Fig. 3B). The increase in the activity of the POD enzyme as well as the increase in H_2O_2 content was a protective measure to degrade the reactive oxygen species (ROS) in the plants (Barbosa *et al*., 2014). PODs are related to the synthesis of lignin and suberin, which increase the hardness of tissues and the production of quinones and active oxygen, which have antibiotic activities (Stout *et al*., 1994).

The activity of the SOD enzyme was reduced in managements 3, 4 and 2, in relation to treatment 1 (control) (Fig. 3C). This enzyme is responsible for the degradation of superoxide ERO in hydrogen peroxide, so it is later degraded by CAT and POD enzymes (Mittler, 2002). An increased SOD and POD activity is directly related to plant tolerance to environmental stresses, such as saline stress (Koca *et al*., 2007). However, when the activity of the enzyme is saturated before reaching the maximum concentration of reactive oxygen species, lipid peroxidation may occur.

The activity of the PAL had a different behavior (Fig. 3D). This enzyme is responsible for catalyzing the conversion of phenylalanine to trans cinnamic acid, the first step for the biosynthesis of phenylpropanoids. This compound is the basis for the synthesis pathway of secondary metabolites that exhibit antioxidant activity, such as flavonoids and tannins (Dias *et al*., 2015). A different behavior can be explained by a different route of action for defending plants against stress. Alternative products, such as chitosan and plant extracts, may increase the activity of phenylalanine-ammonia-lyase (PAL) (Lorencetti *et al*., 2015).

The levels of lipid peroxidation were similar among all treatments (Fig. 3E). Lipid peroxidation is used as a basis to measure the damage caused by the action of reactive oxygen species on the unsaturated lipids of cell membranes. This leads to membrane destruction, failure of cellular mechanisms and, in extreme cases, cell death (Lima and Abdalla, 2001). Therefore,

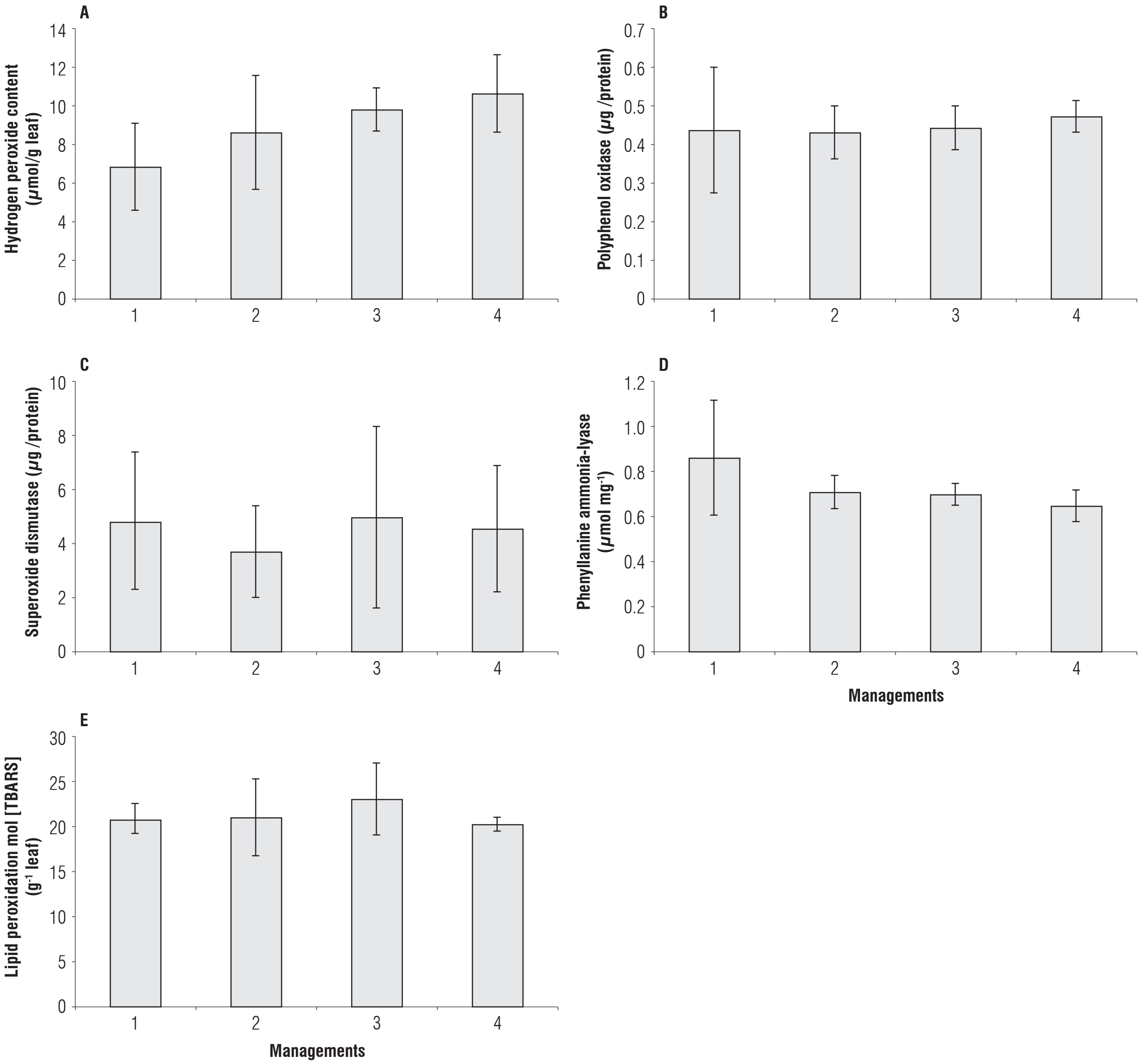

Figure 3. Hydrogen peroxide content (A), polyphenol oxidase activity (B), superoxide dismutase μ^{-1} (C), phenylalanine ammonia-lyase (D), and lipid peroxidation (E) of tomato leaves at 73 DAT, when submitted to different management types for foliar applications of the soil-mineral compound. The vertical bars indicate ± standard error.

because there was no difference between the treatments and the control, there was no cellular damage from the foliar application of the soil-mineral compound.

Silicon increases the synthesis of phenolic compounds in plants and increases the activity of the following enzymes: polyphenol oxidase (PPO), peroxidases (POD) and phenylalanine ammonia-lyase (PAL) (Gomes *et al*., 2005). As a consequence, it tends to reduce the rate of pathogen growth and the incidence of pest insects. The application of Si increased the accumulation of phenolic compounds in walls of epidermal cells of *Triticum aestivum*, and consequently, increased the resistance of plants infected by *Blumeria graminis* f. sp. *tritici* (Bélanger *et al*., 2003).

A PPO plays an important role in plants since it provides resistance to attack from pathogens and diseases. It has also been reported that PPO may exert a direct relationship with photosynthesis since it assists in the maintenance of system homeostasis

(Boeckx *et al*., 2015). This enzyme performs the oxidation of diphenol in quinone, a beneficial compound for the photosystem. In addition, during this process, PPO removes excess O_2 from the system, avoiding the possible formation of superoxide radicals (Boeckx *et al*., 2015).

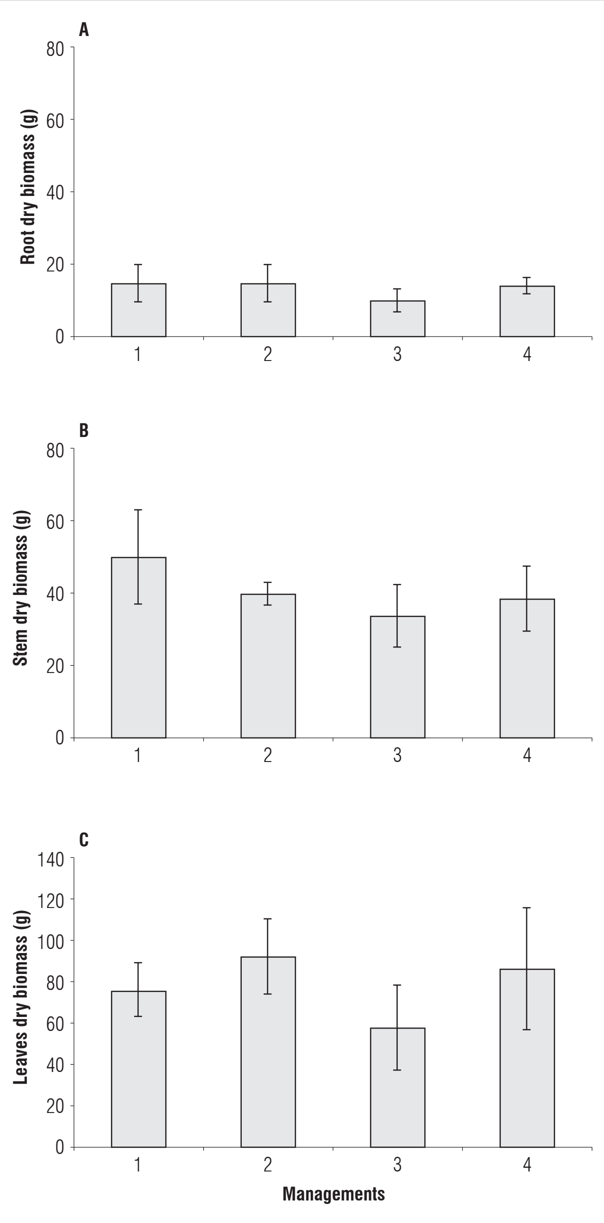

Figure 4. Root (A), stem (B), and leaf (C) biomass of tomato plants when submitted to different management types for foliar applications of the soil-mineral compound. The vertical bars indicate ± standard error.

Although the foliar applied soil-mineral compound had Si, no change in the activity of SOD and PAL enzymes was observed. The interaction of Si with these enzymes is probably more complex and depends on other factors, such as dose, culture, form of application, and interaction with other nutrients in the application, among other factors, which needs more studies to be better understood.

Biomass attributes

About 95% of the dry mass accumulated by plants during their cycle is derived from the photosynthetic activity, and the rest comes from the soil (Benincasa, 2004). The root dry and stem dry biomass did not differ between the management types (Fig. 4AB). There was a trend for a higher dry leaf biomass in management 4, without significant differences (Fig. 4C).

Si, after being absorbed, is translocated and deposited just below the cuticle, forming a double layer of silicon-cuticle. This contributes to protection from abiotic stresses, such as: elemental toxicity, salinity, and frost, among others; as well as protection from biotic stresses, such as pests and diseases (Ranganathan *et al*., 2006). The accumulation of Si makes leaves more upright and rigid and increases the interception of light and, consequently, photosynthetic efficiency (Gonçalves, 2009).

Productivity

All treatments with foliar applications of the soil-mineral compost increased yield per plant and yield. The highest production per plant and productivity were obtained in management 3, with 3.11 kg/plant and 31,152 kg ha^{-1}, respectively (Fig. 5, A and B). The use of the soil mineral compost increased tomato yield by 20% in management 3, a good option for increasing crop productivity.

Si, applied in the form of aluminum silicate on vines (*Vitis vinifera*), contributes to the control of mildew (*Plasmopara viticola*) (Gomes *et al*., 2011). The use of a soil-mineral compost in guava (*Psidium guajava*) contributes to the control of anthracnose (*Colletotrichum gloeosporioides*) and reduced the diameter of colletotricum lesions (*Colletotrichum gloesporioides*) in fruits (Gomes *et al*., 2016). The aluminum oxide (Al_2O_3), silicon dioxide (SiO_2) and sulfur (S) present in the soil-mineral compost inhibited in vitro tests on the

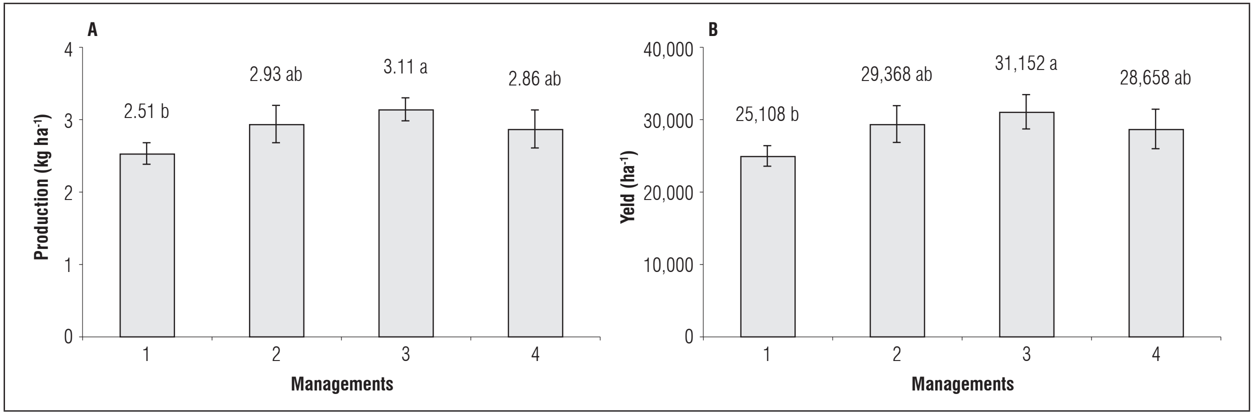

Figure 5. Production per plant (A) and yield (B) of tomato plants when submitted to different management types of foliar applications of the soil-mineral compost. Means with different letters indicate a significant statistical differences according to Tukey test ($P \leq 0.05$) ($n=5$) ± standard error.

mycelial growth of colletotrichum (*Colletotrichum gloesporioides*) (Gomes *et al*., 2016).

We hypothesize that foliar applications of aluminum oxides can trigger the production of reactive oxygen species in plants, which stimulates the production of antioxidant enzymes.

Si was identified as a resistance inducer in some monocotyledons, such as rice (*Oriza sativa*), corn (*Zea mays* L.) and wheat (*Triticum aestivum* L.), which actively absorb and accumulate large amounts of silicon (Liang *et al*., 2015). This was also observed in some dicotyledonous crops, such as cotton (*Gossypium hirsutum*), soybean (*Glycine max* (L.) Merr.), and tomato (*Solanum lycopersicum* L.), and also in some cucurbitaceous species (Liang *et al*., 2015).

Si can favor the upright position of leaves, indirectly favors photosynthesis, and makes the opening and closing of stomata more efficient (Pereira *et al*., 2003). The productivity of sugarcane increased with the foliar applications of Si (Elawad *et al*., 1982). Foliar applications of potassium silicate (K_2SiO_3) increase the chlorophyll content and growth of strawberry plants (*Fragaria* × *ananassa*) (Wang and Galletta, 1998).

The effectiveness of the application of Si in the mitigation of stress depends on the species (Mitani and Ma, 2005) and the level of stress suffered by the plant (Hodson *et al*., 2005). In this experiment, the plants were not subjected to severe stress conditions, which may have contributed to the stress metabolism not responding significantly to the soil-mineral compound treatments. Biochemical and molecular responses using Si occur when the plant is subject to stress conditions (Liang *et al.*, 2015).

Although the treatments with foliar applications did not modify the activity of the evaluated enzymes, the increase in productivity showed that the management types with the compost soil mineral contributed in another way to an increased tomato productivity. We hypothesized that there may have been an increase in proteins, lignins, and efficiency of the photosynthetic activity, but more studies are required for a better understanding.

CONCLUSION

The foliar applications of the soil-mineral compost at doses of 0.5, 0.75, 1.0 and 1.0 kg ha^{-1} at 15, 25, 40 and 60 DAT, respectively, increased the yield of the table tomatoes.

No effect from the foliar applications of the soil-mineral compound was observed on the enzymes related to stress metabolism as a total protein, hydrogen peroxide, nitrate reductase enzyme activity, urease, superoxide dismutase, peroxidase, phenylalanine ammonia lyase and lipid peroxidation under the conditions of this study.

Conflict of interests: this manuscript was prepared and reviewed with the participation of all authors, who declare that there exists no conflict of interest that puts at risk the validity of the presented results.

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Selecting squash (*Cucurbita* sp.) introductions by seed nutritional quality and seed meal

Selección de introducciones de zapallo *Cucurbita* sp. por calidad nutricional en semilla

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Brown seed, characteristic of the cultivar Unapal Abanico-75.

Photo: M.-P. Valdés-Restrepo

ABSTRACT

Squash (*Cucurbita* sp.) is widely used in Colombia as both food and animal feed. However, its seeds are discarded. This study aimed to identify squash genotypes with a high nutritional value in the whole seed meal (WSM) and defatted seed meal (DSM) within a group of 19 introductions (14 of *Cucurbita moschata* and 5 of *C. sororia*). For WSM, 70% of the introductions presented above-average values for extract (36.9%) and crude protein (26.34%); the fiber values were 20.34% neutral detergent fiber (NDF) and 13% acid detergent fiber (ADF). For DSM, 57% of the introductions presented above-average crude protein (43.5%) and 52% above-average crude energy values (4078 cal g^{-1}). Based on the relative feed value (RFV), introductions 1229, 1200, 1201, 1219 and 1206 were selected for WSM, and 1206 1229, 932, 1200, 786 and 954 were selected for DSM. In the selected *C. moschata* introductions, parents with general and specific combining abilities for ether extract and crude protein in WSM or high RFV in DSM should be identified. *C. soraria* introductions 1202 and 954 should be used in crosses that aim to obtain F_2 segregants for seeds with a high oil content and high RFV in DSM.

Additional key words: *Cucurbita moschata*; *Cucurbita sororia*; defatted seed meal; squash seeds; whole seed meal; high forage value.

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RESUMEN

Zapallo (*Cucurbita* sp.) se ha utilizado como alimento en humanos y animales. Sin embargo, sus semillas, son descartadas. Por consiguiente, este estudio tuvo como objetivo identificar los genotipos de zapallo con alto valor nutritivo en harina de semilla entera (HSE) y harina de semilla desgrasada (HSD) dentro de un grupo de 19 introducciones (14 de *Cucurbita moschata* y 5 de *C. sororia*). En cuanto a la HSE, el 70% de las introducciones presentaron valores superiores a la media para el extracto de éter (36.9%) y proteína cruda (26.34%). Con respecto a la HSD, el 57% de las introducciones presentaron proteína bruta por encima del promedio (43.5%) y el 52% tenían valores de energía bruta por encima del promedio (4078 cal g^{-1}). La HSE de zapallo presenta un 20.34% de fibra detergente neutra y un 13% de fibra detergente ácida. Con base en al valor de relativo del forraje (VRF), las introducciones 1229, 1200, 1201, 1219 y 1206 se seleccionaron para HSE y 1206 1229, 932, 1200, 786 y 954 para HSD. En las introducciones seleccionadas de *C. moschata*, la recomendación es identificar a los padres con habilidades combinatorias generales y específicas para el extracto de éter y la proteína bruta en HSE y para una alta VRF en HSD. Las introducciones de *C. soraria* 1202 y 954 deben usarse en cruzamientos que pretenden obtener segregantes de F2 para semillas con alto contenido de aceite y alta VRF en HSD.

Palabras clave adicionales: *Cucurbita moschata*; *Cucurbita sororia*; harina de semilla desgrasada; semillas de zapallo; harina de semilla entera.

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INTRODUCTION

Identification of the place of origin has been the main research topic in Cucurbitas; however, little is known about the seed as a source of energy despite its high content of ethereal extract (Rodríguez *et al.*, 2018). The sororia subspecies includes wild populations with a broad distribution, from Mexico to Nicaragua, which is the wild ancestor of the domesticated species *C. argyrosperma*. The fruits are white with green and have varied sizes. The pericarp is usually coriaceous and is hard to fracture even with a percussion tool, with a bufoid pericarp and bedding (Valdés *et al*., 2014). The mesocarp can be colorless in some introductions; in others, it has an intense yellow color and bitter taste. Some fruits have a salmon-yellow mesocarp, which, upon, contact with air turns from deep green to completely black in a few minutes after removing the seeds, as seen in *C. moschata* (Hidalgo and Vallejo, 2014). *C. moschata* is a domesticated species in South America. Although there is no agreement on the precise area of its domestication, it has been mentioned that northern Colombia is possibly the center of origin (Ortiz *et al*., 2013). The seeds have different shades of coloration in the testa, ranging from havana to coffee with golden edges; the fruits usually have a soft pericarp, which can be smooth or curled, with soft bufoids, sardines, particular colors, one or two colors in bands or spots and variable format. The mesocarp is orange, ranging from pale orange to deep orange. The matrix has an intense orange; some matrices, upon contact with air, become intense green (Hidalgo and Vallejo, 2014).

The economic value of tropical pastures –the basis of nutrition for cattle, goats, and sheep– has been widely documented (McIlroy, 1973; Roy, 2000; Ramírez *et al*., 2012; Sánchez *et al.,* 2015). However, the forage that has been used to feed animals in the tropics lacks protein and energy (Cook *et al*., 2005; Holguín *et al.* 2015) and must be supplemented with agroindustrial raw materials or cereal and legume grains. New genotypes must be identified that can replace the available feed resources to ensure balanced livestock production, enhancing animal performance while reducing the need to use costly imported grains (Ortiz *et al.,* 2013).

Ruminants, for example, are capable of degrading low-quality forage that is rich in plant cell walls, which is not suitable for feeding most monogastric animals that have enzymatic digestive processes (Pereira *et al.,* 2015) because of multiple symbiotic, physical and mechanical associations that occur in the digestive tract, specifically in the rumen (McSweeney and Mackie, 2012). Starch is fermented into volatile fatty acids and nitrogen sources that are degraded to ketoacids and ammonia, the latter being the main source of N for microbial synthesis (Nikkhah, 2015). The intensity of this degradation process varies, depending on the magnitude of the potential degradable fraction and the retention time in the rumen as a function of forage quality, the amount of ammonia in the substrate and the available energy derived from high-quality grains, which degrade more quickly and yield more nutrients for cattle, improving the nutritional efficiency (Olabisi, 2015). Therefore, research is needed on a new source of nutrients for animals, such as Cucurbita Spp seeds.

The whole seed meal (WSM) from squash cultivar Abanico-75 contained 36.25 ± 3.25% ether extract (EE), which was composed of 55.28% unsaturated fatty acids (of which 55.11% was linoleic acid). The defatted seed meal (DSM) contained 51.1 ± 0.95% protein and 4604.66 ± 134.08 kcal kg^{-1} crude energy (CE). The seed starch content ranged from 13 to 28% (Valdés *et al.,* 2014). In 2009 and 2013, the Vegetables Research Group of the Universidad Nacional de Colombia-Palmira campus studied a collection of butternut squash (*Cucurbita moschata* Duch.) genotypes from Central America to determine whether the nutritional composition in the WSM and DSM was sufficiently variable to select superior genotypes for farmers or animal feed (Valdés *et al.* 2014; Ortiz *et al.,* 2013).

The present study aimed to identify squash genotypes with a high nutritional value in WSM and DSM in a group of introductions that belong to the Vegetable Research Program's work collection of the Universidad Nacional de Colombia-Palmira campus.

MATERIALS AND METHODS

Trial site

The experiment was carried out during the first semester of 2017 on the Mario Gonzales Aranda Experimental Farm and in the Seeds and Animal Nutrition Labs of the Universidad Nacional de Colombia,

Table 1. Squash introductions used in the quality screening trial.

Serial	Introduction Code	*Cucurbita* species	Lugar	Municipio	Estado
1	Unapal Abanico-75	*C. moschata*	Palmira	Valle del Cauca	-----
2	419	*C. moschata*	Ocozocoautla	Ocozocoautla de espino	Chiapas
3	1201	*C. moschata*	Tecomatlan	Autlan de navarro	Jalisco
4	786	*C. moschata*	Eji. Plan de juarez	Xilitla	SLP
5	802	*C. moschata*	Eji. huichihuayan	Huehuetlan	SLP
6	1222	*C. moschata*	Teneria del Santuario	Celaya	Guanajuato
7	932	*C. moschata*	Guayabitos	Choix	Sinaloa
8	1219	*C. moschata*	La cienaga	El limon	Jalisco
9	1213	*C. moschata*	Espinal	Ocozocoautla de espino	Chiapas
10	1235	*C. moschata*	El encanto	Las Margaritas	Chiapas
11	1229	*C. moschata*	Col. A López Mateos	Tepalcingo	Morelos
12	1206	*C. moschata*	El chante	Autlan de navarro	Jalisco
13	1205	*C. moschata*	Ahuacapan	Autlan de navarro	Jalisco
14	1200	*C. moschata*	El chante	Autlan de navarro	Jalisco
15	1202	*C. sororia*	El cuastecomate	Ejutla	Jalisco
16	1210	*C. sororia*	------	Amatlan de cañas	Nayarit
17	954	*C. sororia*	Culiacan	Culiacan	Sinaloa
18	1215	*C. sororia*	Ejutla	Ejutla	Jalisco
19	1207	*C. sororia*	El chante	Autlan de navarro	Jalisco
Reference	Abanico-75	*C. moschata*			

SLP, San Luis Potosi.

located in the municipality of Palmira, Colombia (03°30'26.8'' N 76° 18'47.6'' W; average annual temperature, 24°C; 1,000 m a.s.l.; average annual precipitation, 1,000 mm) (Valdés *et al.,* 2010).

Biological study materials

The WSM and DSM came from 19 squash introductions: 14 butternut squash (*Cucurbita moschata*) introductions from Colombia and 5 introductions of a wild Mexican gourd (*Cucurbita sororia*), which belong to the Vegetable Research Program's work collection, Universidad Nacional de Colombia-Palmira Campus (Tab. 1). The WSM and DFM made from the fruit of squash cultivar Unapal Abanico-75 were used as the reference.

Seed treatment protocol. All squash seed samples were lyophilized and minced in a 2-blade Wiley Mill to obtain 1 mm-sized particles. The EE of the ground and lyophilized WSM and DSM was determined using a Soxhlet extractor, preserved separately in 50 ml Eppendorf tubes kept under refrigerated conditions (between 4 and 12°C) for a subsequent quality analysis based on complete proximate analysis, along with fiber analysis using the Van Soest method (Tab. 2).

Table 2. Proximate and fiber analyses of the butternut squash whole seed meal and defatted seed meal based on dry matter content.

Component	Component determined	Method used
Crude protein (CP)	Nitrogen × 6.25	Kjeldahl
Total ashes	Minerals	AOAC 942.05
Ether extract (EE)	Crude fat	Soxhlet
Fiber	Neutral detergent fiber (NDF), acid detergent fiber (ADF), lignin	Van Soest
Crude energy (CE)	Total energy content	Calorimetric pump

Selection of introductions for their nutritional value

The results of the proximate and fiber analyses were used to select the introductions with the highest nutritional value in the WSM and DSM. The weighted selection index (WSI) was used (Roy, 2000; Valdés *et al.,* 2014) as follows: (1), where $WSI_{(k)}$ was the standardized selection index for the k-th introduction (k: 1,2,3…), P_i was the weighted factor assigned to i-th descriptor (i: 1, 2, 3…), $\overline{X}_{i(k)}$ was the average of the i-th descriptor in the k-th introduction, $\overline{X}_{g(i)}$ was the overall average of the collection for the i-th descriptor, and $S_{g(i)}$ was the overall standard deviation of the i-th descriptor considering the k introductions.

$$WSI_{(k)} = \sum_{1}^{19} Pi \frac{(\overline{X}_{i(k)} - \overline{X}_{g(i)})}{S_{g(i)}} \tag{1}$$

The WSI value was calculated for each of the 19 introductions based on the following characteristics: ash content, crude protein (CP) and EE in the WSM and ash content, CP and CE in the DSM. The weighting factor Pi was assumed to be 0.33 and was equal for all variables, based on the assumption of equality of relative importance when studying an introduction for animal nutrition purposes.

Selecting introductions by relative feed value

The protocol of Hoffman and Combs (2014) was followed to determine the relative feed value (RFV) of each introduction. The fiber analysis, in terms of neutral detergent fiber (NDF) and acid detergent fiber (ADF), was used to determine the feed value and forage quality. The following general model was used:

$$RFV = (DDM \times DMI) / 1.29 \tag{2}$$

where DDM was digestible dry matter = 88.9 - (0.779 × % ADF) and DMI was dry matter intake (% liveweight) = 120 / (% NDF).

The data were processed using Excel and subsequently analyzed using the SAS (statistical analysis software) statistical package (SAS, 2013).

RESULTS AND DISCUSSION

Proximate characterization of the squash whole seed meal and defatted seed meal

The dry matter content of the WSM was higher than that of the DSM, probably because the DSM components (sugars, starches) are fat-free and, as a result, are not protected from atmospheric humidity, possibly expressing some sort of hygroscopic ability (Tab. 3 and 4).

In general, the average ash and CP contents of the WSM were lower than those of the DSM, while the CE was higher in the WSM than in the DSM. This was due to the fact that oil, the largest source of energy, was eliminated from the WSM during the ether extraction, whereas in the DSM, the mass ratios favored the remaining metabolites, especially starch and CP (Tab. 3 and 4).

The overall mineral content, represented by the ash content, in the WSM and DSM in this study was higher than the ash content in the squash WSM (3.9%) reported by Ortiz *et al.* (2009) and that of peanut seed meal (2.6%) reported by Pascual *et al.* (2006) (Tab. 3 and 4). The reference material, a WSM made from the fruit of cultivar Abanico-75, presented above-average mineral ash content values (6.18–7.62%), as compared with the WSM (4.13%) and DSM (5.31%) (Tab. 3).

The three introductions with the higher EE contents were 1200 (43.61%), 1219 (42.03%) and Unapal Abanico-75 (41.86%). Based on this information and reports by Ortiz *et al.* (2009), squash seed can be considered an oleaginous raw material.

Of the 19 studied introductions, 70% presented an above-average EE content (36.9%) in the seeds (Tab. 3). According to Younis *et al*. (2000), the EE of squash seeds is higher than that of sunflower seeds (36%), except in the case of squash introduction 802 (30.43%), agreeing with results found by Roy (2000) when compared with maize (5% EE) and linseed (20.7% EE).

Table 3. Nutritional value and weighted selection index (WSI) for whole seed meal of 19 squash introductions and cultivar Abanico-75 (the latter used as the reference).

Introduction	Dry Matter (%)	Ash (%)	Ether extract (%)	Crude protein (%)	Crude energy (cal g^{-1})	WSI	Ranking
1205	94.17	4.41	35.96	23.99	6075.62	-0.1022	12
1207	94.79	4.46	38.77	26.45	5935.76	0.2457	8
1202	93.85	4.12	37.84	29.91	5760.64	0.3211	7
1213	94.29	4.31	34.75	30.0	5691.34	-0.2040	14
1206	94.58	3.05	33.75	28.0	5740.3	-0.8258	19
1229	95.03	4.27	41.37	27.12	6097.11	0.3325	6
1201	93.57	4.35	38.9	30.22	6523.15	0.4998	4
1200	93.62	4.13	43.61	29.2	5747.29	0.5691	2
419	92.31	3.62	37.89	21.33	5002.31	-0.5839	17
1210	93.12	3.38	37.03	26.87	5778.83	-0.2950	16
932	93.37	4.69	34.84	27.31	5882.01	0.2365	9
1219	94.26	4.27	42.03	29.35	6112.84	0.5396	3
802	93.42	4.62	30.43	24.98	5657.46	-0.1844	13
1222	94.23	3.62	41.56	27.76	5288.96	0.0952	10
Abanico-75-S*	93.38	3.07	41.86	21.81	5948.52	-0.6129	18
1235	93.08	4.77	39.43	28.4	5880.92	0.5722	1
Abanico-75-F**	88.45	6.18	10.01	12.68	3818.87	-1.3925	20
786	93.93	4.47	38.9	27.8	4405.45	0.3631	5
954	93.31	3.77	38.38	27.8	5915.85	0.0193	11
1215	93.41	3.22	41.07	25.94	5926.01	-0.2543	15
Mean	93.5	4.13	36.91	26.34	5659.46		
LSD (5%)	0.89	0.47	4	2.678	408.34		
CV %	1.45	17.45	19.26	15.41	10.93		

* Whole seed meal made from the fruit of cultivar Abanico-75 (reference). ** Defatted seed meal made from the fruit of cultivar Abanico-75 (reference).

Of the studied squash introductions, 70% presented an above-average CP content in the seeds (average = 26.34%) (Tab. 3). The CP values were similar to or higher than those for peanut (*Arachis hypogea*), 26.0%; Canavalia grain (*Canavalia ensiformis*), 16-23%; cotton seed (*Gossypium* spp.), 12-22%; and Nima bean (*Phaseolus vulgaris*), 16-20%. For the seed CE, 65% of the introductions presented above-average values (5659.46kcal kg^{-1}), surpassing that of sunflower seed (5,353 kcal kg^{-1}) (Arija *et al.,* 1999) (Tab. 3).

The WSI for the nutritional value of the squash seeds showed that 57% of the introductions were above-average in several of the studied variables, with 1235, 1200, 1219 and 1201 ranking first (Tab. 3).

Of the introductions included in this study, 57% presented above-average CP values (43.5%) in the DSM, presenting higher values than palm kernel cakes (14%), cotton (29.5%), rapeseed (34.5%), copra (21%) (Chatterjee and Walli, 2002) and *Jatropha curcas* fruits (Rodriguez *et al*., 2016). In addition, the CP value in the DSM of introductions 1205 (46.16%), 1229 (52.48%) and 1222 (52.22%) as well as that of cultivar Abanico-75 (51.33%) was higher than that of soybean meal (46%) (Tab. 4).

For the CE in the DSM, 52% of the introductions presented above-average values (4,078 cal g^{-1}). Introduction 1201 presented the highest energy content in the WSM (6,523.15 cal g^{-1}) and introduction 1202

Table 4. Nutritional value and weighted selection indexes (WSI) for defatted seed meal of 19 squash introductions and cultivar Abanico-75 (the latter used as the reference).

Introduction	Dry matter (%)	Ash (%)	Crude protein (%)	Energy (cal g^{-1})	WSI	Ranking
1205	89.91	5.36	46.16	3828.14	-0.5603	19
1207	91.64	5.63	41.31	4107.12	0.1055	7
1202	90.1	5.2	45.82	4290.07	0.6107	4
1213	90.21	5.53	42.76	4035.63	-0.0683	11
1206	90.41	3.6	40.63	4134.69	-0.5465	18
1229	87.08	5.36	52.84	4195.82	0.6674	3
1201	89.95	5.76	39.81	4092.93	0.0580	8
1200	89.98	5.05	40.48	4197.76	0.1181	6
419	90.56	4.73	37.61	4167.96	-0.1762	15
1210	91.66	4.59	39.77	4053.16	-0.4542	17
932	91.78	5.68	44.37	3935.31	-0.2272	16
1219	90.1	5.51	44.8	4053.37	0.0462	9
802	90.2	6.41	38.96	3954.64	-0.1190	13
1222	91.43	4.47	52.22	4149.74	0.2139	5
Abanico-75-S*	91.84	4.03	51.33	4129.52	-0.0245	10
1235	90.17	6.82	49.82	4129.76	0.8853	1
Abanico-75-F**	89.73	7.62	11.16	3825.62	-1.0532	20
786	89.75	5.97	49.78	4220.64	0.8339	2
954	90.2	4.62	51.89	3996.41	-0.1573	14
1215	89.92	4.38	49.73	4074.61	-0.1087	12
Mean	90.33	5.31	43.56	4078.64		
LSD (5%)	0.6	0.63	6.01	81.18		
CV%	1.16	17.97	20.92	3.017		

* Whole seed meal made from the fruit of cultivar Abanico-75 (reference). ** Defatted seed meal made from the fruit of cultivar Abanico-75 (reference).

presented the highest energy content in the DSM (4,90.07 cal g^{-1}) (Tab. 4).

Introductions 1235, 786, 1229 and 1202 ranked first based on the WSI, presenting outstanding nutritional values (Tab. 4).

Based on the data collected in this study, all of the WSMs and DSMs presented higher nutritional values than the raw materials on the market. This study aimed to determine which introductions could serve as potential parents in future breeding efforts and selection processes conducted by the university's Vegetable Research Program. Based on the WSI, those introductions presenting the best proximate performance in terms of WSM were 1235, 1200, 1219, 1201, 954 and 786; in terms of the DSM, introductions 1235, 786, 1229, 1202 and 1222 had the best performance. Likewise, introductions 1235 and 786 ranked first in both the WSM and DSM, based on the comprehensive weighted value.

Fiber quality of whole seed and seed meal of squash introductions

Both food and animal feed industries require raw material with minimal values of NDF, ADF and lignin, particularly in the case of animal nutrition, where the high digestibility of nutrients in ruminants and monogastric animals that present enzymatic digestive processes is associated with a low NDF and ADF with respect to total fiber content. The RFV assigned to the WSM and DSM in Tab. 5 and 6 assumed that the high RFVs mean that these introductions have a high biological value. Based on the foregoing, the best introductions in terms of RFV for the WSM were 1229, 1200, 1201, 1219 and 1206 (Tab. 5).

Table 5. Relative feed value (RFV) of whole seeds of 19 squash introductions and cultivar Abanico-75 (the latter used as the reference).

Introduction	Neutral detergent fiber (%)	Acid detergent fiber (%)	Dry matter consumption[1] (% liveweight)	Digestible dry matter[2] (%)	RFV[3]	Ranking
1205	25.6	18.46	4.69	74.52	270.78	12
1207	23.16	17.39	5.18	88.76	356.53	7
1202	38.23	27.03	3.14	88.69	215.80	16
1213	22.75	15.46	5.27	88.78	363.01	6
1206	21.94	17.83	5.47	88.76	376.34	5
1229	18.76	11.82	6.40	88.81	440.36	1
1201	20.17	15.75	5.95	88.78	409.44	3
1200	19.92	15.43	6.02	88.78	414.59	2
419	31.31	23.77	3.83	88.71	263.58	13
1210	26.59	17.08	4.51	88.77	310.54	9
932	28.71	22.09	4.18	88.73	287.49	10
1219	21.03	13.53	5.71	88.79	392.77	4
802	39.22	31.5	3.06	88.65	210.27	19
1222	38.45	30.01	3.12	88.67	214.51	18
Abanico-75-S*	29.75	13.74	4.03	78.20	244.51	14
1235	33.58	27.45	3.57	67.52	187.03	20
786	30.59	13.7	3.92	78.23	237.89	15
954	25.16	18.46	4.77	74.52	275.52	11
Abanico-75-F**	22.71	6.64	5.28	83.73	342.96	8
1215	30.59	23.22	3.92	70.81	215.34	17
Mean	27.41	19.01	4.60	84.06	301.46	
LSD 5%	4.23	4.27	0.67	4.75	52.55	
CV %	23.43	34.06	22.37	8.56	26.42	

[1] Estimated as 120 / (% neutral detergent fiber). [2] Estimated as 88.9 – (0.779 x % acid detergent fiber). [3] Estimated as (digestible dry matter x dry matter intake) / 1.29. * Whole seed meal made from the fruit of cultivar Abanico-75 (reference). ** Defatted seed meal made from the fruit of cultivar Abanico-75 (reference).

As indicated above, after the seed oil extraction, the DSM acquired a special biologic value because of its high protein content. However, what determines whether the RFV of the DSM is apt for cattle nutrition is the level of fiber (NDF or ADF). Introductions 1229, 932, 1200, 786 and 954 ranked first for this variable (Tab. 6).

Based on the selected introductions, the recommendation is to identify *C. moschata* parental materials with both a general combining ability and a specific combining ability for EE and CP in the WSM and for a high RFV in both the WSM and DSM (Ortiz *et al.,* 2013).

A crossbreeding process should be carried out with *C. sororia* introductions 1202 and 954 to obtain superior F_2 segregants for oleaginous and protein grain (Ortiz *et al.* 2013).

CONCLUSIONS

The squash WSM and the DSM meal derived thereof presented a high nutritional value with the potential to replace protein and oleaginous grains in ruminant feed.

Of the introductions submitted to proximate analysis to determine nutritional value, 10% of the *C. sororia* introductions (954 and 1215) and 26% of the *C. moschata* introductions (786, 1200, 1219, 1229 and 1235) ranked high in terms of quality.

Based on the RFV, introductions 1229, 1200, 1201, 1219 and 1206 were selected for the WSM, and 1206, 1229, 932, 1200, 786 and 954 were selected for the DSM.

In the case of *C. moschata* introductions 1200, 1201, 1206, 1213, 1219 and 1229, parents with both general

Table 6. **Relative feed value (RFV) of defatted seed meal made from 19 squash introductions and cultivar Abanico-75 (the latter used as the reference).**

Introduction	NDF (%)	ADF (%)	Dry matter consumption[1] (% liveweight)	DDM[2] (%)	RFV[3]	Ranking
1205	18.95	17.72	6.33	75.10	368.64	11
1207	22.46	13.99	5.34	78.00	323.06	18
1202	35.25	25.26	3.40	69.22	182.68	20
1213	20.7	10.53	5.80	80.70	362.64	12
1206	19.68	15.67	6.10	76.69	362.51	13
1229	13.58	8.38	8.84	82.37	564.25	2
1201	19.97	10.85	6.01	80.45	374.74	9
1200	17.16	9.73	6.99	81.32	440.83	4
419	21.36	12.6	5.62	79.08	344.42	15
1210	20.51	16.35	5.85	76.16	345.44	14
932	16.21	10.23	7.40	80.93	464.43	3
1219	20.1	7.37	5.97	83.16	384.86	7
802	29.26	14.9	4.10	77.29	245.73	19
1222	21.86	13.01	5.49	78.77	335.18	16
Abanico-75-S*	19.55	11.87	6.14	79.65	379.01	8
1235	19.69	12.46	6.09	79.19	374.14	10
786	16.84	19.29	7.13	73.87	408.07	5
954	18.78	10.51	6.39	80.71	399.80	6
Abanico-75-F**	12.33	5.4	9.73	84.69	638.97	1
1215	22.59	13.13	5.31	78.67	323.96	17
Mean	20.34	12.96	6.20	78.80	381.16	
LSD 5%	3.28	2.96	0.92	2.30	64.49	
CV %	24.45	34.63	22.63	4.43	25.65	

[1] Estimated as 120 / (% neutral detergent fiber). [2] Estimated as 88.9 - (0.779 x % acid detergent fiber).

[3] Estimated as (digestible dry matter x dry matter intake) / 1.29. * Whole seed meal made from the fruit of cultivar Abanico-75 (reference). ** Defatted seed meal made from the fruit of cultivar Abanico-75 (reference). NDF, neutral detergent fiber. ADF, acid detergent fiber. DDM, Digestible dry matter.

and specific combining abilities for oil and CP contents in the WSM and for high RFV in both the WSM and DSM should be identified.

A crossbreeding process should be carried out with *C. sororia* introductions 1202 and 954 to obtain F_2 segregants for seeds with a high oil value in the WSM, as well as for a high RFV in the DSM.

Conflict of interests: this manuscript was prepared and reviewed with the participation of al authors, who declare that there exists no conflict of interest that puts at risk the validity of the presented results.

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Diazotrophic bacteria in the growth of micropropagated ornamental pineapple

Bacterias diazotróficas en el crecimiento de la piña ornamental micropropagada

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Ornamental pineapple cv. Vitória.

Photo: A.B. Silva

ABSTRACT

Ornamental pineapple is a hardy plant with significant landscaping value. Tissue culture of plants is viable for producing plants with a high phytosanitary quality. However, one of the difficulties with this cultivar is the acclimatization process, which is slow and can cause losses. The objective of the present study was to verify the potential of inoculation with diazotrophic bacteria for in *vitro* and *ex vivo* growth of ornamental pineapple. A group of diazotrophic bacterial strains selected at the Universidade José do Rosário Vellano (UNIFENAS) was prioritized in this study, and the treatments included bacterial strains UNIFENAS (100-13, 100-60, 100-68, 100-153, 100-167 and 100-198). These strains were evaluated in terms of their capacity to produce indole 3-acetic acid. Subsequently, plants were cultivated in a medium composed of MS medium salts (1/4), adding 1 mL of the bacterial strain. In the control treatment, the plants were maintained in 2 mL of MS medium. 7 days after inoculation, the plants were transplanted into the MS, where they were maintained for 30 days. After *in vitro* cultivation, the plants were transferred to pots containing commercial Plantmax® substrate and maintained under these conditions for 60 days. The diazotrophic bacteria were able to synthesize auxins, and their inoculation promoted greater growth *in vitro* and *ex vitro* in the plants. In the acclimatization phase, the plants inoculated with UNIFENAS strains (100-60, 100-68 and 100-153) promoted a higher shoot growth, chlorophyll content and nitrate reductase enzyme activity.

Additional key words: *Ananas*; *in vitro*; bacterial strains; acclimatization; pigments; nitrate reductase activity.

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RESUMEN

La piña ornamental es una planta rústica y de alto valor paisajístico. El cultivo de tejidos de la especie se muestra viable, produciendo plantas con alta calidad fitosanitaria. Una de las dificultades de ese cultivo es el proceso de aclimatación, que ocurre lentamente y puede causar pérdidas. El presente estudio tuvo como objetivo verificar el potencial de inoculación de las bacterias diazotróficas en el crecimiento *in vitro* y *ex vitro* de la planta de la piña ornamental. Un grupo de cepas de bacterias diazotróficas seleccionadas en la Universidade José do Rosário Vellano (UNIFENAS) fueron usadas en el estudio y las cepas bacterianas UNIFENAS 100-13, 100-60, 100-68, 100-153, 100-167 y 100-198, constituyeron los tratamientos. Se evaluaron las cepas con relación a la capacidad de producir ácido indol-3-acético. Posteriormente, las plantas fueron cultivadas en medio MS (1/4) y 1 mL de la cepa bacteriana. En el tratamiento control se mantuvieron las plantas con 2 mL de medio MS. Después de 7 días de la inoculación, las plantas fueron trasplantadas a MS, donde permanecieron por un período de 30 días. Después del cultivo *in vitro*, las plantas fueron transferidas a materas con el substrato comercial Plantmax®, donde se mantuvieron por 60 días en estas condiciones. Las bacterias son capaces de sintetizar auxinas y su inoculación promueve mayor crecimiento de las plantas *in vitro* y *ex vitro*. En la fase de aclimatación, las plantas inoculadas con cepas UNIFENAS 100-60, 100-68 y 100-153, promovieron un mayor crecimiento de brotes, un mayor contenido de clorofila y una actividad de la enzima nitrato reductasa.

Palabras clave adicionales: *Ananas*; *in vitro*; cepas bacterianas; aclimatación; pigmentos; actividad nitrato reductasa.

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INTRODUCTION

Bromeliads are found in tropical regions, with around 70% of the genus having been identified and 40% of the species being native to Brazil. Ornamental pineapple belongs to the Bromeliacae family and is a species of great economic value, the third most sold bromeliad in the world. It is cultivated in Thailand, Costa Rica, Brazil, the Philippines, Indonesia and India (FAO, 2016).

Ananas comasus var. bracteatus is a native species widely used in landscaping to outline garden areas and flowerbeds (Oliveira *et al*., 2010), which is perennial and easily grown, and is appreciated for its beautiful leaves and flowers. One of the issues with its large-scale use, however, is the difficulty in finding seedlings with a high genetic and phytosanitary quality that meets the demands of the consumer market.

Conventional propagation methods for bromeliads generate a low number of shoots, as well as high indices of spreading disease (Matos *et al*., 2009). In this context, alternative methods such as stem sectioning (Freitas *et al*., 2012), propagation of the fruit crown (Santos *et al*., 2011) and micropropagation (Baldotto *et al*., 2010) are being investigated to try to minimize these problems.

Bromeliad micropropagation techniques produce healthy and standardized plants (Matos *et al*., 2009; Baldotto *et al*., 2010). However, plants obtained through *in vitro* cultivation have deficient anatomical and physiological characteristics, such as low quantities of cerinas, heterotrophic metabolism, thinner cuticles and external periclinal walls of the epidermal cells, low stomatic density (Barboza *et al*., 2006), poorly functioning roots and inactive photosynthetic structures (Souza *et al*., 2009). During acclimatization, they can present low survival rates (Hazarika, 2006), increasing the price of plants produced using this technology, which hampers their uptake by ornamental plant and floral producers.

A reduction of losses during the acclimatization phase can be achieved using better acclimatization periods (Berilli *et al*., 2011), leaf fertilization (Bregonci *et al*., 2008) and inoculation of micropropagated plants with diazotrophic bacteria, which have presented positive results in terms of adaptation to environmental changes by seedlings from *in vitro* pineapple cultivation (Baldotto *et al*., 2010).

Diazotrophic bacteria present beneficial effects on plant growth, such as biological fixation of nitrogen (Li *et al*., 2008) and solubilization of phosphate. They are also antagonistic to pathogenic species, produce plant hormones (indole-3-acetic acid – AIA) and promote plant growth (Moreira and Siqueira, 2002; Moreira *et al*., 2010). The AIA produced by these bacteria can increase the length and number of radicular hairs, increasing the exploration area of roots, thereby providing greater nutrient and water absorption and tolerance to low soil humidity conditions (Ryan *et al*., 2008; Moreira *et al*., 2010; Cassán *et al*., 2014).

Oliveira *et al*. (2006) related that results demonstrate the feasibility of the inoculation technology using diazotrophic bacteria in micropropagated sugarcane and plants grown in soils with low to medium levels of fertility. Dias *et al*. (2009), using diazotrophic bacteria strains, verified promoted root and plant shoot development. The plant growth promotion correlated with IAA production and phosphate solubilization. Bacterial effects could potentially be harnessed to promote plant growth during seedling acclimatization in strawberry.

Inoculation with diazotrophic bacteria can be a viable acclimatization strategy for pineapple plants propagated *in vitro*, producing hardier plants adapted to field conditions. Therefore, the present study aimed to determine the potential of diazotrophic bacteria for *in vitro* and *ex vitro* growth of ornamental *Ananas comousus* var. bracteus pineapple plants.

MATERIAL AND METHODS

In vitro culture

The present study was conducted in the Plant Biotechnology Laboratory of the Universida de José do Rosário Vellano (UNIFENAS), Alfenas-MG and established June, 2016. The plants were obtained with pineapple axillary buds culture (*Ananas comosus* var. bracteatus L.), which were inoculated in MS medium salts (Murashige and Skoog, 1962): 1.0 mg L^{-1} of BAP and 30 g L^{-1} of sucrose, solidified with 6 g L^{-1} of agar and pH adjusted to 5.8 before autoclaving at 121°C for 20 min. Plants in the third subcultivation were stored in a growing room for 60 d with a temperature of 24±2°C, 16 h photoperiod and photosynthetic photon flux density of 36 μmol m^{-2} s^{-1}.

Six diazotrophic bacterial strains belonging to the collection of the Agricultural Microbiology Laboratory of the UNIFENAS were isolated from soil samples and *Brachiaria decumbens* plant tissues, collected from soil located in southern Minas Gerais (Tab. 1). These strains have been tested for their potential to promote the growth of plants by Florentino *et al*. (2017) and Terra *et al*. (2019).

The bacterial strains were preserved in water according to the Romeiro (2001) methodology and reactivated and cultivated in a liquid FAM medium (Magalhães and Dobereiner, 1984) for 3 d, enough time to reach the log growth phase, around 10^9 UFC/mL. Prior to the inoculation with the bacteria together with the explant, the capacity of the strains (Tab. 1)

Table 1. Identification, medium used for bacteria isolation and morphological characteristics of strains cultured in FAM medium containing bromothymol blue as pH indicator.

Strains	Culture medium of origin	Morphological characteristics in FAM medium		
		pH	Color	EPS
UNIFENAS 100-13	JNFb	Acid	Yellow	High
UNIFENAS 100-60	JNFb	Acid	Yellow	Medium
UNIFENAS 100-68	LGI	Acid	Yellow	Medium
UNIFENAS 100-153	FAM	Acid	Yellow	Medium
UNIFENAS 100-167	NFb	Acid	Yellow	Low
UNIFENAS 100-198	LGI	Acid	Yellowish	Medium

JNFb, Johanna nitrogen fixing bacteria medium; LGI, Lipman Glicose Ivo medium; FAM, initials used by the developer of medium (Magalhães and Döbereiner, 1984); NFb, nitrogen fixing bacteria medium; EPS, production of exopolysaccharides.

to produce 3-indoleacetic acid (AIA) in Dygs medium, both with and without (100 μg mL^{-1}) Tryptophan (Trp) was evaluated, according to the methodology described by Pedrinho *et al*. (2010). The experiment design was completely randomized, consisting of six bacterial strains in combination with tryptophan (presence/absence), totaling 12 treatments with four repetitions.

Inoculation of diazotrophic bacteria

The plants established in the previous phase were standardized with approximately 2 cm of length, with an aerial part and root system, inoculated in a solid medium containing ¼ of the concentration of the MS medium salts, with 5 g L^{-1} sucrose and 7 g L^{-1} agar, and maintained under these conditions for 30 d.

The treatments were composed of different diazotrophic bacterial strains: UNIFENAS 100 (13, 60, 68, 153, 167 and 198) and a control treatment. The experimental design was completely randomized, containing seven treatments with three repetitions and three plants per parcel.

Diazotrophic bacteria were inoculated together with the plants *in vitro* for a period of 7 d, with the application of 1 mL of cultivation medium with the bacteria added to 1 mL of the previously described MS culture medium (¼), totaling 2 mL of solution per container for the different treatments. In the control treatment, only 2 mL of MS medium (¼) were added. The cultivation was conducted in a growing room at a temperature of 25°C, with a 12-h photoperiod and light intensity of 36 μmol photons m^{-2} s^{-1}.

Acclimatization

After the *in vitro* culture, the plants were transferred to containers (100 cm^3) with the commercial Plantmax® substrate and maintained in an arco model greenhouse with plastic covering and shade cloth siding (50% shading), with a temperature between 16 and 29°C±1°C and 70% UR. The plants were maintained under these conditions for a period of 60 d.

Evaluations

IAA Quantification. The indole acetic acid (IAA) concentration was evaluated using the colorimetric quantitative method (Gordon and Weber, 1951) during the log phase for bacterial growth, presenting approximately 10^9 UFC/mL. The estimation of the IAA quantification during the *in vitro* cultivation of diazotrophic bacteria was realized with the help of the standard-curve previously obtained with the sterilized Dygs medium and with the known IAA concentrations (0, 25, 50, 75 and 100 μg mL^{-1}). The absorbance reading was realized using a spectrophotometer with a 5353 nm.

Phytotechnics. The height of the aerial part, number of leaves, root system length and plant dry mass were evaluated.

Chlorophyll content and nitrate reductase enzyme activity. For the chlorophyll content analysis, two fully expanded leaves were collected by repetition. 0.1 g of leaf was macerated in 5 mL of 80% acetone. The extract was filtered through fiberglass, and the volume was completed with 10 mL of 80% acetone. The readings were realized at 663 and 647 nm with a light absorption spectrophotometer (A) (Arnon, 1949). For the calculation of chlorophyll (μg chlorophyll/mL), the following equations were used: $chlorophyll\ a = (12.25 \times A663) - (2.79 \times A647)$; $chlorophyll\ b = (21.50 \times A647) - (5.10 \times A663)$; $total\ chlorophyll = chlorophyll\ a + chlorophyll\ b$.

The Nitrate reductase enzyme activity (ANR) was determined using the methodology proposed by Cataldo (1975). Leaves were cut into small pieces, and 200 mg were placed in a 15 mL test tube with a stopper that contained 4 mL of KNO_3 0.25 M in phosphate buffer. Subsequently, 1 mL of alpha-napthylamine and 1 mL of sodium acetate buffer were added, completing the volume with 50 mL of distilled water. The reading was realized with a Spectrophotometer adjusted to 540 nm. Both evaluations were realized at the end of plant acclimatization.

The data were submitted to analysis of variance (ANOVA) using the statistical program Sisvar 5.3 (Ferreira, 2011), with the values compared using the Scott-Knott test at 5% probability.

RESULTS AND DISCUSSION

The IAA production was directly affected by the interaction ($P \leq 0.05$) of the factors (bacterial strains and tryptophan). The use of tryptophan (TRP) in the culture medium promoted greater indole acetic acid (IAA) production, mainly with the UNIFENAS

Table 2. Indol acetic acid (IAA, μg mL^{-1}) production by bacterial strains in Dygs medium, with and withou tryptophan.

Medium	Bacterial strains					
	100-13	100-60	100-68	100-153	100-167	100-198
A TRP*	0.91±0.03 Aa	0.33±0.01 Bb	0.47±0.1 Bb	0.42±0.1 Bb	1.51±0.1 Aa	0.50±0.1 Bb
P TRP	1.26±0.40 Aa	1.28±0.22 Aa	0.94±0.1 Ab	1.33±0.4 Aa	1.42 ±0.2 Aa	1.46±0.1 Aa

Means with different capital letters in a column and lower case in a row indicate a significant statistical difference according to the Scott-Knott test ($P \leq 0.05$) ($n=4$)±standard error. *A TRP, absence of tryptophan; P TRP, presence of tryptophan.

100-60, 100-68, 100-153 and 100-198 strains, as compared with the strains cultivated in the culture medium without TRP (Tab. 2).

Biosynthesis of auxins by bacteria occurs via different metabolic pathways (Spaepen *et al*., 2007), with TRP being the main precursor for IAA synthesis – a fact that explains the high indole values detected in the culture mediums that had TRP in comparison with those that did not, except for the 100-167 UNIFENAS and 100-13 UNIFENAS strains, which produced high IAA concentrations both with and without TRP (Tab. 1). Similar results were observed by Baldotto *et al*. (2010), who found higher IAA synthesis values in bromeliad seedlings cv. Vitória when adding TRP to the culture mediums. Studies developed by Pedrinho *et al*. (2010) and Florentino *et al*. (2017) showed that the Ab-V5 strain produced a greater quantity of IAA when cultivated in medium containing TRP (Tab. 2).

The results for the bromeliads cultivated *in vitro* and inoculated with different bacterial strains showed greater growth in the aerial part (LPA) of the plants cultivated *in vitro with* the UNIFENAS 100-13 and

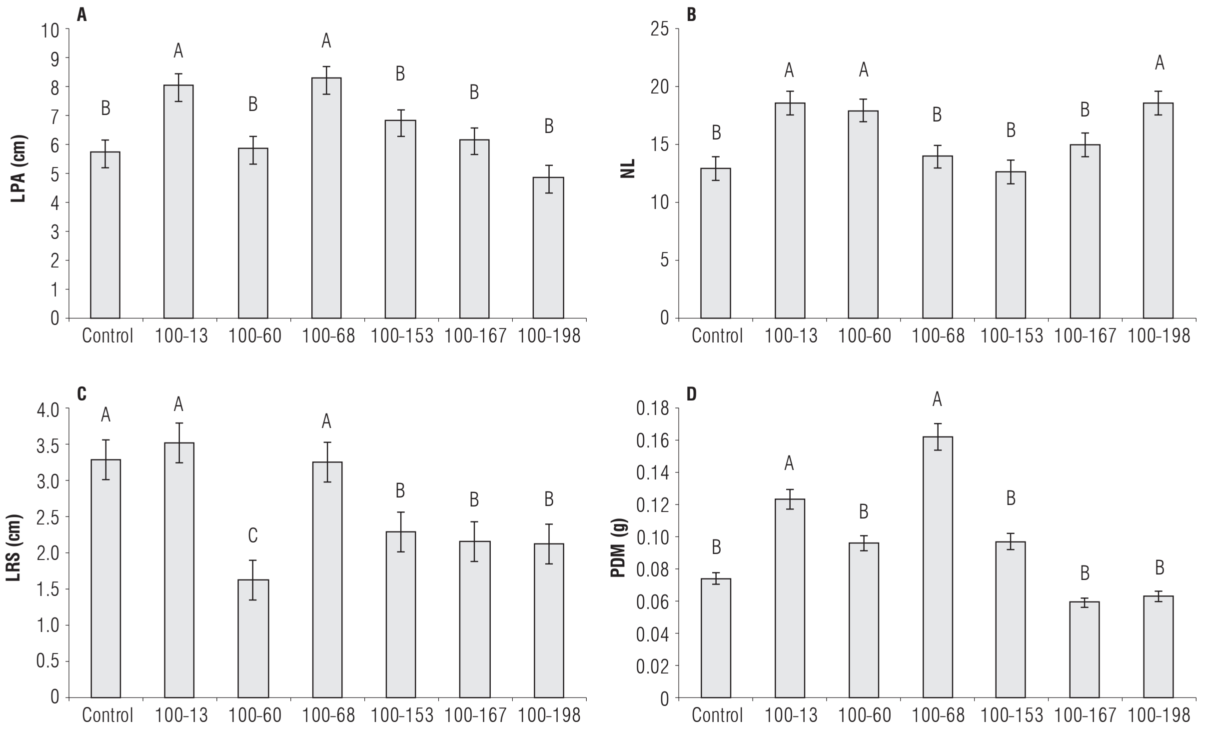

Figure 1. A) length of aerial part (LAP); B) length of root system (LRS); C) number of leaves (NL); D) plant dry mass (PDM) of ornamental pineapple plants cultivated *in vitro* 30 d with diazotrophic bacteria. Means with different letters indicate significant statistical difference according to the Scott-Knott test ($P \leq 0.05$) ($n=4$)±standard error.

UNIFENAS 100-68 strains, 2.5 cm larger than plants cultivated in the control treatment (Fig. 1A). This greater growth of the aerial part led to greater accumulation of dry mass in plants cultivated *in vitro* and inoculated with the UNIFENAS 100-13 and 100-68 strains (Fig. 1D).

The plants inoculated with the UNIFENAS 100-13, UNIFENAS 100-60 and UNIFENAS 100-198 strains presented a larger number of leaves (NL) (Fig. 1B). The size of the root system (LRS) of the *in vitro* bromeliad plants was directly affected by the bacterial strains, with UNIFENAS 100-13 and UNIFENAS 100-68 strains providing greater growth. However, they did not differ from the control treatment, which was greater than the other treatments (Fig. 1C).

Of the strains tested, it was found that inoculation with UNIFENAS 100-13 led to a significant effect on all the evaluated parameters, LPA, NL, LRS and PDM, which could be related to IAA production by this strain, both with and without TRP (Tab. 1). However, it was observed that the other strains, such as UNIFENAS 100-68, UNIFENAS 100-60 and UNIFENAS 100-198 also contributed significantly to *in vitro* cultivation of ornamental pineapple, suggesting a need for further research taking into consideration the possibility of co-inoculation.

During the acclimatization process in the *ex vitro* cultivation, the strains directly affected plant growth (Fig. 2). The UNIFENAS 100-167 strain provided greater growth for the aerial part (LPA) (Fig. 2A). The UNIFENAS 100-60, UNIFENAS 100-68, UNIFENAS 100-153 strains promoted dry mass accumulation (PDM) (Fig. 2AD), which was greater than in the other treatments (Fig. 2D). The inoculation of the plants with better strains resulted in an increase of 1.5 and 5.0 cm for LPA, accumulating 30 to 60% more PDM than in the plants submitted to the control treatment (Fig. 2AD).

The NL and LRS were also affected by inoculation in plants with different strains (Fig. 2, B and C). A greater LRS was observed in the plants inoculated with the UNIFENAS 100-167 strain. For NL, inoculation of

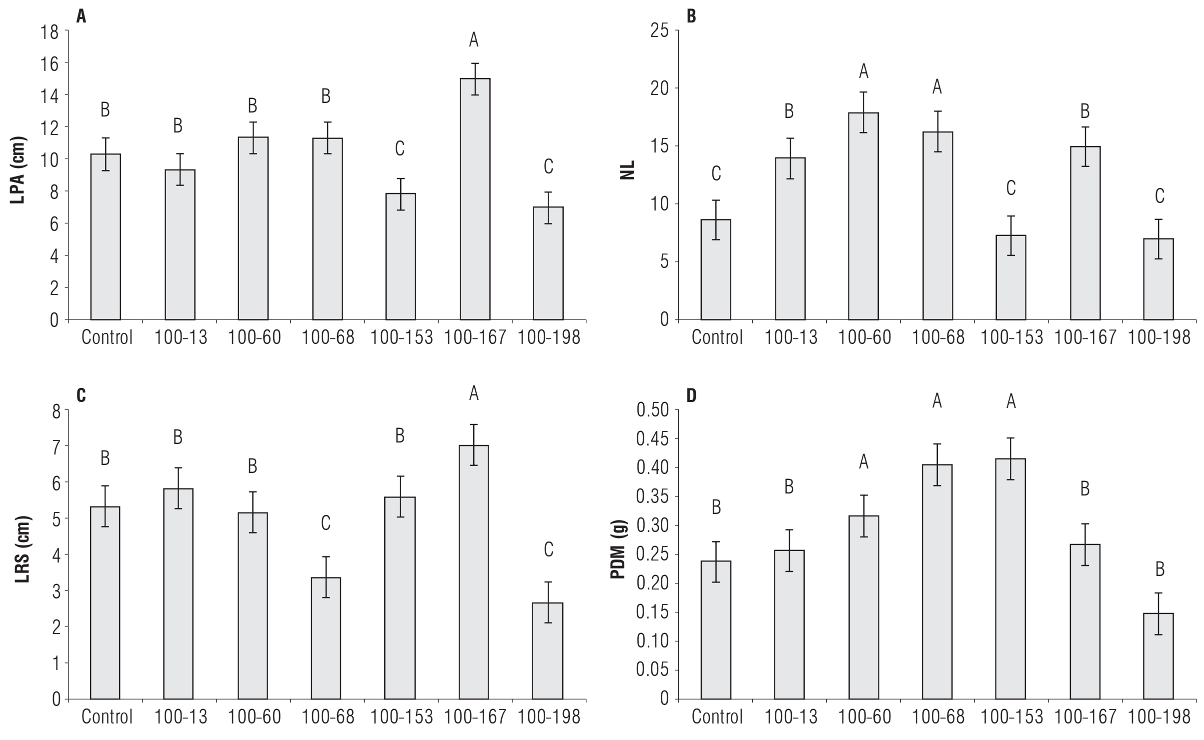

Figure 2. **A) length of aerial part (LAP); B) length of root system (LRS); C) number of leaves (NL); D) plant dry mass (PDM) of ornamental pineapple plants cultivated *ex vitro* (acclimatization) 60 d with diazotrophic bacteria. Means with different letters indicate a significant statistical difference according to the Scott-Knott test ($P \leq 0.05$) ($n = 4$) ± standard error.**

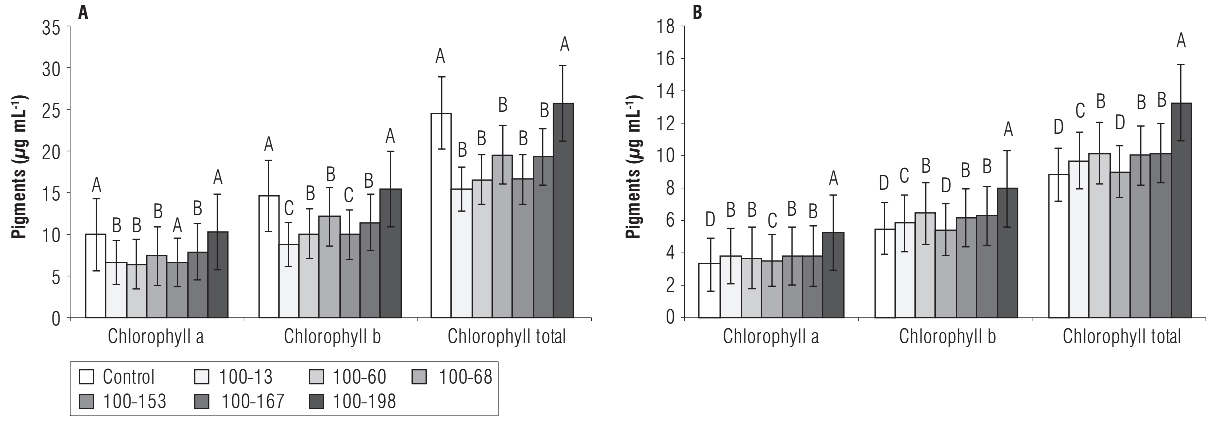

Figure 3. Content of pigments in ornamental pineapple plant inoculated with diazotrophic bacteria *in vitro* (A) and *ex vitro* (B) conditions. Means with different letters indicate a significant statistical difference according to the Scott-Knott test ($P \leq 0.05$) ($n = 4$) ± standard error.

plants with the UNIFENAS 100-60 and UNIFENAS 100-68 strains presented a greater number of leaves (Fig. 2B). Inoculation with bacteria led to greater dry mass and root numbers in acclimatized bromeliad cv. Vitória plants (Silva *et al*., 2016), which was observed for some strains in the present study (Fig. 2).

The promotion of plant growth through inoculation with diazotrophic bacterial strains has already been observed in some agricultural species, such as tomato (Szilagyi-Zecchin *et al*., 2015), rice (Sabino *et al*., 2012), and lettuce (Schlindwein *et al*., 2008; Florentino *et al*., 2017). The beneficial effects of inoculation with these bacteria are related to the fixation of nitrogen, solubilization of phosphate, their antagonistic action against pathogenic species and production of plant hormones such as auxins, all of which promote plant growth (Moreira *et al*., 2010).

Generally, the pigment levels were greater in the *in vitro* culture than in the ex *vitro* cultivation (Fig. 3). This may be related to the lower light intensity during cultivation in the growing room (*in vitro*), making the plants invest more in biosynthesis of pigments, seeking to compensate for the reduction in photosynthesis as a result of the low light intensity. Amâncio *et al*. (1999) and Carvalho *et al*. (2001) reported that an increase in light intensity during acclimatization diminished the pigment levels in grape vines in an in *vitro* culture.

The UNIFENAS 100-198 strain and control treatment presented higher a, b and total chlorophyll levels in the *in vitro* culture than in the other treatments (Fig. 3A). During the acclimatization process of the *ex vitro* plants, similar to what was observed with plants cultivated *in vitro*, the UNIFENAS 100-198 strain presented higher a, b and total chlorophyll levels. However, the control treatment of ex vitro plants presented lower a, b and total chlorophyll levels (Fig. 3B). Inoculation with bacterial strains promoted higher a, b, and total chlorophyll levels than in the non-inoculated plants (Fig. 3B).

The higher photosynthetic pigment levels in the plants inoculated with different bacterial strains when compared with the control plants (Fig. 3) could be related to nitrogen, probably as a result of the biological fixation of nitrogen (Li *et al*., 2008). Nitrogen is a nutrient positively correlated with an increase in pigment levels in the leaves (Argenta *et al*., 2001; Lima *et al*., 2009). In this research, it was observed that the contribution of bacteria promoted plant growth by providing IAA and nitrogen to plant metabolism, resulting in the biomass accumulation observed in the plants during acclimatization (Fig. 2).

The UNIFENAS 100-60, 100-68 and 100-153 bacterial strains promoted nitrate reductase enzyme activity (ANR) (Fig. 4). Nitrate reductase is linked to a reduction of nitrate to nitrite that is subsequently transformed into ammonia and finally assimilated in glutamine by glutamine synthetase (Taiz *et al*., 2017).

Donato *et al*. (2004) and Marcos *et al*. (2016) indicated that the influence of bacteria on nitrogen metabolism is via an increase in ANR, which increases the entry of nitrate and, consequently, promotes an increase in the nitrogen levels in the plant with a greater resulting growth. This may have positively influenced the growth of the plants inoculated with the UNIFENAS 100-60, 100-68 and 100-153 strains (Fig. 4), which presented a greater ANR, as well as a greater dry mass accumulation (PDM) (Fig. 2D). The greater ANR activity could be considered for both the biological fixation of nitrogen and the promotion of greater nitrate absorption by the bacteria (Bashan and Levanony, 1990).

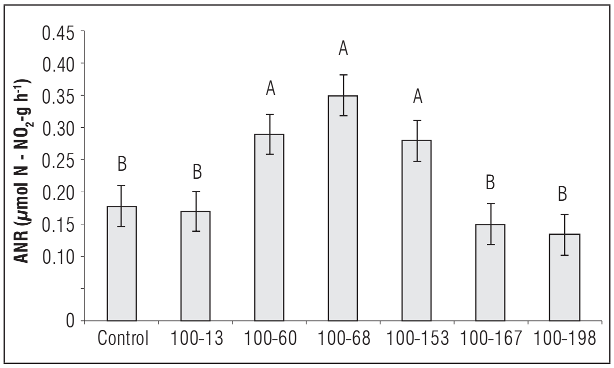

Figure 4. Nitrate reductase enzyme activity (ANR) in *ex vitro* ornamental pineapple plants with diazotrophic bacteria. Means with different letters indicate a significant statistical difference according to the Scott-Knott test ($P \leq 0.05$) ($n = 4$) ± standard error.

CONCLUSION

Diazotrophic bacteria are capable of synthesizing auxins (IAA), and their inoculation in plants promotes greater growth during in *vitro* cultivation and acclimatization phases.

Conflict of interests: the manuscript was prepared and reviewed with the participation of all the authors, who declare that there exists no conflict of interest that puts at risk the validity of the presented results.

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It is possible to maintain productivity and quality standards in carnation with less nitrogen in the fertigation formula

Es posible mantener estándares de productividad y calidad en clavel con menos nitrógeno en la fórmula de fertirriego

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Plants of standard carnation cv. Don Pedro crop, cultivated under plastic greenhouse conditions at Centro Agropecuario Marengo, Universidad Nacional de Colombia.

Photo: A.P. Baracaldo

ABSTRACT

Although nitrogen is the most widely used fertilizer in agriculture, it contaminates the surface and groundwater through leaching. A decrease in the concentration of total nitrogen and changes in the ammonium:-nitrate ratio can provide information for a better use of this nutrient. The objective of this study was to evaluate the effect of a decrease in total nitrogen and an increase in the percentage of $N\text{-}NH_4^+$ on indicators of growth, productivity, quality and nitrogen use efficiency (NUE) in carnation cultivation. In the Centro Agropecuario Marengo at the Universidad Nacional de Colombia, two concentrations of total nitrogen were evaluated (200-140 mg L^{-1} in the vegetative phase and 160-112 mg L^{-1} in the productive phase) with three ratios of $N\text{-}NH_4^+$:$N\text{-}NO_3^-$ (5:95, 15:85 and 25:75) in standard carnation plants cv. Don Pedro grown in substrate. In both fertigation formulas, similar productivity and qualities were obtained, and the formula with less total N provided better NUE, mitigating the negative impact of this nutrient on the environment. Likewise, the ammoniacal component played a preponderant role: the number of flowering stems per plant decreased as the ammoniacal component increased, similar to that observed with the percentage of flowering stems in the 'Select' quality grade.

Additional key words: *Dianthus caryophyllus* L.; cut flowers; soilless cultivation; growth rates; ammonium:nitrate ratio.

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RESUMEN

Aunque el nitrógeno es el fertilizante más usado en la agricultura contribuye a contaminar aguas superficiales y subterráneas a través de su lixiviación. Una disminución en la concentración del nitrógeno total y cambios en la relación amonio:nitrato pueden proveer información acerca de una mejor utilización de este nutriente. El objetivo de este estudio fue evaluar el efecto de la disminución del nitrógeno total y el aumento del porcentaje de N-NH_4^+ sobre indicadores del crecimiento, la productividad, la calidad y la eficiencia del uso del nitrógeno (EUN) en el cultivo de clavel. Para lo cual, en el Centro Agropecuario Marengo de la Universidad Nacional de Colombia se evaluaron dos concentraciones de nitrógeno total (200-140 mg L^{-1} en fase vegetativa y 160-112 mg L^{-1} en fase productiva) con tres relaciones de N-NH_4^+:N-NO_3^- (5:95, 15:85 y 25:75) en plantas de clavel estándar cv. Don Pedro sembradas en sustrato. En ambas fórmulas de fertirriego se obtuvieron productividades y calidades similares y en aquella fórmula con menos N total mejor EUN, mitigando el impacto negativo de este nutriente en el medio ambiente. Así mismo, el componente amoniacal juega un papel preponderante: el número de tallos florales por planta disminuiría en la medida que se aumenta el componente amoniacal, de manera similar a lo observado con el porcentaje de tallos florales en grado de calidad *Select*.

Palabras clave adicionales: *Dianthus caryophyllus* L.; flores de corte; cultivo sin suelo; índices de crecimiento; relación amonio:nitrato.

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INTRODUCTION

The use of nitrogen fertilizers is excessive worldwide, causing pollution problems (Cameron *et al.*, 2013; Schipper *et al.*, 2010; Zhang *et al.*, 2011; Daza *et al*., 2015). It is estimated that, in 2020, approximately 201.7 million tons of NPK-based fertilizers will be used, of which 58.9% will correspond to nitrogen (FAO, 2017). The application of N worldwide has increased more than eight-fold since 1961 (Kant, 2018). In Colombia, the consumption of these agricultural inputs has increased significantly since 2000 despite the fact that no significant increase was observed in the agricultural use area (ICA and MADR, 2015). Locally, fertilization in soilless cultivation systems is based on empirical practices, which generally leads to producers overestimating the needs of plants and applying excessive amounts, with a consequent waste of nutrients and money and risk of contamination for water courses and groundwater. One of the most important objectives for the development of sustainable agriculture is increasing crop yield and quality while using less nitrogen fertilizers and, thereby, improving Nitrogen Use Efficiency (NUE) (Good *et al.*, 2004; Lupini *et al.*, 2017).

The importance of nitrogen for plant development, its essentiality, the compounds it is a part of (Kiba *et al.*, 2011), its influence on metabolic processes (Jin *et al.*, 2015), its role in water use efficiency (Ucar *et al.*, 2017), and the symptoms of its deficiency and toxicity (Gárate and Bonilla, 2013) are widely described in the literature as are the metabolic processes of nitrogen assimilation (Maldonado *et al.*, 2013), the influence of environmental factors and species (Cabrera, 2006), and its mobility, storage, enzyme complexes, organelles and energy sources involved in reduction to ammonium (Barker and Bryson, 2007; Kant, 2018).

Plants have developed mechanisms to modulate efficiency of N uptake in response to factors, such as the availability and form of N present in the soil solution as well as to the N requirement of the plants during the life cycle (Hawkesford *et al.*, 2012).

Strategies for mitigating the impact of nitrogen as a contaminant include lowering the total nitrogen input and increasing the percentage of ammonium. For the efficient use of this nutrient, Kumar *et al.* (2016) showed that, with 0, 50, 100, 150 and 200 mg L^{-1} of N in carnation plants cv. Red Corso, the values of the evaluated variables (number of flower buds per plant, number of flowers per plant, flower size, flower weight, stem length and flower duration) increased significantly up to 150 mg L^{-1}. In several crops, combinations of both forms (N-NH_4^+ and N-NO_3^-) usually result in higher growth than when only one of the forms is used (Vojtíšková *et al.*, 2004; Li *et al.*, 2007). In anthurium, Dufour and Guerin (2005), when using NH_4^+: NO_3^- ratios of 0.25, 0.37 and 0.51 in the nutrient solution, found that increasing the ratio to 0.37 of the total N improved the growth, development and plant yield. Khalaj *et al.* (2017) evaluated the effect of NO_3^-: NH_4^+ (100: 0, 80:20, 60:40, 40:60) ratios in the fertigation solution on gerbera cvs. Stanza and Double Dutch. The 80:20 ratio presented higher values in the evaluated variables (number of leaves per plant, number of flowers, diameter of the stem and flower, fresh and dry mass of roots and shoots and vase life), and, with the 40:60 ratio, the plant growth and biomass were significantly reduced. With the increase in the concentration of ammonium, the carotenoid and chlorophyll contents increased as well as the catalase and peroxidase activity in cells. For a given dose of N and irrigation regime, according to Bar-Yosef (2008), the aforementioned ratios affect several factors in the soil solution and crop: i) nitrogen uptake efficiency; ii) decreased uptake rates for Ca^{+2}, Mg^{+2} and K^+ as the result of competition with NH_4^+ uptake; iii) NH_4^+ decreases the pH of the solution while NO_3^- increases it; and iv) excessive uptake of NH_4^+, particularly at temperatures higher than 28°C in the root zone, is detrimental to the development of roots.

Tabatabaei *et al.* (2006) found in a hydroponic strawberry crop that high NH_4^+ and NO_3^- ratios in the fertigation solution always reduced the yield and that the $25NH_4^+$:$75NO_3^-$ ratio increased the yield by 38% and 84% in cvs. Camarosa and Jungle, respectively. The higher yield with this ratio resulted from increases in the size, length and fresh mass of the fruits; however, the 0 to 75% increase in the NH_4^+ ratio significantly reduced the concentration of fruit Ca and postharvest life of both cultivars. Apparently, a greater foliar area and photosynthesis rate with the 25:75 N ratio were the reasons for the increases in productivity and plant growth. For Roosta (2014), the adverse effects of alkalinity on SPAD values and the maximum quantum yield of PSII (Fv/Fm) were alleviated with increases in the proportion of NH_4^+ in the nutrient solution, increasing the number of fruits and yield in strawberry cv. Camorosa. Among the treatments studied by Abasi *et al*. (2016) in a hydroponic cultivation of tulip cvs. Apricot Parrot and Daytona with a $NH_4^+/NH_4^+ + NO_3^-$ ratio of 0.38 in the nutritive solution, they found the maximum concentrations of Ca and Mg, floral longevity, dry mass and optimal N, P, K. For these reasons, specific studies are required for each species in order to find the best NH_4^+: NO_3^- ratio.

The objective of this study was to evaluate the effect of the lesser rate of total nitrogen and an increase in the percentage of N-NH_4^+ on indicators of growth, productivity, quality and nitrogen use efficiency in standard carnation crop cv. Don Pedro.

MATERIALS AND METHODS

This research was carried out at the Centro Agropecuario Marengo (CAM) at the Universidad Nacional de Colombia, Bogota campus, located in the municipality of Mosquera (Cundinamarca) at 4°42'N and 74°12' W, at 2540 m a.s.l., with an average temperature of 12.4°C, relative humidity between 76 and 89% and annual average rainfall of 1,124 mm (Ordoñez and Bolivar, 2014). During the study, the average temperature and relative humidity inside the greenhouse were 17.5°C and 76%, respectively.

For the test, a traditional greenhouse with a flexon-type metal structure (Acuña and Ortiz, 2004) was used, with passive lateral and zenithal ventilation, in which two warehouses, 9×70 m (630 m^2), were fitted. Six wooden beds (7.0×0.7 m) were built, raised 0.4 m from the ground, with a double container, each measuring 0.25 m wide and 0.2 m deep, and a leachate collection tank at the end of each bed. Standard carnation (*Dianthus caryophyllus* L.) cv. Don Pedro cuttings were used at a density of 15.5 plants per m^2 in an open substrate cultivation system. The substrate consisted of a mixture of 50% raw rice husk + 15% burnt rice husk + 25% reused burnt rice husk + 10% compost, obtained from rose and carnation crop residues (v/v/v/v). The initial EC and pH of this mixture were 0.5-1.0 dS m^{-1} and 6.8 -7.0, respectively. The agronomic crop management described in Flórez *et al.* (2006a) was used.

Six treatments were established, corresponding to modifications in the N content of the conventional formula, commercially applied in the carnation crop (200-160 mg L^{-1} of total N for the vegetative and productive phases of the crop, respectively, maintaining a NH_4^+:NO_3^- ratio equal to15:85). In this formula, the total N was reduced by 30%, and the NH_4^+:NO_3^- ratio was decreased or increased by 10% (Tab. 1). In the fertigation solutions, the EC remained between 1.5 and 2.5, and the pH was between 5.5 and 6.0. The concentrations of the elements (mg L^{-1}) in the vegetative and reproductive phases were: P, 30; K, 150; Ca, 120; Mg, 40; Fe, 3; Cu, 1; Zn, 0.5; B, 1; Mo, 0.1; and S, between 2 and 116 mg L^{-1} because it was used for the stoichiometric balance of the NH_4^+ in the formula. In order to calculate the nutritional solutions, the contribution of water (EC = 0.6 and pH = 7.0) and Mn in the burnt rice husk substrate (Florian-Martínez and Roca, 2011) were taken into account.

For the analysis of growth at 16, 21 and 26 weeks after sowing (WAS) in the production cycle, three destructive samples were carried out in order to measure the variables leaf area and dry mass of root and shoot, separated into dry mass of stems, leaves and flower buds. The leaf area (cm^2) was determined with LI-COR model LI-3100. Based on these data and in accordance with Flórez *et al.* (2006b), the Relative Growth Rate (RGR), the Leaf Area Index (LAI), the Specific Leaf Area (SLA), the Leaf Mass Ratio (LMR) and the Net Assimilation Rate (NAR) were calculated. From 10 WAS, six plants were randomly selected biweekly per treatment for continuous growth measurements. The number of stems per plant was counted, and the length and diameter of the stem, the number of leaves, and the length and diameter of the flower bud were measured on the middle stem of each plant.

The productivity and quality of the carnation flower stalks were recorded for each of the treatments. Productivity was expressed as the number of flowering stems produced per m^2 of greenhouse area, and quality was the percentages of stems according to the parameters of the select, fancy, standard and national grades. This classification was determined, among other characteristics, with the length and strength of the stems, the size and opening point of the flowers, defects in quality attributes or parameters and the presence of pests or diseases, as described by Reid and Hunter (2000) and Escandón (2009).

To determine the Nitrogen Use Efficiency (NUE) in the samples of complete plants by treatment at 16, 21 and 26 WAS, the content of N in the vegetal tissue was determined. The analyses were performed in the Laboratorio de Aguas y Suelos de la Facultad de Ciencias Agrarias at the Universidad Nacional de Colombia, according to the procedures described by Carrillo *et al.* (1994): N (total nitrogen) micro-Kjeldahl methodology. The NUE was calculated taking into account the dry mass accumulation and nitrogen uptake, agreeing to Good *et al.* (2004); according to the following formula: NUE = dry mass of complete plant (g)/nitrogen content in complete plant (g).

Statistical analysis

A completely randomized experimental design was used, with a factorial arrangement of two levels of total nitrogen and three NH_4^+:NO_3^- ratios (Tab. 1). The six treatments had three replicates, and the experiment unit consisted of 2 m of bed with 46 plants. The inferential analysis was carried out with analysis of variance (Anova) and multiple comparison Tukey test, with a level of significance $P \leq 0.05$. Normality and homogeneity of variance in the residuals were verified by means of the Shapiro-Wilk and Levene tests, respectively, to validate the adjusted model. All analyses were performed with the statistical software SAS v. 9.1.

Table 1. Nitrogen concentrations and ratios evaluated in the fertigation formula used in the cultivation of carnation cv. Don Pedro grown in substrate.

Treatments		NH_4^+:NO_3^-	Vegetative phase			Productive phase		
			NH_4^+	NO_3^-	Total N	NH_4^+	NO_3^-	Total N
		(%)	(mg L^{-1})					
5% N-NH_4^+		5:95	10	190	200	8	152	160
15% N-NH_4^+		15:85	30	170		24	136	
25% N-NH_4^+		25:75	50	150		40	120	
30% less N	5% N-NH_4^+	5:95	7	133	140	5.6	106.4	112
	15% N-NH_4^+	15:85	21	119		16.8	95.2	
	25% N-NH_4^+	25:75	35	105		28	84	

RESULTS AND DISCUSSION

Continuous growth analysis

Among the treatments evaluated, the formula with less total N and a lower percentage of NH_4^+ (140-112N-5% N-NH_4^+) presented a number of stems per plant significantly higher than the formula with more total N, with the highest percentages of ammoniacal component; meanwhile, no significant differences were found for the variables length and diameter of the flowering stem (Tab. 2). There were no significant statistical differences in the variables number of leaves (average of 29.1 leaves per stem), length (between 42 and 53 mm) and diameter (average of 22 mm) of the floral bud (data not shown). It was evident that it is feasible to maintain quality attributes and even improve productivity with a more conservative fertigation formula for the nitrogen component.

This finding is consistent with the significant increases in productivity and quality variables reported by Kumar *et al.* (2016) with up to 150 mg L^{-1} of N in carnation plants cv. Red Corso. For NO_3^-: NH_4^+ ratios, similar to that reported here, Khalaj *et al.* (2017) obtained the highest values for variables evaluated in Gerbera cvs. Stanza and Double Dutch with the lowest percentage of ammonium, i.e. the 80:20 ratio. On the other hand, Dufour and Guerin (2005) found improvements in development and yield in anthurium with increases in the NH_4^+:NO_3^- ratio, up to 0.37 of the total N.

This study confirmed that concentrations of 140-112 mg L^{-1} of N in the vegetative and reproductive

Table 2. Length, diameter and number of the flowering stems per plant of standard carnation cv. Don Pedro planted in substrate and subjected to a decrease in total N and increases in the ammoniacal component in the fertigation formula.

Treatments		Week after sowing								
		10	12	14	16	18	20	22	24	26
Total N (mg L^{-1})	N-NH_4^+ (%)	Stem length (cm)								
200-160*	5	12.5 a	16.9 ab	26.6 a	37.5 a	56.8 a	76.5 a	82.6 a	88.3 a	92.8 a
	15	14.1 a	17.0 ab	28.0 a	42.7 a	66.0 a	81.3 a	86.6 a	86.6 a	87.5 a
	25	14.3 a	18.3 ab	27.8 a	42.8 a	63.5 a	78.8 a	85.7 a	87.2 a	88.0 a
140-112*	5	13.9 a	18.7 ab	28.3 a	42.2 a	62.0 a	79.5 a	87.5 a	88.6 a	88.6 a
	15	14.0 a	20.5 a	30.1 a	45.4 a	62.8 a	79.4 a	84.7 a	90.4 a	91.8 a
	25	13.4 a	15.7 b	23.2 a	39.5 a	60.9 a	77.8 a	85.3 a	87.8 a	89.0 a
Standard error		0.36	0.41	0.77	1.37	1.98	1.17	1.16	1.08	1.25
Total N (mg L^{-1})	N-NH_4^+ (%)	Stem diameter (mm)								
200-160*	5	5.1 a	5.6 a	6.3 a	6.4 a	6.4 a	6.2 a	6.4 a	6.5 a	6.2 a
	15	5.8 a	6.5 a	7.1 a	7.4 a	7.3 a	7.0 a	6.8 a	6.9 a	6.9 a
	25	5.2 a	5.9 a	6.8 a	7.1 a	7.1 a	7.0 a	6.8 a	6.8 a	6.8 a
140-112*	5	4.3 a	5.5 a	6.8 a	7.3 a	7.2 a	6.9 a	6.4 a	6.4 a	6.4 a
	15	5.6 a	6.1 a	6.6 a	7.0 a	6.8 a	6.7 a	6.7 a	6.8 a	6.6 a
	25	5.3 a	6.2 a	6.8 a	7.3 a	7.1 a	6.9 a	6.6 a	6.6 a	6.5 a
Standard error		0.15	0.10	0.09	0.10	0.10	0.12	0.09	0.09	0.11
Total N (mg L^{-1})	N-NH_4^+ (%)	Number of stems per plant								
200-160*	5	8.3 a	8.5 ab	8.8 ab	9.2 ab	9.2 ab	8.8 ab	8.8 ab	8.8 ab	8.8 ab
	15	7.7 a	7.8 b	7.8 b	7.8 b	7.8 b	7.8 b	7.8 b	7.8 b	7.8 b
	25	7.8 a	8.2 ab	8.2 ab	8.2 b	8.2 b	8.2 b	8.2 b	8.2 b	8.1 b
140-112*	5	10.0 a	10.3 a	10.3 a	10.7 a	10.7 a	10.7 a	10.5 a	10.5 a	10.4 a
	15	9.0 a	9.0 ab	9.0 ab	9.2 ab	9.3 ab	9.3 ab	9.3 ab	9.3 ab	9.2 ab
	25	7.8 a	8.2 ab	8.2 ab	8.5 ab	8.5 ab	8.5 ab	8.5 ab	8.5 ab	8.4 ab
Standard error		0.23	0.23	0.23	0.23	0.22	0.23	0.22	0.22	0.22

* The first concentration is the one used in the vegetative phase and the second in the productive phase.

Means followed with different letters indicate significant differences by Tukey test ($P \leq 0.05$).

phases, respectively, are adequate for normal plant development and that the number of stems per plant is significantly lower in treatments with a higher concentration of total N, possibly because of a higher percentage of N-NH_4.

Growth rates and indexes

Although the calculated rates and indexes LAI, LMR, SLA, RGR and NAR did not obtain significant statistical differences in any of the three samples in each of the evaluated treatments, the trends of these variables to elucidate the physiological behavior of the submitted plants are presented to the treatments (Fig. 1).

As expected, the LAI presented a pattern of gradual growth in all treatments, with a tendency for better yield in the treatments with a lower amount of total N, independent of the NH_4^+ concentration. When the foliar area intercepts the maximum photosynthetically active radiation, the optimal LAI is

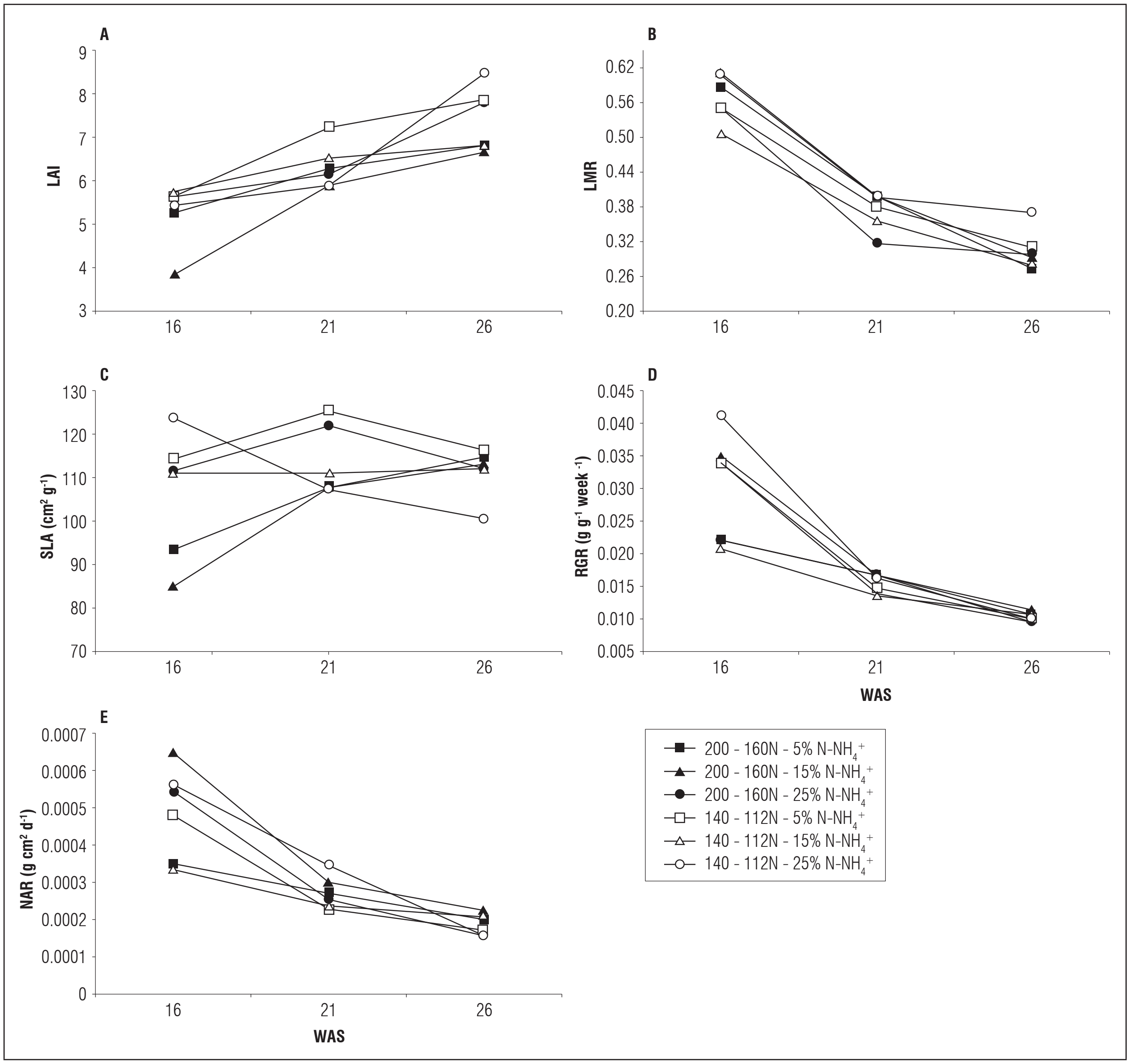

Figure 1. Behavior of standard carnation cv. Don Pedro planted in substrate and subjected to increases in the ammoniacal component of two fertigation formulas. (A) Leaf Area Index - LAI, (B) Leaf Mass Ratio - LMR, (C) Specific Leaf Area - SLA, (D) Relative Growth Rate - RGR, and (E) Net Assimilation Rate - NAR. WAS = weeks after sowing.

obtained (Hunt, 1978); Criollo and García (2009), for radish, and Carranza *et al.* (2009), for lettuce, obtained maximum LAI values of 3.4 and 6.8, respectively. When Cárdenas *et al.* (2006) took into account the leaf area of entire carnation plants cv. Nelson grown on substrate, they reported maximum LAI values between 4.4 and 4.8; meanwhile, for the same cv. at the second harvest peak, Baracaldo *et al.* (2010) reported maximum values between 7.5 and 8.7, similar to those obtained in the present study. Therefore, LAI values depend, among other factors, on the type of plant, variety, phenological stage of development and agronomic management. When plants use N better, the metabolism of carbon is optimized because these processes modulate each other (Maldonado *et al.*, 2013), and leaf area affects the photosynthetic carbon gain, which is reflected in the growth rate of the plant (Taiz and Zeiger, 2002) through synthesis of new biomass.

In all treatments, there was a decrease in the LMR from the beginning of the trial, which indicated an inversion of photoassimilates in the formation of other plant structures. The highest value of this variable was seen in the treatments 200-160-15% N-NH_4^+ (commercial formula) and 140-112N-25% N-NH_4^+ at 16 WAS; although the latter treatment had a higher proportion of ammonium, the accumulation of dry leaf mass was not affected.

In the formulas with more nitrogen, a tendency for greater translocation of dry mass was observed, in comparison with the treatments using the most conservative formulas. When increased to 25% of N-NH_4^+ in the formula with high N, there was a decrease in the amount of biomass that is redirected to other structures of the plant. Therefore, it was inferred that, in the 200-160N-5% N-NH_4^+ treatment, the plants would direct more dry mass for the formation of flower buds, as observed at 26 WAS (Tab. 3). The marked decrease in the LMR from 16 to 21 WAS in all treatments coincided with the maximum AGR for the formation of flower buds (18 and 19 WAS). This relationship slowed down between 21 and 26 WAS when these structures reached their definitive development.

Table 3. Dry mass of carnation plants cv. Don Pedro grown on substrate, with decrease of the total N and increases in the component of N-NH_4^+ in the formula of fertigation.

Week after sowing	Treatment		Dry mass (g)				
	Total N (mg L^{-1})	N-NH_4^+ (%)	Leaves	Stems	Root	Flower bud	Total
16	200 - 160*	5	16.9	11.1	1.0		29.0
		15	13.5	7.9	0.7		22.1
		25	15.0	12.4	0.8		28.2
	140 - 112*	5	15.4	11.7	0.9		28.0
		15	15.5	14.2	0.9		30.6
		25	12.9	7.7	0.6		21.2
21	200 - 160*	5	17.6	25.8	1.7	3.3	48.4
		15	16.4	23.1	0.9	0.9	41.4
		25	15.1	27.7	1.3	2.3	46.5
	140 - 112*	5	17.2	25.6	1.6	1.1	45.4
		15	18.0	28.3	1.4	1.8	49.4
		25	16.4	22.3	1.8	1.3	41.8
26	200 - 160*	5	17.7	33.1	2.9	12.3	65.9
		15	17.7	32.1	2.3	8.2	60.3
		25	20.9	35.2	2.6	7.6	66.3
	140 - 112*	5	20.2	35.0	2.4	10.8	68.4
		15	18.3	36.9	2.1	8.0	65.4
		25	25.8	33.5	2.1	11.2	72.6

* The first concentration is the one used in the vegetative phase and the second in the productive phase.

Although in treatments 200-160N-5% N-NH_4^+ and 200-160N-15% N-NH_4^+, the SLA started low at 16 WAS (Fig. 1C); treatments 200-160N-25% N-NH_4^+ and 140 -112N-5% N-NH_4^+ had the same response profile, that is, they had a greater leaf area in relation to their dry mass, becoming sources of carbohydrates for sink organs between 16 and 21 WAS. The other treatments, although they were sources of assimilates, exported them less efficiently: in the 140-112N-15% N-NH_4^+ treatment, what was synthesized was exported without affecting the SLA, and, in the 140-112N-25% N-NH_4^+, less than what was synthesized was translocated, reducing the SLA and maintaining a good amount of the biomass it synthesized in the leaves (Tab. 3). A high SLA indicates greater foliar area with light leaves and, consequently, greater capacity to capture light and produce carbohydrates. An SLA increase response indicates a constant partitioning of carbohydrates to sinks with high demand. With the most conservative fertigation formulas for total N, the ammoniacal component played a preponderant role, where the highest percentage negatively affected the SLA, as seen in the 140-112N-25% N-NH_4^+ treatment (Fig. 1C).

For RGR, two response groups were observed (Fig. 1D): in the group of treatments with a higher RGR, 140-112N-25% N-NH_4^+ stood out, which means that it was the most efficient treatment in the accumulation of new biomass to the system. This was reflected in leaves with the lowest SLA towards 26 WAS (Fig. 1C). This was explained by a reduction in the leaf area and an increase in the biomass, as shown in Tab. 3, verifying that, in this treatment, the plants invested more dry mass in the leaves and flower buds at the expense of the stems and roots. In the second group, with a lower initial RGR (200-160N-5% N-NH_4^+ and 140-112N-15% N-NH_4^+), regardless of the total N concentration, were not found the highest percentages of ammonium, which would stimulate the accumulation of new initial biomass. According to Grime and Hunt (1975), the highest RGR value was obtained in plants grown under conditions of greater fertility. In gerbera, an increase in the proportion of NH_4^+ in the nutrient solution resulted in larger fresh and dried masses in the shoots and roots, with higher values in the 80:20 (NO_3^-:NH_4^+) ratio (Khalaj *et al.*, 2017).

For Baracaldo *et al.* (2018), a reduction of total nitrogen and an increase in the NH_4^+: NO_3^- ratio in the fertigation formula modified nutrient contents in carnation cv. Don Pedro plant tissue: with the lower content of total N, the contents of Cu and Zn were significantly increased; the increase in NH_4^+ significantly raised the contents of N and Zn and reduced Mg. The highest Mg content was observed at 16 and 21 WAS in treatments with the lowest percentage of NH_4^+ (5%). Thus, from the nutritional point of view, the better yield in the conservative treatments for total nitrogen was understandable, with a lower percentage of ammonium.

Similar to RGR, the same response groups, possibly associated with the percentage of ammonium (Fig. 1E), were evident for NAR. For this parameter, the highest initial NAR value was observed in treatment 200-160N-15% N-NH_4^+. NAR represents the photosynthetic efficiency and decreases in the course of the development of the flowering stem, behavior that is associated with the leaf area existing in the first days after the pinch, when the leaves are more exposed to radiation and are more efficient at the assimilation of CO_2. At the end of the cycle, similar values were seen in all treatments, with the smallest increases in total dry mass resulting from, among other factors, processes of foliar senescence, high planting density and plant architecture, which generate leaf shady, which causes a reduction in photosynthetic efficiency (Fig. 1E).

Productivity and quality

Taking into account the nitrogen factor, no statistically significant difference was found between the 200-160N and 140-112N treatments, with productivity averages of 83.5 and 87.9 stems/m^2 of greenhouse area, respectively; it follows that the same level of productivity could be achieved with less total nitrogen in the formula. However, the increases in N doses used by Thakulla *et al.* (2018) in exotic carnation var. Chabaud had a positive effect on most of the parameters attributed to growth and flowering, with the exception of the maximum dose, which delayed the time to flowering. For this variable, the minimum time was obtained with 300 kg ha^{-1} of N, while the maximum plant height, the length of the flowering stem and the number of branches were obtained with a rate of 400 kg ha^{-1} of N.

On the other hand, there was a decrease in the percentage of stalks in the Select quality grade as the percentage of ammonium increased, with a consequent increase in the percentage of flowering stems in the Fancy quality grade (Fig. 2).

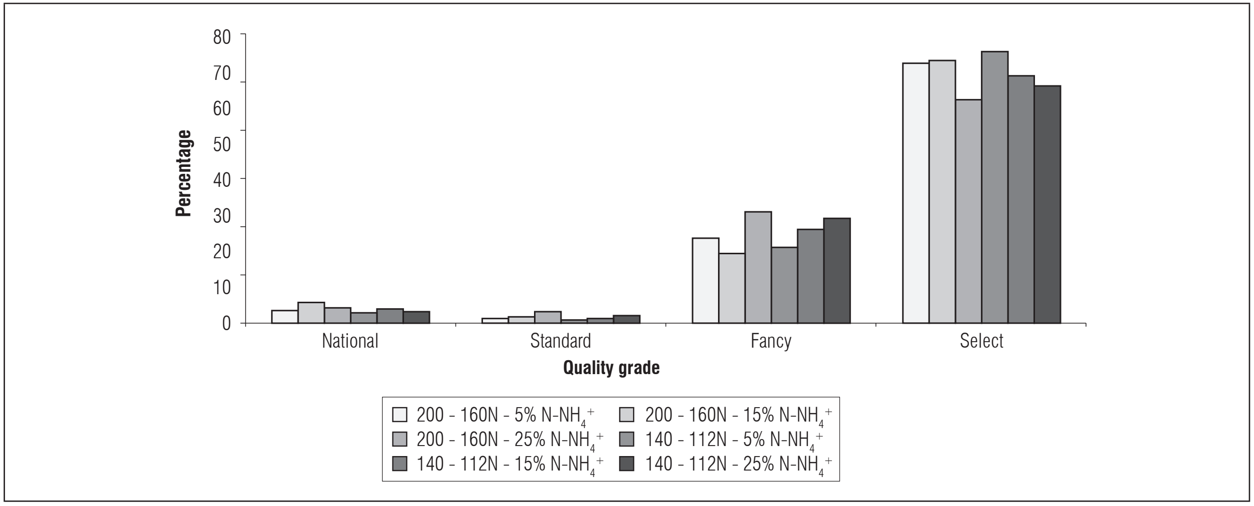

Figure 2. **Quality of standard carnation flowering stems cv. Don Pedro planted in substrate subjected to different nitrogen fertilization treatments.**

Although there would not be a statistical difference, productivity would increase to four more stems per m^2 with a higher degree of quality and the economy in the cost of the fertilizer and in the environmental impact would result in higher profits for producers.

Achievements in productivity and quality based on particular NH_4^+:NO_3^- ratios were addressed by Tabatabaei *et al.* (2006) in strawberries, based on better calcium-based nutrition and increases in leaf area and photosynthesis rate, and better SPAD values and quantum yield of PSII (Fv/Fm) (Roosta, 2014), or, in tulips, with maximum concentrations of Ca and Mg, flower longevity, dry mass and optimal N, P, K (Abasi *et al.*, 2016).

Nitrogen use efficiency - NUE

At 21 WAS, the plants of the 140-112 N treatment presented a significantly higher NUE value compared to the treatment with the highest N concentration, indicating that the gain of dry mass resulted from a more efficient use of N (Fig. 3). Dry mass production is closely related to NUE. In this experiment, no statistically significant differences were found in the dry mass values (Tab. 3), hence the plants fertilized with the lowest concentration of N had higher NUE values, in a manner consistent with that reported by Good *et al.* (2004) and Lupini *et al.* (2017).

With the onset of the reproductive phase, according to Kant (2018), there is a parallel breakdown of proteins in the process of foliar senescence, and the released amino acids and other N compounds must be efficiently directed towards sink organs. This step is a critical component to improving NUE and thus avoiding wasted proteins and amino acids.

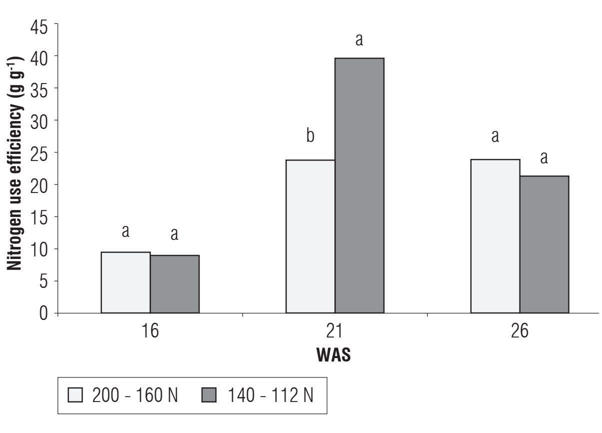

Figure 3. **Efficiency in the use of nitrogen in standard carnation plants cv. Don Pedro planted in substrate under different nitrogen fertilization treatments. Means followed with different letters indicate significant differences by Tukey test (*P*≤0.05).**

In oats with applications of 80, 100 and 120 kg ha^{-1} of N, it was found that NUE decreased significantly with increasing doses of N, with values of 38.3, 34.7 and 30.2 kg of grain/kg of N applied, respectively (Rahman *et al.*, 2011). This coincides with that obtained in this study, where the higher concentrations of N (200 and 160 mg L^{-1}) had a lower NUE than the lowest concentration. To achieve a greater NUE in carnation cv. Gaduina, a constant release of the N incorporated in the soil is required (Muthukrishnan *et al.*, 2014).

CONCLUSIONS

In both fertigation formulas:

- similar productivity and qualities were obtained, and the formula with less total N had better NUE, mitigating the negative environmental impact of this nutrient.
- the ammoniacal component plays a preponderant role: the number of flowering stems per plant decreased as the ammonium component increased, similar to that observed with the percentage of flowering stems in the Select quality grade.

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Conflict of interests: the manuscript was prepared and reviewed with the participation of the authors, who declare that there exists no conflict of interest that puts in risk the validity of the presented results.

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Impact of phosphorus and luminosity in the propagation, photochemical reactions and quality of *Lippia alba* (Mill.) N.E.Br. seedlings

Impacto del fósforo y luminosidad en la propagación, reacciones fotoquímicas y calidad de plántulas de *Lippia alba* (Mill.) N.E.Br.

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Propagation of *Lippia alba* by cutting.

Photo: C.C. Santos

ABSTRACT

Lippia alba (Mill.) N.E.Br. (lemongrass) is a spice and medicinal plant species, with fledgling studies related to mineral nutrition and luminosity on foliar and vegetative responses. Thus, the aim of this study was to assess the foliar aspects and production of *L. alba* under two light conditions (full sun and 50% shading) and four levels of phosphorus-P (0, 150, 300 and 450 mg kg^{-1}). The *L. alba* seedlings presented changes in physiological indices and photochemical responses based on chlorophyll-*a* fluorescence. The phosphate fertilization helped mitigate the light stress for the synthesis of chlorophylls. The greatest leaf biomass production occurred with addition of P. The principal components analysis explained 74% of the variability, with leaf number, initial fluorescence and specific leaf area in principal component 1 (PC 1) and bud number, survival and leaf area in PC 2. Two groups formed in the cluster analysis, with lower distances between P300 full sun and P450 shading (2.31). The seedlings cultivated under full sun presented a higher survivability and seedling quality. The production of *L. alba* seedlings should be done under full sun with the addition of 450 mg kg^{-1} of phosphorus.

Additional key words: acclimatization; chlorophyll-*a* fluorescence; medicinal plant; mineral nutrition.

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RESUMEN

Lippia alba (Mill.) N.E.Br. (hierba de limón) es una especie de interés medicinal y aromática, con estudios incipientes en relación a la nutrición mineral y a la luminosidad en las respuestas foliares y vegetativas. El objetivo del trabajo fue evaluar los aspectos foliares y la producción de plántulas de *L. alba* bajo dos condiciones de luminosidad (pleno sol y 50% de sombreamiento) y cuatro niveles de fósforo (0, 150, 300 y 450 mg kg^{-1}). Las plántulas presentaron alteraciones en los índices fisiológicos y respuestas fotoquímicas basadas en la fluorescencia de la clorofila-*a*. La fertilización fosfatada contribuyó en la mitigación del estrés luminoso en la síntesis de clorofilas. La mayor producción de biomasa de las hojas ocurrió con la adición de P. El análisis de componentes principales explicó el 74% de la variabilidad donde fueron características representativas el número de hojas, la fluorescencia inicial y el área foliar específica dentro del componente principal 1 (PC 1) y el número de brotes, supervivencia y el área foliar en el PC 2. En el análisis de conglomerados, se constató la formación de dos grupos, con distancias más bajas entre 300 pleno sol y 450 sombreamiento (2.31). Las plántulas cultivadas a pleno sol presentaron mayor capacidad de supervivencia y calidad. La producción de plántulas de *L. alba* debe ser realizada bajo pleno sol con adición de 450 mg kg^{-1} del fósforo.

Palabras clave adicionales: aclimatización; fluorescencia de la clorofila-*a*; planta medicinal; nutrición mineral.

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INTRODUCTION

Lippia alba (Mill.) N.E.Br. (lemongrass, Verbenaceae) is a plant of medicinal interest, and tea made from its leaves has a soothing effect (Hohlenwerger *et al.*, 2017). It can also be used as a natural preservative (Machado *et al*., 2011). The essential oil from its leaves has shown an antifungal activity against *Aspergillus, Fusarium, Penicillium* and *Trichoderma* (Glamočlija *et al*., 2011). There are few studies on cultivation practices that must be established during the production of seedlings propagated with stem cuttings.

The abiotic factors that may influence the vegetative propagation of seedlings include light availability in the cultivation environment, which may affect the morphophysiological responses of plants since it acts directly in the process of photochemical and biochemical reactions of photosynthesis (Leal *et al*., 2015).

However, light intensity may cause damage to the photosynthetic apparatus (Díez *et al*., 2017; Taiz *et al*., 2017) by changing the carboxylation speed of Ribulose 1.5 bisphosphate carboxylase/oxygenase, the assimilation of CO_2 and, consequently, the production of photoassimilates for seedling formation. The plants have foliar functional strategies based on chlorophyll-*a* fluorescence in photosystem II (PS II), enabling the efficient use of light (Jaimez *et al*., 2018; Jiménez-Suancha *et al*., 2015). Therefore, these photochemical parameters may be used in assessing the integrity of photosynthetic machinery under ecophysiological disorders as a result of light stress.

In addition to light, another limiting factor for plants is soil in tropical regions, such as soil in Cerrado, which is highly weathered, has high levels of iron and aluminum oxides, and has high acidity (Souza *et al*., 2013; Islas-Espinoza *et al*., 2014), limiting the availability of some nutrients, particularly phosphorus (P). Greater attention is required for the low availability in soil in the tropics because of the greater fixation in soil (Quesada *et al*., 2011; Gérard *et al*., 2016).

Generally, P-deficient plants may suffer damage in diffusive and non-diffusive processes of photosynthetic metabolic pathways through a reduction in consumption and regeneration of Rubisco and production of ATP and NADPH (Andrade *et al*., 2018), which may cause instability in the photochemical process in PS II. Thus, phosphate fertilization is an important agronomic practice since it may assist in photochemical stability (Carstensen *et al*., 2018) and biomass increases in plants (Kuwahara *et al*., 2016).

We tested the hypothesis that P may contribute to the production and quality of seedlings, mitigate damage and increase photochemical reactions in PS II under light stress. This study associated the mineral nutrition and the ecophysiology of the plants in order to assess the photochemical and vegetative response and quality of *L. alba* seedlings produced with stem cuttings under different light availability and phosphate fertilization.

MATERIAL AND METHODS

Collection of plant material and preparation of cuttings

This species was identified and a voucher was deposited at the Herbarium DDMS of the Federal University of Grande Dourados (UFGD), under number 5226. The collection of plant material was performed from plant matrices in the Horto de Plantas Medicinais (22º11'43.7"S and 54º56'08.5"W, 452 m a.s.l.) of UFGD, in good phytosanitary conditions. The cuttings were removed from the median portion of the branches, standardized as 20 cm in length, mean diameter of 2.45 mm, and a pair of leaves at the apex; $^1/_3$ of the cuttings was buried in the substrate.

Studied Factors and experiment design

This experiment was performed from October to December of 2017 in the Faculty Agrarian Science (22º11'43.7"S e 54º56'08.5"W, 452 m a.s.l.) of UFGD in Dourados, Mato Grosso do Sul, Brazil. The climate of the region is classified as Am (Alvares *et al*., 2013) with an annual average rainfall over 1,500 mm. The factors consisted of two light conditions (full sun and 50% shading) and four levels of phosphorus in the form of simple superphosphate - 18% of P_2O_5 (0, 150, 300 and 450 mg kg^{-1}). The treatments were displayed in a 2×4 factorial scheme, in randomized blocks, with four replicates. The experiment unit consisted of five 500 mL plastic containers, with one cutting each.

Shading was simulated using polypropylene black screen with 50% retention of light. The soil was classified as Dystroferric Red Latosol (Santos *et al.*, 2018), clay texture, with the following chemical attributes: pH $CaCl_2$ = 4.76; P= 0.5 mg dm^{-3}; Ca = 1.04 cmol$_c$ dm^{-3}; K = 0.06 cmol$_c$ dm^{-3}; Mg = 0.12 cmol$_c$ dm^{-3}; Al = 1.2 cmol$_c$ dm^{-3}; H+Al = 7.71 cmol$_c$ dm^{-3}; sum of bases = 1.22 cmol$_c$ dm^{-3}; cation exchange capacity = 8.93 cmol$_c$ dm^{-3} and base saturation = 13.68. No soil correction was performed. Base fertilization was performed in coverage with ammonium sulphate (20% N) and potassium chloride (60% K_2O). The cultivation practices consisted of daily irrigation at 70% of the substrate's field capacity.

Chlorophyll-*a* fluorescence

At 30 and 60 days after burial (DAB) of cutting, the emission of chlorophyll-*a* fluorescence was assessed by subjecting the leaves to darkness for 30 min, using leaf clips, between 8:00 and 10:00 am. The initial (F_0) and maximum (F_m) chlorophyll-*a* fluorescence and photochemical efficiency of photosystem II (F_v/F_m) were measured under flash of 1,500 µmol m^{-2} s^{-1} with a portable fluorometer (OS-30P; Opti-Sciences Chlorophyll Fluorometer, Hudson, NY). The variables fluorescence ($F_v = F_m - F_0$), efficiency of absorbed energy conversion (F_v/F_0), non-photochemical maximum yield (F_0/F_v) and electron transport rate (ETR) were estimated (Baker, 2008).

Quantification of photosynthetic pigments

At 60 DAB, fully expanded leaves were collected, with 1 g macerated in 8 mL of acetone (80%). Afterwards, the samples were centrifuged for 10 min at 3000 rpm, and the absorbance reading was taken at the wavelengths of 470, 645 and 663 nm using a spectrophotometer. The contents of chlorophylls *a*, *b*, total (*a* + *b*) and carotenoids were estimated (Arnon, 1949; Lichtenthaler and Buschmann, 2001).

Growth indicators

After 65 DAB, the survival, length of the aerial part (distance from the collet to the inflection of the highest leaf), collar diameter - CD (± 1.0 cm above the substrate level), leaf thickness, and number of buds and leaves were recorded. Seedlings were removed from the containers, washed and assessed for length of the largest root and rooted cuttings (emission of adventitious root of at least 5.0 cm). The leaf area was also assessed (LA) using an area integrator (area meter LI-COR 3100 C; Licor, Lincoln, NE).

Biomass, physiological and quality indices

Fresh material from the aerial and root parts were submitted to forced air circulation in an oven at 60±5ºC to obtain the dry mass. Using the data for the LA and leaves and total dry biomass (LDM and

TDM, respectively), the physiological indices of leaf area ratio (LAR = LA/LDM), specific leaf area and mass (SLA = LA/TDM and SFM = LDM/TDM, respectively) were calculated (Benincasa, 2003). From the data for total dry biomass, height/diameter ratio (RHD) and aerial part/root ratio (APRR), the Dickson quality index (DQI) was estimated (Dickson *et al*., 1960).

Statistical analysis

The rooting and survival data were transformed into arcsine of $\sqrt{x + 0.5}$ and subjected to normal distribution with the Shapiro-Wilk test for normalization. The data for chlorophyll-*a* fluorescence were assessed in plots subdivided in time. All data were subjected to analysis of variance (ANOVA), and, when significant according to the F test, the averages were compared with Student's *t* test for the luminosity and evaluation periods, along with regression analysis for phosphorus ($P \leq 0.05$), using SISVAR.

Additional multivariate analysis of principal components was carried out with variance and co-variance arrays. A cluster analysis was also performed using the complete linkage method to describe the similarity between the factors, and the grouping was performed with the classical method using Euclidean distances.

RESULTS

The greatest seedling survival and collet diameter were observed under full sun (Fig. 1A and B). The greatest height/diameter ratio (RHD) occurred in the shaded seedlings (Fig. 1C). The leaf number (LN) was influenced by the factors independently, with higher amounts under the shaded environment (Fig. 1D), without adjustment to phosphate fertilization (Tab. 1).

The characteristics height, rooting, length of the largest root, stem fresh and dry mass, root fresh mass and total mass were not influenced by the factors ($P > 0.05$), with averages of 22.4 cm, 76%, 12.0 cm, 2.22, 1.04, 0.96 and 6.30 g/plant, respectively. The number of buds, chlorophyll *b* and fresh and dry mass of the leaves were influenced only by phosphorus, in

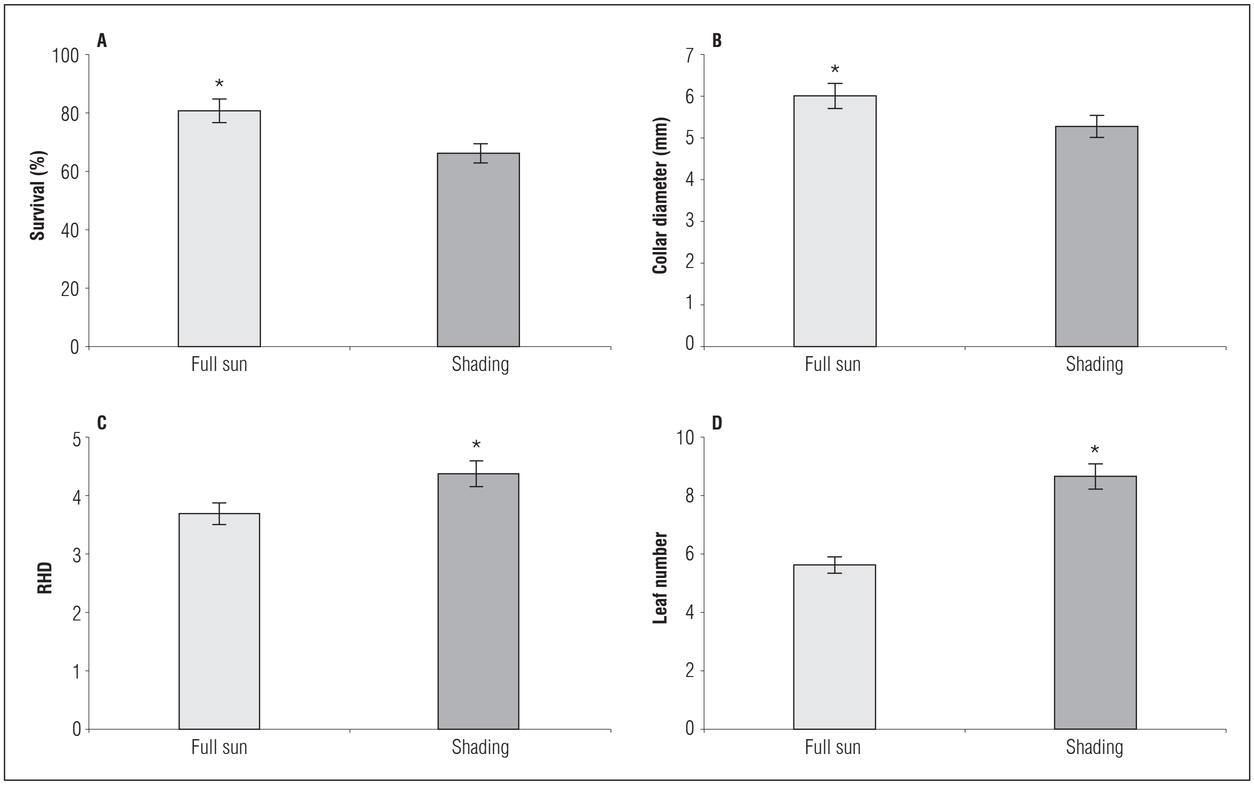

Figure 1. Survival (A), collar diameter (B), height/diameter ratio – RHD (C) and leaf number – LN (D) in *L. alba* seedlings produced under light ambience. * significant difference according to the Student's *t* test ($P \leq 0.05$).

Table 1. Leaf and budding numbers, chlorophyll *b*, variable fluorescence, leaf fresh and dry mass in *L. alba* seedlings produced with different phosphorus doses.

Characteristic	Equation	R^2
Leaf number (LN)	$\hat{y}=\bar{y}=$ 7.18 leaves/plant	W/adjust
Budding number (BN)	$\hat{y}=\bar{y}=$ 6.34 budding/plant	W/adjust
Chlorophyll *b*	$\hat{y}=\bar{y}=$ 9.31 μg cm^2	W/adjust
Variable fluorescence	$\hat{y} = 0.40832 + 0.00054{*}P - 0.00001{*}P^2$	0.98
Leaf fresh mass	$\hat{y} = 0.1564 + 0.0013{*}P - 0.00002{*}P^2$	0.98
Leaf dry mass	$\hat{y} = 0.14551 + 0.00079{*}P$	0.93

* significant difference ($P \leq 0.05$).

which the data for LN, NB and chlorophyll *b* had no adjustment to the mathematical models (Tab. 1) because of the low value of the determination coefficient (R^2).

Chlorophyll *a* and total showed the same trend in the shaded environment, reducing the levels with increasing doses, with maximum levels (17.62 and 28.19 μg cm^2) and minimum levels (7.74 and 10.21 μg cm^2) without and with (450 mg kg^{-1}) P, respectively (Tab. 2). On the other hand, under full sun, chlorophyll *a* and carotenoids had higher contents (9.96 and 1.84 μg cm^2, respectively) with the addition of 450 mg kg^{-1} of P.

Under the shaded environment, the data for F_v/F_m and F_0/F_v had no adjustment to the mathematical models (Tab. 2). Under full sun, the highest ratio was 0.750 and 0.338 electrons quantum^{-1}, under 450 mg kg^{-1} of P, respectively. The higher F_v/F_m values were observed at 60 DAB in full sun as a result of the lowest non-photochemical yield (F_0/F_v) (Tab. 3). The greatest F_0 occurred in the leaves of shaded seedlings (0.162 electron quantum^{-1}) (Fig. 2A) and at 30 DAB (0.187 electrons quantum^{-1}) (Fig. 2B).

The maximum F_v 0.415 electron quantum^{-1} was found with the addition of 27 mg kg^{-1} of P (Tab. 1). The shaded seedlings had higher F_v and F_m values at 30 DAB, with a reduction of values at 60 DAB in both environments (Tab. 4). The ETR had lower values at 30 DAB (Tab. 4). The greatest ETR occurred in the leaves of seedlings cultivated under full sun at 60 DAB as a result of the higher incident light.

In the shaded environment, the largest LA was 16.43 cm^2 under 450 mg kg^{-1} of P. Under full sun, the maximum leaf area (11.85 cm^2) occurred in seedlings cultivated with 228.31 mg kg^{-1} of P (Tab. 5). The leaf

Table 2. Chlorophyll *a*, total chlorophyll, carotenoids, photochemical efficiency of the photosystem II (F_v/F_m), maximum non-photochemical yield (F_0/F_v) and efficiency of absorbed energy conversion (F_v/F_0) in *L. alba* seedlings produced with phosphorus under light environment.

<table>
<tr><th colspan="2">Chlorophyll *a* (μg cm^{-2})</th></tr>
<tr><th>Full sun</th><th>Shading</th></tr>
<tr><td>$\hat{y} = 5.57350 + 0.00975{*}P$
$R^2 = 0.56$</td><td>$\hat{y} = 17.64521 - 0.02201{*}P$
$R^2 = 0.90$</td></tr>
<tr><th colspan="2">Total chlorophyll (*a* + *b*) (μg cm^{-2})</th></tr>
<tr><th>Full sun</th><th>Shading</th></tr>
<tr><td>$\hat{y} = \bar{y} = 16.73$
W/ adjust</td><td>$\hat{y} = 33.33526 - 0.05138{*}P$
$R^2 = 0.89$</td></tr>
<tr><th colspan="2">Carotenoids (μg cm^{-2})</th></tr>
<tr><th>Full sun</th><th>Shading</th></tr>
<tr><td>$\hat{y} = 0.58725 + 0.00279{*}P$
$R^2 = 0.75$</td><td>$\hat{y} = \bar{y} = 1.51$
W/adjust</td></tr>
<tr><th colspan="2">Photochemical efficiency of the photosystem II (F_v/F_m)</th></tr>
<tr><th>Full sun</th><th>Shading</th></tr>
<tr><td>$\hat{y} = 0.71932 + 0.00007{*}P$
$R^2 = 0.57$</td><td>$\hat{y} = \bar{y} = 0.749$
W/adjust</td></tr>
<tr><th colspan="2">F_0/F_v (electrons quantum^{-1})</th></tr>
<tr><th>Full sun</th><th>Shading</th></tr>
<tr><td>$\hat{y} = 0.39187 - 0.00013{*}P$
$R^2 = 0.50$</td><td>$\hat{y} = \bar{y} = 0.337$
W/adjust</td></tr>
<tr><th colspan="2">F_v/F_0 (electrons quantum^{-1})</th></tr>
<tr><th>Full sun</th><th>Shading</th></tr>
<tr><td colspan="2">30 DAB</td></tr>
<tr><td>$\hat{y} = 2.0480 + 0.00192{*}P$
$R^2 = 0.80$</td><td>$\hat{y} = \bar{y} = 2.958$
W/adjust</td></tr>
<tr><td colspan="2">60 DAB</td></tr>
<tr><td>$\hat{y} = \bar{y} = 3.642$
W/adjust</td><td>$\hat{y} = \bar{y} = 3.261$
W/adjust</td></tr>
</table>

* significant difference ($P \leq 0.05$).

Table 3. Photochemical efficiency of the photosystem II (F_v/F_m) and maximum non-photochemical yield (F_0/F_v) in *L. alba* seedlings produced with phosphorus under light environment, at 30 and 60 days after burial (DAB).

	F_v/F_m		F_0/F_v	
	(electrons quantum^{-1})			
Environment	30 DAB	60 DAB	30 DAB	60 DAB
Full sun	0.699 aB	0.774 aA	0.438 aA	0.284 bA
Shading	0.740 aA	0.759 aA	0.353 aB	0.312 aA
C.V. (%)	7.26		11.37	

Means followed by the same lower case letter in the rows, for day after burial, and upper case in the columns, for light environment, do not differ according to the Student's *t* test ($P \leq 0.05$).

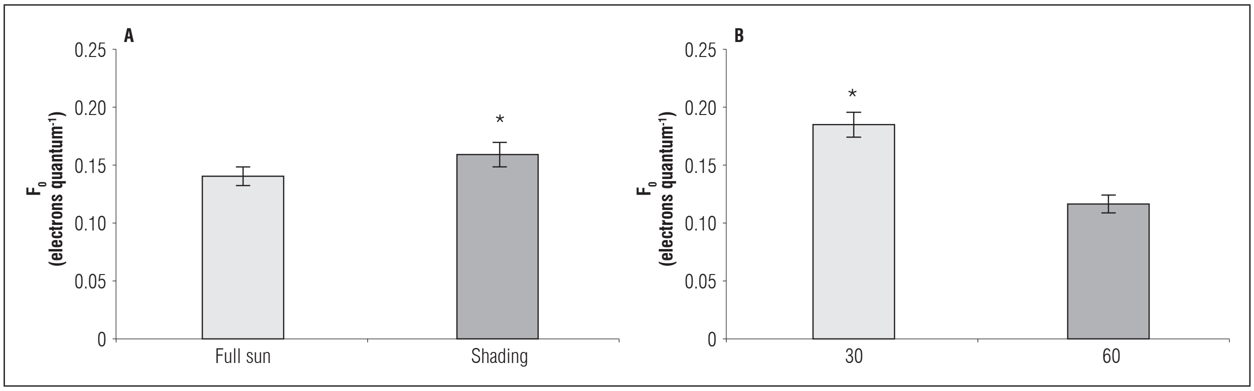

Figure 2. Initial fluorescence – F_0 in leaves of *L. alba* seedlings produced under phosphorus rates and light environment (*a*), at 30 and 60 days after burial (*b*). *significant difference according to the Student's *t* test ($P \leq 0.05$).

Table 4. Maximum (F_m) and variable (F_v) fluorescence and electron transport rate (ETR) in leaves of *L. alba* seedlings produced with phosphorus under light environment at 30 and 60 days after burial (DAB).

Environment	F_m		F_v		ETR	
	(electrons quantum^{-1})					
	30 DAB	60 DAB	30 DAB	60 DAB	30 DAB	60 DAB
Full sun	0.611 aB	0.503 bA	0.433 aB	0.395 aA	248.38 bA	275.29 aA
Shading	0.768 aA	0.543 bA	0.572 aA	0.415 bA	107.94 aB	110.78 aB
C.V. (%)	8.33		10.22		6.12	

Means followed by different lower case letters in a row, for day after burial, and upper case letters in the columns, for light environment, indicate significant differences according to the Student's *t* test ($P \leq 0.05$).

thickness data were not adjusted, and the sun leaves had higher values. The data for root dry mass and APRR of the seedlings under full sun were not adjusted to the mathematical models, but, under the shading, the higher mean values were 0.462 g/plant and 0.44 with 450 mg kg^{-1} of P. The fresh and dry biomass of the leaves were influenced only by phosphorus, with higher values (0.177 and 0.0501 g/plant) with the addition of 32.5 and 450 mg kg^{-1} of P, respectively (Tab. 1).

Table 5. Leaf area, thickness, root dry mass and aerial part/root ratio in *L. alba* seedlings produced with phosphorus under light environment.

Leaf area – LA (cm^2 per plant)	
Full sun	Shading
$\hat{y} = 7.68317 + 0.03653^{*}P - 0.00008^{*}P^2$ $R^2 = 0.86$	$\hat{y} = 8.50657 + 0.01762^{*}P$ $R^2 = 0.72$
Leaf thickness – LT (mm)	
Full sun	Shading
$\hat{y} = \bar{y} = 0.33$ W/adjust	$\hat{y} = \bar{y} = 0.26$ W/adjust
Root dry mass – RDM (g per plant)	
Full sun	Shading
$\hat{y} = \bar{y} = 0.537$ W/adjust	$\hat{y} = 0.21012 + 0.00056^{*}P$ $R^2 = 0.60$
Aerial part/root ratio – APRR	
Full sun	Shading
$\hat{y} = \bar{y} = 0.093$ W/adjust	$\hat{y} = 0.03605 + 0.0009^{*}P$ $R^2 = 0.63$

* significant regression ($P \leq 0.05$)

The greatest LAR (2.027 cm^2 g^{-1}) and SLM (0.0458 g cm^{-2}) occurred in the shaded seedlings (Fig. 3A and B) as a result of the greatest leaf thickness (Tab. 5). The greatest SLA was 48.54 cm^2 g^{-1} in the seedlings produced in the shaded environment (Fig. 3C). For the DQI, the greatest value (1.70) was observed in the seedlings cultivated under full sun (Fig. 3D), regardless of phosphorus.

The principal components analysis explained 74% of the variability, in which PC1 and PC2 contributed 41.05% and 32.05%, respectively, of the remaining variance of the characteristics in the L. *alba* seedlings (Fig. 4A). In the cluster analysis, two groups were formed for the P dose (G1 and G2), with six subgroups (Fig. 4B). The subgroups with lower distances between P dose and light environment (S - shading and PS - full sun) were 300 PS and 450 S (2.31), followed by 450 S and 450 PS (4.61) and 300 S and 0 S (6.65).

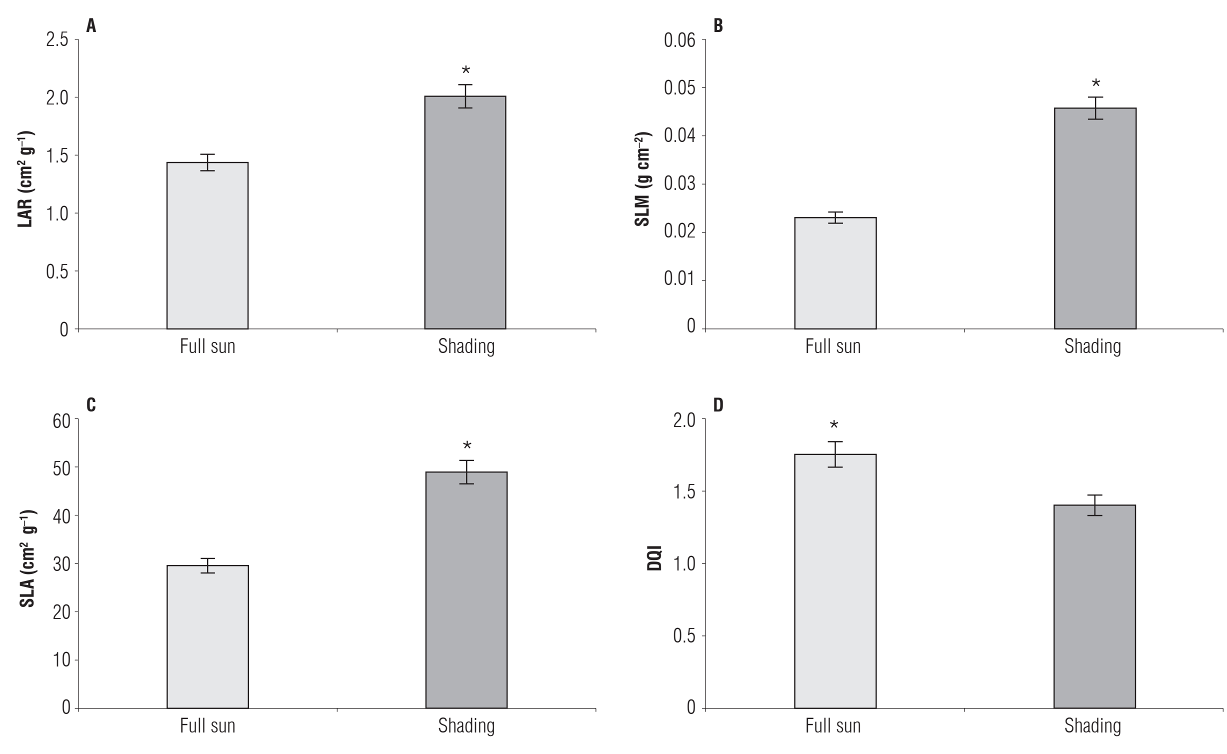

Figure 3. Leaf area ratio – LAR (A), specific leaf mass – SLM (B), specific leaf area – SLA (C) and Dickson quality index – DQI (D) in *L. alba* seedlings under light environment at 65 days after burial. *significant difference according to the Student's *t* test ($P \leq 0.05$).

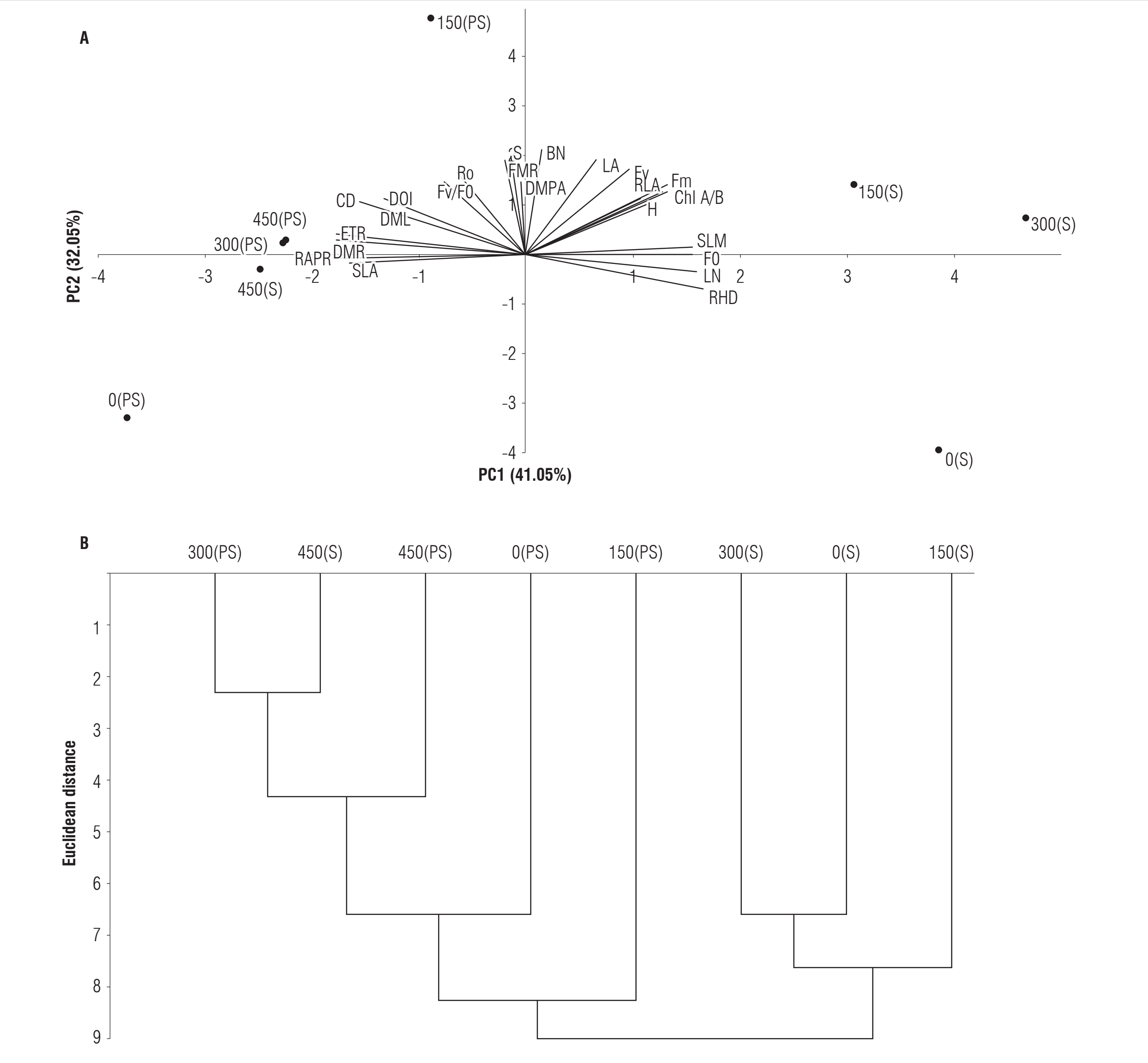

Figure 4. **Principal components analysis (A) and dendrogram of similarity, based on Euclidean distances (B) of characteristics evaluated in the *L. alba* seedlings produced with phosphorus doses (0, 150, 300 and 450 mg kg^{-1}) and light environment (S - shading and PS - full sun).**

DISCUSSION

The greatest survival was associated with the amount of photoassimilates as a function of greater incident light. Seedlings under excessive light lose more water through transpiration because of an increased temperature, with the greater diameter allowing a greater ability to transport water and photoassimilates to other organs in order to maintain turgidity (Taiz *et al*., 2017). The greatest RHD occurred in the shaded seedlings, but the values did not indicate that there was etiolation in the seedlings under limited light.

Generally, plants under shading tend to expand the number of leaf limbs, enabling greater light interception (Oliveira *et al*., 2017) and maximizing photosynthesis. These characteristics are important in the vegetative propagation of the species since the greater the amount of buds, the greater the formation of leaves, contributing to photoassimilate distribution to developing organs.

The reduction of pigments, except carotenoids, in leaves under full sun can be explained by photoinhibition of chlorophylls as a result of excessive light (Fu *et al*., 2012). However, shaded leaves tend to increase the content of chlorophyll and, thereby, maximize the photosynthetic capacity (Díez *et al*., 2017). Similar results were observed by Soares *et al*. (2017) when assessing young *Tamarindus indica* L. plants under shading, which presented higher concentrations of chlorophylls.

An increase in chlorophyll content is desirable because it is responsible for capturing light photons and transmitting energy to reaction centers (Monteiro *et al*., 2018), and carotenoids are responsible for chlorophyll photoprotection and reduction of membrane harm (Taiz *et al*., 2017), maximizing the photosynthetic capacity. Under full sun cultivation, phosphate fertilization can be a potential mitigation strategy for ecophysiological disorders from light stress in the synthesis of chlorophylls.

P, in the form of Pi, is responsible for the control of enzymatic reactions and metabolic regulation in the cytosol and chloroplast (Hawkesford *et al*., 2012). Higher photochemical indicators, especially the F_v/F_m ratio, with phosphorus result because P favors the speed of ATP synthesis, contributing to the export of protons to the chloroplast stroma and thylakoid lumen (Carstensen *et al*., 2018), favoring the maintenance of electron mobility in PS II. Increasing F_0/F_v can be a photoprotective mechanism of the photosynthetic apparatus for excessive incident radiation (Blind *et al*., 2018), i.e. a greater use of electrons produced as a result of higher efficiency in absorbed energy conversion (F_v/F_0), regardless of the shading level during this period (60 DAB) (Tab. 2).

The higher F_0 at 30 DAB is desirable in order to mitigate damage in the photosynthetic apparatus (Fig. 2B). Fu *et al*. (2012) described a higher F_0 in *Lactuca sativa* L. plants cultivated under 100 μmol m^{-2}s^{-1} (low irradiance) at the initial stage of the experiment, stating that this mechanism can mitigate an increase in reactive oxygen and D1 protein degradation. As for phosphorus in F_v, P is involved in phosphorylation reactions and pyrophosphate release, acting in the activation of catalytic enzymes, forming ATP (Hawkesford *et al*., 2012, Xing and Wu 2014; Andrade *et al*., 2018) and favoring photochemical stability because F_v is related to the ability to transfer electrons.

The results for F_m and ETR is important in the photochemical process since larger values favor the flow of chlorophyll molecules between acceptors in photosystems (Farias *et al*., 2016). The higher photochemical efficiency in PS II effectively contributes to maintaining the integrity of the photosynthetic apparatus and increasing vegetative characteristics.

Plants exposed to low luminosity enhance the expansion of leaves (Gondim *et al*., 2018) as a strategy for use of light. Increasing APRR and RDM may be associated with the fact that under high irradiance, substrate and leaves tend to lose more water through evapotranspiration and leaf transpiration. Thus, an increase in these characteristics under full sun promotes water absorption for the maintenance of metabolic processes and nutrient transport (Sarto *et al*., 2018).

The responses of plants to abiotic variants can change according to the species. Oliveira *et al*. (2017), when assessing the physiological and productive aspects of *Origanum vulgare* L. plants, found greater root biomass under shaded cultivation. On the other hand, there was greater biomass allocation in *Physalis minima* L. seedlings under full sun (Silva *et al.* 2016), similar to *L. alba* seedlings (Fig. 3).

P plays an important role in plant biomass allocation and is a structural component of nucleic acids, phospholipids and ATP formation, favoring primary metabolism reactions and constituting ~ 0.2% of plant mass (Kuwahara *et al*., 2016). For seedling production in *Dalbergia nigra* Benth., it was found that the addition of 500 mg kg^{-1} of P favored an increase in biomass (Carlos *et al*., 2018), similar to the *L. alba* seedlings in this study (Tab. 1).

LAR and SLM show greater biomass allocation through leaf area with greater thickness (Oliveira *et al*., 2016). These authors found that *Melissa officinalis* L. plants, at 120 d after transplanting, showed a greater SLM when cultivated under full sun. For LAR, Ribeiro *et al*. (2018) found higher values in *Pogostemon cablin* cultivated under shaded conditions. The increase in SLA under this condition indicated the adaptive ability of leaf tissues in optimizing light capture (Guzmán *et al*., 2016; Liu *et al*., 2016) because it promotes CO_2 assimilation and stomatal control (Gommers *et al*., 2013). However, leaves with a smaller thickness are less heavy. Similar results were observed in *Enterolobium contortisiliquum* (Vell.) Morong

(Souza *et al*., 2017) and *Colocasia esculenta* L. Schott (Gondim *et al*., 2018). Both authors described higher values in plants under artificial shading.

The DQI demonstrated that this species has survivability and stability capacity when exposed to high irradiance, an important fact since the initial cultivation under this condition reduced the acclimatization period (rustification) of the seedlings, i.e. these will be less susceptible to weather under field conditions, such as excessive sunlight. The use of DQI has been constant when assessing seedling quality since it is an easy implementation analysis, performed by calculating the morphometric stability level, distribution and biomass allocation.

In PC 1, the characteristics that showed positive scores in descending order were RHD (0.271), LN (0.263), F_0 and SFM (0.255), and the characteristics with negative scores were APRR (-0.292), ETR and RDM (-0.288) and SLA (-0.270), which were the most similar. In PC 2, the components that most contributed with positive factorial scores were the number of buds (0.352), survival (0.330), leaf area (0.319) and F_v (0.289), with no negative absolute scores > 0.30. The cluster analysis consisted of sample classification in order to verify the similarity within the groups and between-group heterogeneity, considering all evaluated characteristics (Araújo *et al*., 2013). Thus, there was greater heterogeneity between the luminous environments, with G1 comprising the association of P with shading, except for 450 S and G2 under full sun.

CONCLUSION

The *Lippia alba* seedlings responded positively to the phosphate fertilization when vegetative propagation was used with stem cuttings. The association of 450 mg of phosphorus with the cultivation under full sun contributed substantially to mitigating ecophysiological disorders in the photosynthetic apparatus as the result of light stress, providing greater photochemical stability, survival and quality in the *Lippia alba* seedlings. No acclimatization process was required.

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Conflict of interests: The manuscript was prepared and reviewed with the participation of the authors, who declare that there exists no conflict of interest that puts at risk the validity of the presented results.

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Phenotypic performance of four stevia genotypes in the Alto Vale do Itajaí region, Brazil

Rendimiento fenotípico de cuatro genotipos de estevia en la Región del Alto Vale do Itajaí, Brasil

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***Stevia rebaudiana* (G12) plants in field experiment.**

Photo: R.J. Debarba

ABSTRACT

An evaluation of four stevia genotypes for biomass yield, stevioside and rebaudioside A content and yield under decreasing photoperiod conditions was carried out in the Alto Vale do Itajaí region, located in the State of Santa Catarina (SC), Brazil. This field experiment was conducted at Site São Miguel, a farm located in the city of Lontras (SC), Brazil, under conditions of decreasing photoperiods, with a variation of 13.72 h of light at experiment implantation to 12.57 h of light at the end of the evaluations. The treatments consisted of four genotypes (G4, G8, G9 and G12) provided by EMBRAPA-CENARGEN. A randomized complete block design with four treatments (stevia genotypes) and four replications was used. Each plot consisted of 21 plants, and the floor area had five plants. G12 had the highest leaf dry weight (LDW), total leaf area, leaf area index, leaf area ratio and specific leaf area of all the genotypes. G4 and G12 were equal for LDW and were higher than the other genotypes, with yields of 755.6 and 836.4 kg ha^{-1}, respectively. The stevioside content was highest in G12 (200.07 mg g^{-1}). G8 and G9 were similar for rebaudioside A content (64.77 and 49.05 mg g^{-1}, respectively). The rebaudioside A: stevioside ratio was highest in G8 (0.44 g g^{-1}). No genotype had a rebaudioside A: stevioside ratio suitable for industry requirements.

Additional key words: photoperiodicity; physiological response; genotypes; sweeteners; *Stevia rebaudiana* (Bert.) Bertoni; stevioside; rebaudioside.

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RESUMEN

La evaluación de cuatro genotipos de estevia para la producción de biomasa, el contenido y rendimiento de esteviósido y rebaudiósido A, se llevó a cabo en la región de Alto Vale do Itajaí, estado de Santa Catarina, SC (Brasil), en condiciones de disminución de fotoperíodo. El experimento se realizó en el campo de la Granja São Miguel, Lontras, SC, Brasil, bajo condiciones de disminución del fotoperíodo, con una variación de 13.72 h de luz, en el momento de la implantación del experimento, a 12.57 h de luz al final de las evaluaciones. Los tratamientos fueron cuatro genotipos (G4, G8, G9 y G12), procedentes de EMBRAPA-CENARGEN. Se utilizó un diseño de bloques completos al azar con cuatro tratamientos (genotipos de estevia) y cuatro repeticiones. Cada parcela constaba de 21 plantas y el área del piso por cinco plantas. G12 tuvo el mayor peso seco de la hoja (PSH), área foliar total, índice de área foliar, relación de área foliar y área foliar específica de todos los genotipos. G4 y G12 presentaron similitud en PSH, siendo superiores a los demás, con producción de 755,6 y 836,4 kg ha^{-1}, respectivamente. El contenido de esteviósido fue mayor en G12 (200.07 mg g^{-1}). G8 y G9 fueron iguales para el contenido de rebaudiósido A, presentando 64,77 y 49,05 mg g^{-1}, respectivamente. La relación rebaudiósido A: esteviósido fue mayor en el genotipo G8 (0,44 g g^{-1}). Ningún genotipo evaluado bajo las condiciones productivas del Alto Vale do Itajaí presentó la relación rebaudiósido A: esteviósido apropiada requerido para la industria.

Palabras clave adicionales: fotoperiodicidad; respuesta fisiológica; genotipos; edulcorantes; *Stevia rebaudiana* (Bert.) Bertoni; esteviósido; rebaudiósido.

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INTRODUCTION

The demand for sweeteners for dietary and pharmacological purposes is growing. Stevia (*Stevia rebaudiana* Bert.) contributes to the supply of natural and non-carcinogenic sweeteners (Anton *et al*., 2010). Stevia (Asteraceae), native to Brazil and Paraguay, has been used for its medicinal and dietary properties, derived from its glycosides stevioside and rebaudioside A, both of which are 300 times sweeter than sucrose (Espita *et al*., 2009).

The relationship between stevioside and rebaudioside A is an important variable for analysis in new stevia genotypes. According to Mota *et al*. (2015), the industry prefers genotypes that have a ratio close to 1 g g^{-1} because stevioside has a low water solubility and bitter residual taste, while rebaudioside A is more soluble and has no residual taste.

Stevioside and rebaudioside A are more concentrated in leaves and are diterpene glycosides that are synthesized in the same mevalonate pathway as gibberellic acid (Jarma *et al*., 2010).

The cultivation of stevia in Brazil is incipient and does not meet the growing domestic demand. Brazil imported more than US$8 million and exported US$2.7 million in stevia in 2013 (MDIC, 2019).

Stevia has potential for cultivation in the Alto Vale do Itajaí region, Santa Catarina. The increasing demand for glycosides warrants studies for the selection of new genotypes with productive potential, local adaptation, and stevioside and rebaudioside A ratios required by the industry (Mota *et al*., 2015).

In southern Brazil, studies by the Universidade Federal do Parana (UFPR) with unevaluated genotypes provided by EMBRAPA - CENARGEN collected in different regions of Brazil, showed that stevia can be grown in the Curitiba region, State of Parana (Francisco, 2015), justifying exploratory research on the genotypes provided by EMBRAPA - CENARGEN in areas with different soils and climates, such as those in the Alto Vale do Itajaí region (SC).

Because of the lack of agronomic information and genotypes adapted to the Alto Vale do Itajaí region (SC), this study aimed to evaluate four different stevia genotypes provided by EMBRAPA - CENARGEN in terms of dry weight yield, glycoside content and yield, and rebaudioside A: stevioside ratio.

MATERIAL AND METHODS

This study was carried out at Sítio São Miguel, located in the city of Lontras (SC), Brazil (27º 9’ 58” S, 49º 32’ 31” W, altitude of 475 m). The climate was Cfa according to the Köppen classification; climate data during the experiment were determined (Tab. 1).

The soil in the experiment area was classified as Argissolo Amarelo distrófico típico (EMBRAPA, 2006). Soil analysis data were collected (Tab. 2).

The soil was corrected according to laboratory analysis results. The corrections were based on the Fertilization and Liming Manual for the states of Santa Catarina and Rio Grande do Sul (SBCS and CQFS, 2016). The soil was corrected with phosphorus and potassium fertilization when preparing the beds, 15 d prior to transplanting the seedlings. A rotary tiller was used to prepare the soil of the experiment area.

The fertilization was carried out with doses (kg ha^{-1}): 90 N, 68 P_2O_5 and 110 K_2O, supplied by urea, superphosphate and potassium chloride, respectively. A split application of urea was done at transplanting and 30 d after transplanting, according to Lima Fiho (2004). The genotypes used in this study were supplied by EMBRAPA - CENARGEN (Brasilia, DF). The genotypes have distinct morphological and productive characteristics, as evidenced by previous studies at UFPR (Francisco, 2015).

The seedlings were obtained from branches of mother plants located at Fazenda Canguiri - UFPR – Pinhais (PR), Brazil, based on the methodology of Carvalho and Zaidan (1995) and using IBA (Indol butyric acid) at a concentration of 2000 mg L^{-1}. The seedlings were produced in commercial substrate (Macrofértil®) on the premises of the Instituto Federal Catarinense (IFC) - Rio do Sul (SC) campus. At transplanting, the seedlings were subjected to apical bud breaking, according to EMBRAPA (2004), which consisted of cutting the seedlings at a height of 5 cm from the soil.

The seedlings were transplanted in beds prepared with a raised bed planter. The experiment was installed on December 12, 2014. The spacing used was 0.25 x 0.50 m - 80,000 plants/ha (SBCS and CQFS, 2016).

The treatments consisted of Genotype 4 (G4), Genotype 8 (G8), Genotype 9 (G9) and Genotype 12 (G12). A randomized complete block design with four treatments (stevia genotypes) and four replicates was used. Each plot consisted of 21 plants, with 5 plants per useful area. Plants were harvested when they were at 5% flowering (Lima Fiho, 2004), which occurred at different times for each genotype.

The following characteristics were evaluated: leaf dry weight (LDW), stem dry weight (SDW), branch

Table 1. Temperature, humidity and photoperiod during the experiment. Rio do Sul (2014/2015).

Climate data	December	January	February	March
Maximum temperature (ºC)	30.7	30.9	30.6	26.4
Minimum temperature (ºC)	13.9	15.9	15.6	12.2
Average temperature (ºC)	20.5	20.5	20.9	18.3
Relative air humidity (%)	89	91	93	94
Photoperiod (h)	13.72	13.68	13.24	12.57

Source: IFC (2015).

Table 2. Soil chemical characteristics of the experiment area.

pH SMP	Al^{+3}	$H^+ + Al^{+3}$	Ca^{+2}	Mg^{+2}	CEC	K^+	P	OM	V
	cmol dm^{-3}					mg dm^{-3}		%	%
6.0	0.3	4.5	2.9	2.0	9.42	32.0	10.8	1.3	52.19

dry weight (BDW), total dry weight (TDW), main branch length, number of secondary branches, number of tertiary branches, leaf area (LA), leaf area index (LAI), specific leaf area (SLA), leaf area ratio (LAR), leaf weight ratio (LWR), stevioside and rebaudioside A yield, stevioside and rebaudioside A contents, and rebaudioside A: stevioside- ratio. The plants of the useful plot were cut 5 cm from the ground (Lima Fiho, 2004) and taken to the Plant Physiology Laboratory of IFC (Rio do Sul campus), where the leaves were separated from the stems. The LDW and SDW were determined on a digital scale after drying the leaves and stems at 50 ºC until constant weight was reached (Espita *et al.,* 2009). A tape measure was used to determine the height of the main branch. The number of secondary and tertiary branches was counted.

The leaf area (LA), leaf area index (LAI), specific leaf area (SLA), leaf area ratio (LAR) and leaf weight ratio (LWR) were determined using the methodologies described by Cunha *et al.* (2010) using an artisanal leaf disc cutter with an area of 10 mm, precision electronic scale (Gehaka® AG 220S) and air circulation oven (ACB Labor®).

To quantify the stevioside and rebaudioside A yield, samples of 1 g of leaf tissue were collected from plants in the useful area of each treatment. A leaf tissue sample was randomly collected from the LDW of the plants that made up the useful area of the plot. We used the extraction and quantification methodology described by Kolb *et al.* (2001), with modifications. The modification consisted of using 0.3 mL of the sample extracted from the leaves of each treatment, adding 0.7 mL of HPLC grade acetonitrile. Dry leaf tissue samples were placed in 250 mL Erlenmeyer flasks with 100 mL of 70% ethanol. The solution was heated to 70 °C and stirred for 30 min. After cooling, a 10 mL aliquot was filtered (quantitative filter paper and a 0.22 μm nylon syringe filter). We used 0.3 mL of the sample extracted from the leaves of each treatment by adding 0.7 mL of HPLC grade acetonitrile. From this dilution, 20 μL were injected for further analysis in a High Performance Liquid Chromatograph (Shimadzu CBM-10A) containing a Phenomenex Luna® 5 μm NH2 100 Å, 250 x 4.6 mm column. Elution was at room temperature in isocratic mode using a mixture of acetonitrile-distilled water (80:20, v/v) as a solvent and a flow rate of 2 mL min^{-1}. Detection was done by UV at 210 nm with sensitivity adjusted to 0.04 AUFS. Readings were taken in triplicate. The quantification of each metabolite was obtained by converting the area of the curve corresponding to retention time using a previously established calibration curve. The standard solution for obtaining the calibration curve was 1.0 g L^{-1} stevioside and rebaudioside A in methanol (Kolb *et al*., 2001). The conversion was expressed in mg g^{-1}.

The results were subjected to analysis of variance using Assistat 7.7 beta (Silva and Azevedo, 2009). Treatment variances were initially assessed for homogeneity with Bartlett's test. All variables showed homogeneous variances, and the effects of the treatments were tested with the F test. Means were compared with the Tukey test at 5%.

RESULTS AND DISCUSSION

Plant growth

G12 and G4 had the highest LDW of all the genotypes (Tab. 3). G12, G8 and G4 were equal for TDW, all of which were higher than G9. There were no statistical

Table 3. Total dry weight (TDW), stem dry weight (SDW), leaf dry weight (LDW), secondary branches (number of secondary branches), tertiary branches (number tertiary branches) and length of main branch (main stem length) of stevia genotypes grown in the Alto Vale do Itajaí region. Rio do Sul (2016).

Genotypes	TDW (kg ha^{-1})	SDW (kg ha^{-1})	LDW (kg ha^{-1})	Secondary branches	Tertiary branches	Main stem length (cm)
G4	1335.00 ab	579.40 a	755.60 a	3.10 b	47.50 bc	54.26 a
G8	1572.00 ab	1028.80 a	543.20 b	4.50 a	88.95 a	60.80 a
G9	845.20 b	495.80 a	349.40 c	3.25 b	38.80 c	41.35 b
G12	1874.40 a	1038.00 a	836.40 a	3.15 b	56.80 b	60.45 a
SE	119.63	91.10	52.53	0.16	4.99	2.20

Means followed by different letters indicate significant statistical differences according to the Tukey test at ($P \leq 0.05$) ($n=4$). SE, standard error.

differences among the genotypes in terms of SDW yield (Tab. 3). G12, G8 and G4 were equal for main stem length, all of which were greater than G9 (Tab. 3). G8 had the highest number of secondary branches and tertiary branches of all the genotypes.

LDW yield becomes more relevant as glycosides are found in greater amounts in leaves (Jarma *et al.,* 2010). LDW yields ranged from 349.40 kg ha^{-1} (G9) to 836.40 kg ha^{-1} (G12) with an overall mean of 621.15 kg ha^{-1}. These yields were lower than those found by Hastoy *et al.* (2019), Parris *et al.* (2016), Serfaty *et al.* (2013) and Espita *et al.* (2009).

In evaluating the same genotypes used in this study, Francisco (2015) found LDW yields of 4031.8 (G9) and 3733.1 kg ha^{-1} (G12) in two harvests in the first year of cultivation. The lower yields found in this study could be explained by the genotype-environment interaction, where the photoperiod conditions observed by Francisco (2015) were favorable to genotype growth. Reduced yield is associated with the reduction of the vegetative cycle, which is caused by differences in seedling transplantation time (carried out in early December). The vegetative cycle in our study was 43 d shorter than the cycles of other studies, which affected the yields of the genotypes.

The seedlings of the stevia genotypes used in this study were produced by rooting branches. Stevia seedlings are typically produced from seeds (Lima Fiho, 2004). This enables the production and early transplanting of seedlings, which are more conducive to good crop growth (Ceunen and Geuns, 2013; Yoneda *et al*., 2017).

Late planting in this study reduced the crop growth period as a result of decreased photoperiod. As stevia is a short-day crop, it prematurely enters the reproductive phase, reducing the productive capacity of the plant (Ceunen and Geuns, 2013).

The variable LDW can also be influenced by planting density (Munz *et al*., 2018). The higher yields found by Daza *et al*. (2015), >3,500 kg ha^{-1} and Espita *et al.* (2009), 1378 kg ha^{-1}, were a result of the use of more adapted genotypes and plant populations. The highest LDW yield found in G12 was associated with the highest LAI (Tab. 4), and there was a positive correlation between the variables (Tab. 5). A higher LAI increases the interception of photosynthetically active radiation and stimulates crop growth and photoassimilate production (Kumar *et al.,* 2014).

Leaf dry weight (LDW), number secondary branches (secondary branches), number tertiary branches (tertiary branches), total leaf area (TLA), leaf area index (LAI), leaf area ratio (LAR), specific leaf area (SLA), rebaudioside A content (Reb A).

The TDW was influenced by LDW yield, with a positive but not significant correlation between the

Table 4. Total leaf area (TLA), leaf area index (LAI), leaf area ratio (LAR), specific leaf area (SLA) and leaf weight ratio (LWR) of stevia genotypes grown in the Alto Vale do Itajaí region. Rio do Sul, 2016.

Genotypes	TLA (cm^2)	LAI (m^2 m^{-2})	LAR (cm^2 g^{-1})	SLA (cm^2 g^{-1})	LWR (g g^{-1})
G4	719.93 b	5.75 b	42.80 a b	76.67 b	0.56 a
G8	428.01 b c	3.42 b c	23.49 b	63.41 b	0.37 a
G9	284.80 c	2.27 c	28.46 b	65.31 b	0.43 a
G12	1236.72 a	9.89 a	53.74 a	117.97 a	0.45 a
SE	101.14	0.68	3.66	6.53	0.02

Means followed by different letters indicate significant statistical differences according to the Tukey test at ($P \leq 0.05$) ($n=4$). SE, standard error.

Table 5. Correlations between dry weight yield and growth of stevia genotypes.

Indexes	TLA	LAR	SLA	LAI	Secondary branches	Tertiary branches	LDW	Reb A (mg g^{-1})
LDW	0.85**	0.68**	0.62**	0.85**	-0.31^{NS}	0.11^{NS}		0.91**
TDW							0.70**	

^{NS} = not significant; ** significant correlation at 1%.

variables (Tab 5). G9 had a TDW yield of 845.20 kg ha^{-1}, which was equal to G8 and G4, but lower than G12 (Tab. 3). These values are explained by the reduced TLA and LAI of the genotypes (Tab. 4).

There was a low correlation between the LDW yield and number of branches, with a negative, non-significant correlation between the LDW and number of secondary branches (Tab. 5). G8 had the highest number of lateral branches of all the genotypes (Tab. 3). According to Pal *et al.* (2015), the number of branches is associated with a longer growth period of the root system, where cytokines produced at the root apices and radicles stimulate lateral branches.

G8 had a longer production cycle than the other genotypes, and the harvest time occurred at 85 d after transplanting, which was also observed by Francisco (2015). G8, grown in the region of Curitiba, showed harvest conditions 121 d after transplanting (Francisco, 2015).

G12 (Tab. 4) showed the highest TLA, LAI and SLA of all the genotypes and an LAR equal to that of G4, and no differences for LWR were found among genotypes in the means separation test; a similar behavior was found by Francisco (2015). The highest TLA value in G12 was due to the highest LDW value, and there was a high correlation between LDW yield and TLA (Tab. 5).

According to Ceunen and Geuns (2013), LDW yield is directly influenced by the duration of the photoperiod. Longer photoperiods promote greater leaf expansion. An increase in leaf expansion favors total photosynthetic potential, promotes increased production of photoassimilates, and facilitates the partitioning of dry weight to other plant organs.

The genotypes assessed in this study were subjected to the same photoperiod. There was a decreasing photoperiod during the experiment period (Tab. 1), which, according to Francisco (2015) and Ceunen and Geuns (2013), is not favorable for the growth of stevia.

LAI is important for assessing plant response to different environmental conditions. According to Watson (1947), LAI is the unilateral total area of leaf tissue per unit surface area of soil. The genotypes presented distinct LAI values, ranging from 2.27 to 9.89 (m^2 m^{-2}). These values are close to those found by Francisco (2015), which ranged from 3.51 to 7.01 (m^2 m^{-2}). A high LAI is desirable and shows a higher trend in leaf production, soil coverage and capacity to compete for light with weeds (Francisco, 2015). The LAI showed a high correlation with the LDW, which was also observed by Francisco (2015). G12 presented a higher LDW and TLA yield, and consequently a higher LAI.

According to Magalhães (1979), LAR is a measure of the size of the photoassimilatory apparatus and serves as a variable to assess the effects of genotype, climate and management. G12 and G4 need a larger leaf area to synthesize 1 g of DW (Tab. 4). Francisco (2015) reported that genotypes with a low LAR may be associated with genotypes with a higher potential for stevioside and rebaudioside A yield. Thus, G9 and G8 showed a higher photosynthetic efficiency compared to other genotypes (Tab. 4). The high LAR values in G12 and G4 are explained by the high TLA, which influences SLA, and increased SDW yield, showing a high correlation with TDW (Tab. 4).

SLA is the ratio of leaf area to LDW. According to Poorter and Garnier (1999), it is an important physiological index representing leaf biomass allocation per unit of area. SLA is important for evaluating stevia genotypes because it represents the efficiency in synthesizing LDW, and the leaves present a higher concentrations of stevioside and rebaudioside A (Jarma *et al*., 2010). G12 had the highest SLA value of all the genotypes (Tab. 4), which demonstrates that G12 had an increase in the expansion of the surface of the leaf blade, providing the development of thinner and more slender leaves. In our study, the SLA values were lower than those found by Francisco (2015), where values ranged from 10.82 to 17.37 m^2 kg^{-1}. These values are explained by the longer growth period in Francisco's study (2015).

G12 had a higher TLA. Plants with a higher TLA tend to promote shading of the lower canopy leaves. Under conditions of low solar radiation, plants use photoassimilates and other nutrients to increase leaf area, resulting in an increased SLA (Lambers *et al.,* 2008).

According to Magalhães (1979), LWR is important to studying the performance of a cultivar. At the beginning of vegetative growth, plants consist of a large part of leaves, and LWR values are high. However, this ratio decreases over time as the plant develops, and other parts grow at the expense of material imported from plant leaves. There were no differences in the LWR among the genotypes (Tab. 4).

Stevioside and rebaudioside A yield

G12 showed the highest stevioside content and yield (Tab. 6). In terms of rebaudioside A, G8 was equal to G9, both of which were higher than the other genotypes (Tab. 6). G8 had the highest Reb A: St ratio and rebaudioside A yield (Reb A kg ha^{-1}).

LDW is influenced by the photoperiod (Ceunen and Geuns, 2013). Under increasing photoperiod conditions, stevia exhibits an increased LDW accumulation (Ceunen and Geuns, 2013). The genotypes in this study were exposed to a decreasing photoperiod, which indicates that the differences were associated with genetic differences.

The rebaudioside A content in G8 and G9 was equal and higher than those found in the other genotypes (Tab. 6). The values ranged from 20.39 mg g^{-1} (2.03%) in G4 to 64.77 mg g^{-1} (6.47 %) in G8, which were lower than those of Francisco (2015).

The differences between our results and those of Francisco (2015) regarding rebaudioside A content are explained by the reduction of the crop cycle. Francisco (2015) reported that the genotypes showed a 27.9% reduction in stevioside accumulation and a 36.6% reduction in rebaudioside A accumulation under decreasing photoperiod conditions.

The correlation analysis between the morphophysiological characteristics and yield components of stevioside and rebaudioside A showed that the morphophysiological variables showed a high correlation with the stevioside content and yield, while the rebaudioside A content, Reb A: St ratio and rebaudioside A yield were negatively correlated with the LDW, TLA, LAI, SLA and LAR (Tab. 7).

The low rebaudioside A yield had a negative correlation with the LDW and TLA, LAI, SLA and LAR (Tab. 7). This negative correlation may explain the lower production of rebaudioside A in genotypes

Table 6. Result of the comparison of means for the variables associated with the accumulation of glycosides stevioside and rebaudioside A contents.

Genotype	Stevioside-St (mg g^{-1})	Rebaudioside A-Reb A (mg g^{-1})	Reb A: St ratio (g g^{-1})	St yield (kg ha^{-1})	Reb A yield (kg ha^{-1})
G4	176.97 b	20.39 b	0.11 c	133.66 b	15.33 c
G8	145.93 c	64.77 a	0.44 a	79.25 c	35.14 a
G9	128.47 c	49.05 a	0.38 b	44.72 d	17.12 c
G12	200.07 a	26.81 b	0.13 c	167.28 a	22.41 b
SE	7.64	4.87	0.04	12.67	2.06

Means followed by different letters indicate significant statistical differences according to the Tukey test at ($P \leq 0.05$) ($n=4$). SE: Standard error. The results of the stevioside content (Tab. 6) ranged from 128.47 mg g^{-1} (12.8%) in G9 to 200.07 mg g^{-1} (20%) in G12. These values were consistent with Francisco (2015) (stevioside content of 12% in G9 and 14.2% in G12). The stevioside content and yield were highly correlated with LDW (Tab. 7). According to Francisco (2015), genotypes that present a high LDW yield also present a high stevioside yield.

Table 7. Correlations between growth variables with stevioside and rebaudioside A yield and content.

Indexes	LDW	TLA	LAI	SLA	LAR
St (mg g^{-1})	0.98**	0.97*	0.97*	0.88^{NS}	0.93^{NS}
Reb A (mg g^{-1})	-0.73^{NS}	-0.70^{NS}	-0.70^{NS}	-0.63^{NS}	-0.88^{NS}
St (kg ha^{-1})	0.98**	0.90**	0.90**	0.71**	0.78**
Reb A (kg ha^{-1})	0.00^{NS}	-0.08^{NS}	-0.08^{NS}	-0.63^{NS}	-0.38^{NS}
Reb A:St ratio	-0.31^{NS}	-0.80^{NS}	-0.80^{NS}	-0.71^{NS}	-0.93^{NS}

** Significant correlation at 1%, * significant correlation at 5%, ^{NS} not significant.

Leaf dry weight (LDW), total leaf area (TLA), leaf area index (LAI), specific leaf area (SLA), leaf area ratio (LAR), stevioside content (St mg g^{-1}), rebaudioside A content (Reb A mg g^{-1}), stevioside yield (St kg ha^{-1}), rebaudioside A yield (Reb A kg ha^{-1}), rebaudioside A: stevioside ratio (Reb A: St ratio).

with higher growth rates. According to Bondarev *et al.* (2010), the smaller leaves in stevia present an increased density of glandular trichomes and, consequently, a higher rebaudioside A content.

The reb A: St ratio is important for selecting stevia genotypes. According to Mota *et al.* (2011), the ideal Reb A: St ratio for industrial use is 1. The genotypes did not show the desired Reb A: St ratio. G8 had the best Reb A: St ratio of all the genotypes (Tab. 6). The ratios found in this study were lower than those observed by Francisco (2015), Tavarini *et al.* (2015) and Mandal *et al.* (2013).

G12 showed the highest stevioside yield (Tab. 6). Genotypes with a higher LDW yield and higher secondary metabolite concentration (Tavarini *et al*., 2015; Vasilakoglou *et al*., 2016) tend to have better stevioside yield. There is a strong correlation between LDW yield and TLA, LAI, SLA and LAR (Tab. 7) for stevioside yield.

CONCLUSIONS

The G8 genotype had the highest rebaudioside A content, the highest rebaudioside A yield and the best rebaudioside A: stevioside ratio under the productive conditions with a decreasing photoperiod in the Alto Vale do Itajaí region (SC).

The G12 genotype showed the best growth rate, morphophysiological indexes, stevioside content and yield.

No genotype evaluated in this study had a rebaudioside A: stevioside ratio that was suitable for industry requirements.

Conflict of interest: this manuscript was prepared and reviewed with the participation of all authors, who declare that there exists no conflict of interest that puts at risk the validity of the presented results.

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Besides a clear, precise and sequential description of the materials used for the research (plant or animal materials, agricultural or laboratory tools), this section illustrates the procedures and protocols followed, and the experimental design chosen for the statistical analysis of the data (where possible, include them as annexes). In addition, indicate the place of origin and date of the investigation.

Results and discussion

The results shall be presented in a logical, objective, and sequential order, using text, tables and figures. The latter two should be easily understandable and self-explanatory, regardless of a thorough explanation in the text. The graphics should be two-dimensional and prepared in black and white, using varying tones to illustrate variations between columns. Diagram curves must be prepared in black, dashed or continuous lines (- - - - or ———), using the following conventions: ■, □, ◆, ▲, ●, etc. The tables should contain few columns and lines.

The tables should contain few columns and lines. Averages should be accompanied by their corresponding standard error (SE) values. The discussion of results must be complete and exhaustive, explaining the results obtained, limitations and importance, in contrast to the most up-to-date literature on the subject.

Conclusions

This section should summarize in a brief and concise form the most important findings of the research, such as those containing the most significant support in the studied area.

Acknowledgments

When considered necessary, the authors may acknowledge the researchers or entities that contributed - conceptually, financially or practically - to the research: specialists, commercial organizations, governmental or private entities, and associations of professionals or technicians. Include the sponsor of the investigation. In case of projects, the name and identification code.

Conflicts of interest and authorship contributions

Mention possible conflicts of interest (personal or contractual) that place at risk the validity of the results presented, and the individual contributions of the authors in the conception, start-up, execution of the research and writing of the manuscript.

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For short citations located in the text, use the system: author(s), year uniformly. When the publication cited has three or more authors, please mention the name of the first author accompanied by the Latin phrase et al., with the year (e.g. Garcia et al., 2003). When using texts from other authors, use double quotes and mention the location page. It is recommended to paraphrase with several authors and avoid the literal inclusions.

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International unit system (SI) or metric unit system

The *Revista Colombiana de Ciencias Hortícolas* requires the use of SI units (Système international d'unités).

The numbers of multiplication and the negative numbers of the superscripts should be used only in conjunction with SI units (for example, kg ha^{-1}). Do not place non-SI units in SI units, because the units are mathematical expressions. Reorganize the phrase respectively, for example:

- P at 20 g L^{-1}, but not 20 g P L^{-1}, nor 20 g P/L.
- The yield measured in dry mass was 10 g^{-1}, but not 10 g^{-1} of dry mass dd^{-1}.
- The active ingredient was applied at 25 g ha^{-1}, but not 25 g a.i./ha^{-1}.
- Each plant received water at 30 g^{-1} ha, but not irrigation was applied at 30 g H_2O/ha per plant.

The slant line (/) is a symbol of mathematic operation that means "division"; in science, it may be substituted by the word "per" in the meaning of "per each" and indicates rates or degrees. Use the slant lineto connect SI units with non-SI units (for example: 10°C/h or 10 L/plant). Never use the raised period and slant line in the same expression. If you mix SI and non-SI units, use the slant line first and then the word "per" in the second terminus. Never use two or more slashes (/) or the word "per" more than once in the same phrase to avoid redundancy, for example: irrigation/day per plant should be changed to: each plant was irrigated two times per day. For the totally verbal units, use one slash, such as: three flowers/ plant or 10 fruits/branch.

To cite units based on names, use lowercase, such as: "one siemens represents…". However, Celsius units should be written with the first letter capitalized.

Use the same abbreviation or symbol for the singular and plural forms of a unit (for example, 1 kg and 25 kg). Leave a blank space between the numerical value and the symbol (for example, 35 g, but not 35g), except for a percentage or °C, such as: between 14 and 20°C or growth up to 3, 6, and 9 m.

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Fruit section

Vegetable section

Ornamental section

Section on aromatic, medicinal and spice plants

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