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CHLOROPHYLL FLUORESCENCE AS A STRESS INDICATOR IN LULO / SEED DORMANCY AND GERMINATION IN TREE TOMATO AND LULO / EFFECT OF DEFICIT IRRIGATION ON AROMA OF THE PEAR / QUALITY IN HASS AVOCADO: PRE-HARVEST AND HARVEST FACTORS THAT DETERMINE IT / BIOPESTICIDES ASSOCIATED ANNONACEAE / PHYLLOSPHERE MICROBIOME IN HORTICULTURAL CROPS / PLANT DENSITY AND PACLOBUTRAZOL ON PRODUCTION OF INDUSTRIAL TOMATO / SALT WATER AND SILICON APPLICATION ON BEET / ESSENCIAL OIL AFFECTS LETTUCE SEEDS / CHARACTERIZATION OF *ARRACACIA XANTHORRHIZA* GENOTYPES / AGROCLIMATIC ZONING IN THE ALTA GUAJIRA, COLOMBIA / BOVINE MANURE AND ROCK POWDER UNDER BUTTER KALE / GROWTH AND QUALITY OF LISIANTHUS ON RICE HUSK AND LEACHING RECIRCULATION /PRE-DEPULPING AND DEPULPING OF *SYAGRUS ROMANZOFFIANA* SEEDS

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REVISTA COLOMBIANA DE CIENCIAS HORTÍCOLAS
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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 Revista Colombiana de Ciencias Hortícolas, wish to inform our authors and readers that we continue to take giant steps in implementing all necessary changes for maintaining the recognition gained by the Department through Revistas Científicas Colombianas Especializadas – Publindex (Colciencias) in Convocatoria 830 of 2018, which classifies our journal in Category B, and for improving our journal's classification as much as possible. For this reason, the members of the Editorial Committee will strive to ensure that the quality of the published articles will contribute to achieving this important goal. As such, we are very pleased to present to our readers volume 13 number 3 (2019), which contains 14 articles, available completely in English in a digital format with continuous publication, i.e. accepted versions are published subject to change by definitive versions. However, a printed copy of this issue will be maintained for legal and administrative purposes.

In this issue, the Fruit Section has five articles, which include research on physiological responses in a lulo culture under waterlogged conditions, an article on tree tomato seed physiology, the effect irrigation regime on the production of volatiles of the pear, a review that analyzes the concept of quality in Hass avocado and its relationship with pre- and post-harvest factors, an article on research advances and trends in the use of biopesticides synthesized from annonaceous species, and, finally, a review of the potential applications of microbiome from the plant phyllosphere.

The Vegetable section includes various studies on the use of tomato growth regulators for industrial processing, the quality of beet irrigation water and its relationship to the addition of silicon in plants, the effect of *Lipia alba* essential oil on lettuce germination, the morphological characterization of commercial arracacha genotypes, the use agroclimatic zoning as a planning tool in the La Guajira region of Colombia, and the use of bovine mature with rock dust in kale fertilization.

The Ornamental section contains an article on the use of nutrient solutions in recirculating systems with economically important plants cultivated in commercial substrates and an article that presents different methods for depulping queen palm fruits for propagation.

This issue's articles deal with a broad range of topics related to horticultural species used for fresh consumption and also to crops destined for agro-industrial use.

With this content, we hope our readers will take the newly gained knowledge and use it to find concrete solutions for current problems in domestic and global horticultural production.

We ask our authors to continue adjusting the content of their articles to meet our publication standards in order to streamline and update our volumes. Likewise, our authors should continue to send cover images, editable graphics, ORCID, and standardized citations for authors and research evidence; noncompliance with these requirements may result in the return of manuscripts.

Finally, the institutions that edit and finance the RCCH are contemplating fees for our authors starting with the first issue of 2020 in order to raise the funds necessary for the journal's financing, sustainability, and impact.

Diego Miranda

Chief Editor

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Chlorophyll fluorescence and other physiological parameters as indicators of waterlogging and shadow stress in lulo (*Solanum quitoense* var. *septentrionale*) seedlings

Parámetros de fluorescencia de la clorofila y otros parámetros fisiológicos como indicadores del estrés por anegamiento y sombrío en plántulas de lulo (*Solanum quitoense* var. *septentrionale*)

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Experiment with lulo plants in greenhouse under shadow net.

Photo: G. Fischer

ABSTRACT

Climate change has resulted in an increasing frequency of the phenomenon "La Niña," generating prolonged periods of waterlogging and low light. The objective of the present study was to evaluate the effects of two abiotic stresses: shading (65%) and waterlogging, and their interaction on fluorescence parameters of chlorophyll *a* in lulo (*Solanum quitoense* var. *septentrionale*) seedlings. A completely randomized design with a factorial arrangement was implemented. The first factor consisted of two levels of light (with and without shading). The second factor were four levels of duration of the waterlogging period (0, 3, 6 and 9 days), for a total of 8 treatments with three replicates. The response variables were recorded at 6, 12 and 18 days after the application of the waterlogging treatments began. Measurements of relative water content (RWC), electrolyte leakage, chlorophyll content and chlorophyll *a* fluorescence were recorded. The lulo plants appeared to be more susceptible to waterlogging than to shading, with a lower RWC when waterlogged 6 and 9 days,

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presenting damage at the level of photosystem II from day 3, causing a decrease in the chlorophyll content. The plants flooded under shading had a greater tolerance to this factor than those cultivated in full light. The techniques of quantification of the chlorophyll *a* fluorescence, especially the maximum quantum efficiency of the PSII, the effective photochemical quantum yield of PS II and the photochemical quenching were useful tools that characterized the lulo seedlings under stress conditions.

Additional keywords: oxidative stress; hypoxia; light energy dissipation; electrolyte leakage.

RESUMEN

El cambio climático trae como consecuencia el aumento en la frecuencia de fenómenos como los eventos “La Niña”, generando periodos prolongados de anegamiento y sombrío. El objetivo del presente estudio fue evaluar los efectos de dos estreses abióticos sombrío (65%) y anegamiento y su interacción sobre parámetros de fluorescencia de la clorofila *a* en plántulas de lulo (*Solanum quitoense* var. *septentrionale*). Se implementó un diseño completamente al azar con un arreglo factorial. El primer factor consistió en dos niveles de sombrío (sin y con). El segundo factor fueron cuatro niveles (0, 3, 6 y 9 days) de duración del periodo de anegamiento, para un total de 8 tratamientos con tres repeticiones. Las variables de respuesta se registraron a los 6, 12 y 18 días después de iniciada la aplicación de los tratamientos de anegamiento. Se registraron medidas de contenido relativo de agua (CRA), fuga de electrolitos, contenido de clorofilas y fluorescencia de la clorofila *a*. Se encontró que las plantas de lulo son más susceptibles al anegamiento que al sombrío, evidenciado en una reducción del CRA en condiciones de anegamiento durante 6 y 9 días, presentando daño a nivel de fotosistema II a partir del día 3 y causando disminución en el contenido de clorofilas. Las plantas bajo sombrío presentaron mayor tolerancia al anegamiento en comparación a aquellas sin sombrío. Las técnicas de cuantificación de la fluorescencia de la clorofila *a*, especialmente la eficiencia máxima del PSII, la eficiencia real del PSII y el *quenching* fotoquímico, fueron una herramienta útil que permitió la caracterización de plántulas de lulo a condiciones de estrés.

Palabras clave adicionales: estrés oxidativo; hipoxia, disipación de energía lumínica, fuga de electrolitos.

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INTRODUCTION

The lulo plant (*Solanum quitoense* Lam.; Solanaceae) is a tropical shrub from the inter-Andean areas (Huertas *et al*. 2011), whose fruit is desired for its organoleptic characteristics, such as soft and exotic aroma, taste, color and bright appearance in the pulp (Ardila *et al*., 2015). In addition, it has antioxidant properties and a high nutritional potential with its important source of vitamins and minerals (Gancel *et al*., 2008). It is widely consumed in Colombia, desired mainly for its high potential in agroindustry with uses for its pulp, nectars and juices (González *et al*., 2014). It belongs to the group of exotic Solanaceae that enjoys demand in the international market, especially in Europe (Álvarez-Herrera *et al*., 2015; Cruz *et al*., 2007; Orjuela-Castro *et al*., 2017).

In Colombia, two botanical varieties predominate: a) septentrionale, with thorns on the stem and leaves and higher acidity in the fruits; and b) quitoense, without thorns and with sweeter fruits (Bonnet and Cárdenas, 2012). The lulo exhibits its best development in sites with temperatures of 15 to 24°C, with an optimum of 20°C, requires slightly acidic soils, with pH between 5.5 and 6.0, with good moisture retention capacity and deep and good drainage (Gómez *et al*., 2014). It is a plant native to humid forests, adapted to fresh and shaded zones, but, in the course of its domestication, commercial crops have been adapted to areas free from light exposure (Ardila *et al*., 2015; Huertas *et al*., 2011).

Fischer *et al*. (2018) stated that "the environmental conditions of the site (climate and soil) are crucial for the process of crop quality formation" when plants are exposed to multiple factors of abiotic and biotic stress (Visser *et al*., 2015). The Intergovernmental Panel on Climate Change (IPCC) reported that the increase in rainfall as a result of climate change might have a greater impact on tropical regions as a result of the increased world hydrological cycle (Bailey-Serres and Voeseneck, 2008), generating waterlogging conditions as a result of heavy rains and poor drainage and gloomy weather because of the greater presence of clouds. This situation is aggravated on mixed plantations and can negatively affect photosynthesis and the performance of crops (Soleh *et al*., 2018). The lulo was classified as a species susceptible to waterlogging (Flórez-Velazco *et al*., 2015) and moderately susceptible to shaded conditions (Cardona *et al*., 2016).

In general, plants are often limited by a number of stressful factors that occur simultaneously, which makes it difficult to predict their geographical distribution based on physiological responses of an individual factor (Mittler, 2006; Fischer *et al*., 2016; Fischer and Melgarejo, 2020); thus, the effect of a combined stress on plant physiology is likely to be key to understanding the mechanisms of susceptibility to stress under natural field conditions (Casierra-Posada and Cutler, 2017). However, the potential effects of the stress combination may vary depending on the level of each of the individual stresses combined (e.g. severe *vs*. low) and the type of plant or pathogen involved (Mittler, 2006; Villarreal-Navarette *et al*., 2017; Jiménez *et al*., 2015).

Some physiological responses reported about the effects of stress from waterlogging and cloudy conditions suggest that both factors interact in such a way that the adverse effect of the shade is amplified in flooded soils (Laan *et al*., 1990). Waterlogging and shading can affect the growth of plants independently or interact in such a way that a factor reduces or increases the impact of additional factors (Urbas and Zobel, 2000).

The effects of abiotic stress caused by waterlogging and shading, mainly on growth and development factors, have been studied in Solanaceae species, such as the cape gooseberry (*Physalis peruviana* L.) by Aldana *et al*. (2014), tomatoes (*Solanum lycopersicum* L.) by Ezin *et al*. (2010) and Baracaldo *et al*. (2014) and lulos (*Solanum quitoense*) by Flórez-Velasco *et al*. (2015).

It has been shown that the decrease in chlorophyll content under stressful conditions can be considered a typical symptom of oxidative stress as a result of photooxidation of pigments and degradation of photosynthetic pigments (Anjum *et al*., 2011). Chlorophyll fluorescence, which indicates the photosynthetic efficiency of photosystem II (PSII) (Bansal *et al*., 2019), is a highly informative parameter of plant traits under adverse environmental conditions. Nowadays, chlorophyll fluorescence measurements are nearly universally used in ecophysiological plant studies, where, especially under natural and managed growth conditions, plant reactions can be easily understood when factors are changing (Hanelt, 2018).

Generally, environmental stress can be detected early through the quantification of chlorophyll fluorescence since this methodology uses information on the photochemical activity of plants (Marques *et al*., 2017). This is possible because the chlorophyll molecule is fluorescent and, through the dissipation of photons, changes in electron transfer at the level of chloroplast membranes can be detected (Do Nascimento and Marques, 2018). However, studies on the effects of the interaction of two abiotic stresses on the fluorescence in lulo plants are scarce.

Therefore, the objective was to evaluate the effects of two abiotic stresses (shading and waterlogging) and their interaction on physiological parameters such as fluorescence of chlorophyll, relative water content and electrolyte leakage in seedlings of lulo (*Solanum quitoense*) var. septentrionale.

MATERIALS AND METHODS

Plant material and experiment conditions

This study was conducted in a plastic greenhouse at the Faculty of Agricultural Sciences of the Universidad Nacional de Colombia, Bogota, located at 4°35'56'' N and 74°04'51'' W and at 2,556 m a.s.l.

The plant material was lulo seedlings (*Solanum quitoense* var. *septentrionale*) obtained from seeds, with an age of 10 weeks. The seedlings were transplanted into 5 L pots, which contained a substrate composed of quartzite sand and sieved soil at a ratio of 1:1 v/v. The substrate had the following physical and chemical properties: pH 5.2; organic carbon 6.33%; N 0.55%; Ca 6.21 meq/100 g; K 1.7 meq/100 g; Mg

1.42 meq/100 g; Na 0.29 meq/100 g; P 37.2 mg kg^{-1}; Cu 0.20 mg kg^{-1}; Fe 47.0 mg kg^{-1}; Mn 4.57 mg kg^{-1}; Zn 1.45 mg kg^{-1}; B 0.34 mg kg^{-1}; AI 0.23 meq/100 g; CEC 9.94 meq/100 g; and a sandy loam texture (sand 72%, lime 17% and clay 11%).

Experiment design and treatments

A completely randomized design was carried out with a factorial arrangement, where the first factor consisted of the two levels of light conditions: a) without shading (WS); with an average noon radiation of 66,694±10,981 lux; b) shading (SH); with an average shading percentage of 65±4% (radiation of 29,935±3,428 lux), which was done through the installation of a black polyshade, 1.5 m high, covering the seedlings on all sides. The second factor corresponded to the four levels of waterlogging (WA) period (0, 3, 6 and 9 d). To do this, the drainage holes of the materials were closed hermetically, and, through irrigation, the water level was established at 3 cm above the substrate during the experiment. At the end of each period of waterlogging, the drainage holes of the pots were reopened to begin the recovery period. The experiment had a total of 8 treatments with three replicates for each one. Additionally, the response variables were recorded at 6, 12 and 18 d after the application of the waterlogging treatments began.

The mean air temperature for the two light conditions, with and without shading, was 25.3 and 25.6°C, respectively, while the mean relative humidity in the polyshade chamber was higher than that of without shading, with 61.2 and 25.7%, respectively.

Relative water content of leaves

The relative water content (*RWC*) was determined by extracting 1 cm diameter discs, taken from the middle area of the leaf blade of the second fully expanded leaf. The following equation was used to calculate the RWC (1)

$$RWC = [(FW\text{-}DW) / (WT\text{-}DW)] \times 100 \qquad (1)$$

where, *FW* is the fresh weight, *WT* is the weight at turgidity, measured after 24 h of saturation in distilled water at 4°C in darkness and *DW* is the dry weight determined after 48 h of drying in an oven at 80°C.

Electrolyte leakage

The methodology described by Jiang and Zhang (2001) was followed to determine the electrolyte leakage. Ten discs, with a 0.5 mm diameter, were extracted from the middle area of the leaf blade from the second fully expanded leaf (counted from the apical part of the plant) in each treatment replicate. The discs were lightly washed with deionized water and placed in a test tube with 30 mL of deionized water. The tubes were subsequently incubated in a water bath (model B-480; Büchi Waterbath, Flawil, Switzerland) at 30°C for 2 h. The initial electrical conductivity (EC_1) was measured with a conductimeter (Bench Meter, model P700; Oakton Instruments, Vernon Hills, IL). Subsequently, they were incubated again in the water bath, for 15 min at a temperature of 100°C, in order to extract all released electrolytes, and, with a conductivity meter, the final electrical conductivity (EC_2) was measured. The percentage of electrolytes was calculated with the following equation (2)

$$\text{Electrolyte leakage (\%)} = [(CE_1/CE_2)] \times 100 \qquad (2)$$

Chlorophyll content

The chlorophyll content was estimated using a chlorophyllometer (atLEAF, FT Green, Wilmington, DE). The readings were taken on the second fully expanded leaf, taking three data per replicate.

Fluorescence of chlorophyll *a*

The maximum quantum efficiency of photosystem II (PSII) (F_v/F_m), the effective photochemical quantum yield of PSII (Y_{II}), the photochemical quenching (qP) and the non-photochemical quenching (NPQ) were determined at 6, 12 and 18 DAOW, using a Modulated fluorescence chlorophyll meter (MINI-PAM, Walz, Effeltrich, Germany). The leaves were adapted to darkness with clips for 15 min. The maximal fluorescence (F_m) was estimated with a 0.8 s long saturating light pulse (2,600 μmol m^{-2} s^{-1}), with 20,000 Hz frequency. The variable fluorescence (F_v) was estimated with the difference between F_0 and F_m. The potential maximal PSII quantum yield (F_v/F_m ratio) was calculated from the F_v and F_m. The photochemical and non-photochemical quenching were assessed as qP =

$(F_m' - F)/(F_m' - F_0)$, and NPQ = $(F_m - F_m')/F_m'$ according to Schreiber *et al.* (1994).

Statistical analysis

The data were analyzed using Statistix program v. 9.0 (analytical software; Informer Technologies, Tallahassee, FL). When significant differences were obtained in the ANAVA, the Tukey mean comparative test at $P \leq 0.05$ was used.

RESULTS AND DISCUSSION

Relative water content (RWC) of leaves

The RWC expresses the percentage of water content and is relative to the turgidity or total saturation in a given tissue (Melgarejo, 2010). Significant differences were found in the waterlogging factor at the three evaluation moments (Tab. 1). The waterlogging period of 6 d and longer negatively affected the RWC (Tab. 2). Although at 12 and 18 d after the onset of waterlogging (DAOW) the plants in the 6 and 9 d waterlogging treatments had a recovery time (6 and 3 d at the first evaluation point; 12 and 9 d at the second evaluation point, respectively), the values were close to 55 and 65% of the RWC at the two evaluation points, respectively, and were significantly lower than the control treatment (about 80% RWC).

The reduction of the RWC after 6 d of waterlogging was possibly caused by the low absorption and transfer of water to the leaves, negatively affecting cellular turgor (Moreno and Fischer, 2014) as caused by anoxia conditions in the root system (Moreno *et al*., 2019), a situation that can damage numerous metabolism processes in plants (Yan *et al*., 2018). Similarly, six tree species in Bogota constantly decreased their RWC with an increase in waterlogged time between 0 and 28 d (Moreno *et al*., 2019).

Table 1. Summary of analysis of variance of the effect of shading (SH) and waterlogging (WA) treatments on physiological variables of lulo seedlings at 6, 12 and 18 d after the onset of waterlogging (DAOW).

Variable	Abbreviation	6 DAOW			12 DAOW			18 DAOW		
		SH	WA	SH × WA	SH	WA	SH × WA	SH	WA	SH × WA
Relative water content	RWC	NS	*	NS	NS	***	NS	NS	**	NS
Electrolyte leakage		NS	NS	***	***	**	NS	***	NS	*
Chlorophyll content	At-LEAF	*	NS	NS	*	***	**	NS	***	**
Real efficiency of PSII	$Y_{(II)}$	NS	*	NS	NS	***	NS	***	***	NS
Photochemical quenching	qP	***	*	***	NS	**	NS	*	***	NS
Non-photochemical quenching	NPQ	NS	***	**	**	NS	**	***	**	**
Maximum efficiency of PSII	F_v/F_m	**	NS	NS	**	***	*	**	**	NS

NS: non-significant; *significance level $P \leq 0.05$, **significance level $P \leq 0.01$, *** significance level $P \leq 0.001$.

Table 2. Relative water content (RWC) of the lulo seedling leaf under conditions of 0, 3, 6 and 9 d of waterlogging and subsequent recovery of 6, 12 and 18 d (DAOW).

Treatment	RWC (%)		
	6 DAOW	12 DAOW	18 DAOW
Waterlogging (d)			
0	81.45±2.66 a	79.75±2.26 a	79.45±2.97 a
3	75.27±4.27 ab	83.42±2.73 a	74.70±2.51 a
6	70.22±4.31 b	56.73±8.25 b	68.52±3.58 ab
9	71.68±3.85 ab	52.62±2.48 b	60.48±8.17 b
CV (%)	8.68	9.91	10.73

Means with different letters indicate a significant statistical differences according to Tukey test ($P \leq 0.05$) ($n=3$ ± standard error); CV, coefficient of variation.

The plants with up to 3 d of waterlogging (DWA) without any influence on the RWC or recovery time may have activated mechanisms that diminished the water potential in the plant cells through the synthesis of compatible osmolytes, such as proline, soluble sugars, or glycine betaine in order to maintain a water potential gradient that favored water intake (Oh and Komatsu, 2015). On the other hand, and according to Cardona *et al*. (2016), lulo plants waterlogged for 3 d do not suffer damage to their root system, so the absorption and transport of water in this organ is much greater and is related to the high RWC values compared to the 6 and 9 d waterlogged plants, which presented more serious damage, especially through the reduced diameter of the root neck.

Electrolyte leakage

For the loss of electrolytes, significant differences were found at the level of the shading×waterlogging interaction at the three sampling points (Fig. 1).

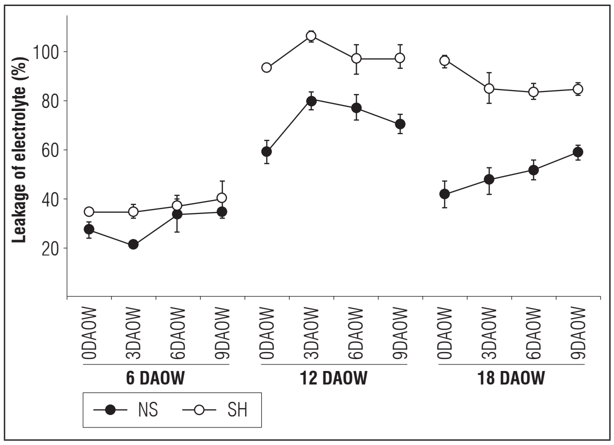

Figure 1. Electrolyte leakage in lulo seedlings under non-shading (NS) and shading (SH) conditions and at 0, 3, 6 and 9 d of waterlogging (DWA) and subsequent recovery of 6, 12 and 18 d after onset of waterlogging (DAOW). The vertical bars indicate ± standard error.

In general, it was observed that the treatments that did not have the additional shade (SH) presented the lowest values for electrolyte leakage. At 6 DAOW, the loss of electrolytes from the seedlings with shading maintained values close to 40%, while the seedlings without shading had values that were on average 35%. At 12 DAOW, the leakage of electrolytes from the seedlings with shadowy conditions was higher than 90%. Also, for the seedlings without shading with 0 d of waterlogging, the electrolyte leakage values were close to 60%, probably because of a mild dry air stress during this evaluation period (Sánchez-Reinoso *et al*., 2019). For the plants with some period of waterlogging stress, these values were close to 80%. At 18 DAOW, the percentage of electrolyte leakage from the plants of the different SH treatments was close to 85%. However, the highest values found in the seedlings of the different WS treatments were observed in those that had 9 d of waterlogging, with about 60% electrolyte leakage, and the lowest values were evidenced in the seedlings with 0 d of waterlogging (about 40% electrolyte leakage). However, the treatment of the lulo seedlings with 9 d of waterlogging reached values of approximately 60% loss of electrolytes.

Obviously, the flooding stress was increased by the conditions of shading, increasing the leakage of electrolytes, which is related to oxidative stress (Moreno *et al*., 2019). Similar results were found by Bansal *et al*. (2019) in black beans, in which the membrane stability decreased as a result of the greater loss of electrolytes generated by the conditions of the lack of oxygen in the radical system (waterlogging for 10 d starting at 30 d after sowing), which was also manifested by cell membrane damage by ROS-induced peroxidation of membrane lipids (Andrade *et al*., 2018).

Chlorophyll content

For the chlorophyll content, significant differences were obtained in the shading×waterlogging interaction at the three sampling points (Fig. 2; Tab. 1). Particularly, the plants in the different shading treatments had lower chlorophyll contents than the shaded plants. Additionally, the chlorophyll values at 6 DAOW were, on average, ~ 50 at-LEAF units. In general, it was evident that, with the increase in the period of waterlogging, the reduction of chlorophyll content was greater. This reduction was more pronounced in the treatments with 6 and 9 d in the last two evaluation points when the plants were unshaded, which reached values close to 30 at-LEAF units. However, the SH plants with 9 d of waterlogging reached the lowest values at 18 DAOW (about ~35 at-LEAF units), followed by SH plants with 6 d of waterlogging (~ 40 at-LEAF units) at the same evaluation point.

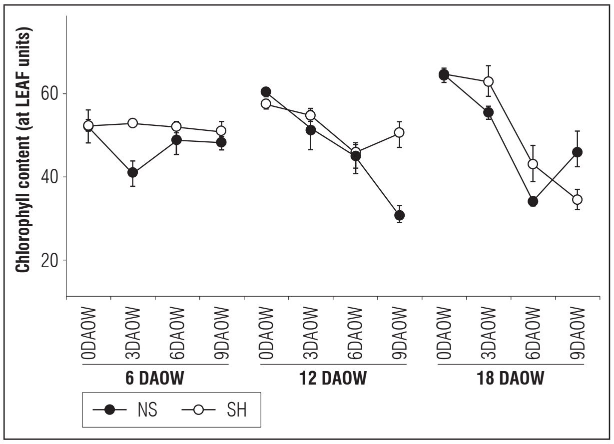

Figure 2. Chlorophyll content in at-LEAF units in lulo seedlings under non-shading (NS) and shading (SH) conditions and at 0, 3, 6 and 9 d of waterlogging (DWA) and subsequent recovery of 6, 12 and 18 d after onset of waterlogging (DAOW). The vertical bars indicate ± standard error.

A decrease in chlorophyll content with the duration of waterlogging stress has been observed in many species, such as the cape gooseberry (Aldana *et al*., 2014), tomatoes (Ezin *et al*., 2010; Baracaldo *et al*., 2014), black beans (Bansal *et al*., 2019), and cabbage (Casierra-Posada and Cutler, 2017), among others, which is manifested by foliar yellowing, followed by wilting, affecting photosynthesis (Wu *et al*., 2015) and consequently reducing the biomass of the lulo plants, such as with a decrease in leaf area and root volume (Cardona *et al*., 2016).

The lower reduction of chlorophyll content in the lulo plants under additional shade as waterlogging continued (Fig. 2) coincided with that observed by Mielke and Schaffer (2009) in the Solanaceae pitanga, taking into account that shaded leaves generally have a higher concentration of chlorophyll and more pigment molecules per electron transport chain than leaves in full light (Kadereit *et al*., 2014). However, Visser *et al*. (2015) found the opposite in *Solanum dulcamara*, which means that the flooded and shaded plants synthesized a smaller amount of chlorophyll than those waterlogged and in full light.

Chlorophyll fluorescence parameters

The results of the maximum quantum efficiency of PSII (F_v/F_m), effective photochemical quantum yield of PSII (Y_{II}), photochemical quenching (qP), and non-photochemical quenching (NPQ) were affected by the waterlogging (Fig. 3). In general, regardless of the duration of waterlogging or the shading condition, there was a tendency towards a reduction of the parameters F_v/F_m, $Y_{(II)}$, and qP. Additionally, the reduction was higher in the seedlings that were not shaded (NS), being lower in those that had 9 d of waterlogging (Fig. 3A, B and C). However, the NPQ tended to increase its values up to 40% with respect to the waterlogging condition, being greater when the lulo plants were exposed to a greater number of days (especially 6 and 9 d of waterlogging). However, at 6 DAOW, the highest values were found in the plants in shading conditions, but, as time progressed, the shaded plants had higher values (Fig. 3D).

For the reduction of the NPQ values at 12 and 18 DAOW, it is supposed that the lulo plants under shady conditions did not have stress conditions, with no need to dissipate energy in the form of heat since lulo plants are native to undergrowth areas (Bonnet and Cárdenas, 2012). This may be related mainly to the fact that the light compensation point may be lower than in other species grown with free exposure (Taiz *et al*., 2018).

A reduction in the maximum quantum efficiency of PSII, when the condition of waterlogging stress is increased, is common in many different plant species (e.g. black beans, Bansal *et al*., 2019; pitanga, Mielke and Schaffer, 2010; and several tree species, Moreno *et al*., 2019). Most likely, the decrease in F_v/F_m in the flooded lulo indicated that it adversely affected the photochemistry of photosynthesis in these stressed plants (Mielke and Schaffer, 2010), while Janowiak *et al*. (2002) observed tomato damage in the light-harvesting complex of PSII in waterlogged plants.

The strong decrease in the F_v/F_m ratio was especially evident in the plants with 9 d of waterlogging and 12 of recovery, with values below 0.7, which indicated severe stress that generated serious damage in PSII (Moreno *et al*., 2019) and showed, according to Ashraf (2012), the inability of the plants to regenerate Rubisco under these stressful conditions. In the other treatments, with levels that exceeded 0.7, the functioning of PSII was not impaired (Bansal *et al*., 2019); rather, there was a dynamic photoinhibition, without presenting real damage to the photosystems (Moreno *et al*., 2019).

The waterlogged and shaded lulo showed a lower reduction in the maximum efficiency of PSII than the

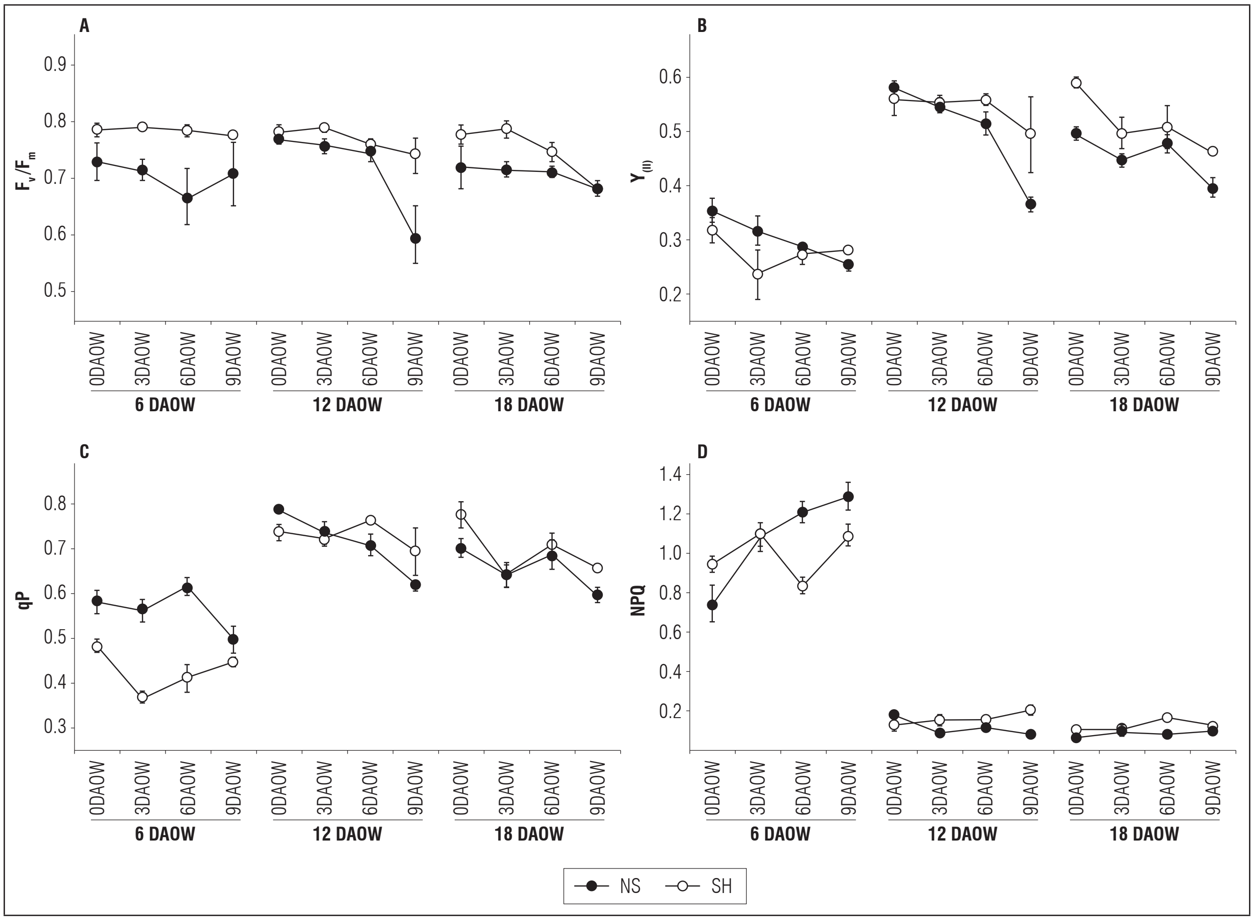

Figure 3. Fluorescence parameters of chlorophyll *a* in lulo seedlings under non-shading (NS) and shading (SH) conditions and at 0, 3, 6 and 9 d of waterlogging (DWA) and subsequent recovery of 6, 12 and 18 d after onset of waterlogging (DAOW). A) Maximum quantum efficiency of PSII (F_v/F_m); B) Effective photochemical quantum yield of PSII (Y_{II}); C) Photochemical quenching (qP); and D) Non-photochemical quenching (NPQ). The vertical bars indicate ± standard error.

plants at full light, a result that Mielke and Schaffer (2010) also observed in pitanga. Supposedly, a higher concentration of chlorophyll, larger grana and a higher proportion of PSII compared to PSI in shaded leaves (Kadereit *et al*., 2014) led to the increase in F_v/F_m in the lulo.

The effective photochemical quantum yield of PSII (Y_{II}) indicates the part of the energy absorbed by chlorophyll, which is associated with PSII and is used in the photochemical activity, evidencing the sum of transported electrons and, thus, becomes an indicator for photosynthesis (Lichtenthaler *et al*., 2005; Jiménez-Suancha *et al*., 2015). Overall, the Y_{II} of the waterlogged lulo plants decreased with the increased stress, as Else *et al*. (2009) observed in tomatoes. With the increase in recovery time (at 12 and 18 DAOW), this stress was reduced.

Photochemical quenching (qP) quantifies the photochemical capacity of PSII and refers to the proportion of excitation energy trapped by open PSII reaction centers (Lichtenthaler *et al*., 2005; Hanelt, 2018). While non-photochemical quenching (NPQ) indicates the activation of non-photochemical processes that lead to the dissipation of non-radiant energy, such as changes in the transthylacoidal pH gradient, photoinhibition, interruption of light collection complexes, and formation of zeaxanthin, among others (Jiménez-Suancha *et al*., 2015; Rohaček, 2002).

The reduction of the qP value in all treatments, especially between 0 and 3 d of waterlogging and also in the comparison between the 6 and 9 d waterlogged plants, indicated that the plants were under stress. Casierra-Posada and Cutler (2017) and Wu *et al*. (2015) observed a reduction of photochemical dissipation in

Triticum aestivum as the result of a reduction in the efficiency of the excited energy collected in the open photosystem II centers. In the lulo, it was observed that the plants tried to tolerate waterlogging in a specific way when they were stressed for 6 d, as seen in the increases in the qP and YII values, mainly in the shading treatments (Fig. 3B and C), but, in the end, they could not resist, as the results showed at 9 DWA, at recovery times of 12 and 18 DAOW. The increase in the non-photochemical dissipation with the increase in the stress duration was observed in the lulo only when the recovery of the plants occurred for 6 d (Fig. 3D), an effect that occurred in tomatoes (Else *et al*., 2009) and manifested mainly in plants under full light (Waldhoff *et al*., 2002); this situation did not occur in the plants with recovery periods of 12 and 18 d.

Interestingly, the qP values were near 0.8 at 0 DWA on 12DAOW; presumedly, the low radiation accompanied by a mild dry air stress condition that occurred in the evaluation period resulted in this value (Sánchez-Reinoso *et al*., 2019).

CONCLUSIONS

In the lulo seedlings, the effect of waterlogging and shading was especially observed in the treatments evaluated at 12 and 18 d after the onset of the treatments, in which a lower light intensity (shade) favored the chlorophyll content and the maximum and real efficiency of photosystem II, but also increased the electrolyte leakage.

The lack of water absorption and translocation that resulted from the waterlogging of the root system reduced the relative water content in the leaves, especially in the plants with a longer waterlogging time (6 and 9 d) and observation (12 and 18 d).

For the chlorophyll *a* fluorescence, the measurements of the maximum quantum efficiency of PSII (F_v/F_m), the effective photochemical quantum yield of PSII (Y_{II}), and the photochemical quenching (qP) proved to be a good indicator of the effects of this double stress on the lulo seedlings.

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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Seed dormancy and germination in tree tomato (*Solanum betaceum* Cav.) and lulo (*Solanum quitoense* Lam.)

Latencia y germinación de semillas de tomate de árbol (*Solanum betaceum* Cav.) y lulo (*Solanum quitoense* Lam.)

ALBA MARINA TORRES-GONZÁLEZ[1, 2]

Lulo and tree tomato fruits.

Photo: A.M. Torres-González

ABSTRACT

Tree tomato (*Solanum betaceum* Cav.) and lulo (*Solanum quitoense* Lam.) fruits enjoy high consumption and commercialization in Colombia. Seed dormancy has been reported for both species, and their propagation depends on seeds. The optimal germination conditions for these species are not well known. Thus, the temperature regimes for the seed germination were based on the mean, minimum and maximum temperatures of the locations where the crops were grown. Germination tests were carried out in four replicates of 50 seeds each on Petri dishes for both crops. Six temperature conditions and four pre-treatments were evaluated to break the seed dormancy for several seed lots. *S. betaceum* and *S. quitoese* exhibited shallow seed dormancy, and less dormancy was detected in the commercialized cultivars, such as *S. betaceum* cv. Tamarillo and *S. quitoense* (*i.e.* common lulo). For both species, the most recently harvested seeds had more germination capacity than the seeds stored for several months at a low seed moisture content (4%) and low storage temperature (20°C). The seed dormancy of *S. betaceum* and *S. quitoense* was broken successfully by applying GA_3 (2,000 mg L^{-1}) or alternating temperatures (*e.g.* 25/15°C). However, both treatments at the same time did not provide an additional benefit to promote seed germination. Potassium nitrate (1%) promoted seed germination in the *S. betaceum* seeds at both constant and alternating temperatures and in the *S. quitoense* seeds, only when alternating temperatures were applied. The application of GA_3 increased the rate of germination more than KNO_3 for both species at all temperatures. Using any of these treatments would work well to break seed dormancy in *S. betaceum* and *S. quitoense*, and the most convenient option could be selected depending upon budget and other resources.

Additional key words: gibberellic acid; potassium nitrate; seed storage; plant propagation.

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RESUMEN

Tomate de árbol (*Solanum betaceum* Cav.) y lulo (*Solanum quitoense* Lam.) son frutas altamente consumidas y comercializadas en Colombia. Latencia de semillas ha sido reportada para ambas especies y su propagación se realiza por semillas. Las condiciones óptimas de germinación no son bien conocidas para estas especies. Así, los regímenes de temperatura para la germinación de las semillas fueron usados con base en la temperatura media, mínima y máxima de las localidades donde crecen estos cultivos. Se realizaron pruebas de germinación en cajas de Petri, con seis temperaturas y cuatro pre-tratamientos que fueron probados para romper la latencia de varios lotes de semillas. Los resultados muestran que las dos especies tienen latencia, en menor medida en los cultivares más comercializados, *S. betaceum* cv. Tamarillo y *S. quitoense* (*i.e.* cultivar común). Para ambas especies, las semillas recién cosechadas tuvieron mayor promoción de la germinación que las almacenadas por varios meses con bajo contenido de humedad de la semilla (4%) y baja temperatura de almacenaiento (20°C). La latencia de las semillas de *S. betaceum* y *S. quitoense* se rompió exitosamente con la aplicación de GA_3 (2,000 mg L^{-1}) o temperaturas alternadas (*e.g.* 25/15°C). Ambos tratamientos al mismo tiempo no tuvieron un beneficio adicional para promover la germinación. KNO_3 (1%) promovió la germinación de semillas de *S. betaceum* a temperaturas constantes y alternadas, y de semillas de *S. quitoense* solamente a temperaturas alternadas. El pre-tratamiento con GA_3 incrementó la velocidad de germinación más que KNO_3, para ambas especies y en todas las temperaturas. Se sugiere usar cualquiera de estos tratamientos que funciona bien para romper la latencia de semillas de *S. betaceum* and *S. quitoense*, y la opción más conveniente puede ser seleccionada dependiendo del presupuesto y otros recursos.

Palabras clave adicionales: ácido giberélico; nitrato de potasio; almacenamiento de semillas; propagación vegetal.

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INTRODUCTION

Solanaceae is one of the main plant families of economic importance in Colombia because it provides several food species. Among the edible Solanaceae species, tree tomato (*Solanum betaceum* Cav. = *Cyphomandra betaceum* (Cav.) Sendtn.) and lulo (*Solanum quitoense* Lam.) fruits enjoy high consumption and commercialization in Colombia. Despite being cultivated species, the germplasm of both species studied with COSII molecular markers have a high population structure, which could be the result of low genetic migration between planted populations (Enciso-Rodríguez *et al*., 2010).

S. betaceum is a domesticated species grown throughout the Andes between 600 and 3,200 m a.s.l. (Bohs, 2015). Moreover, *S. betaceum* is grown for commercial purposes in New Zealand with the name "Tamarillo", in the Mediterranean area and in Germany (Samuels, 2015). Thus, the name "Tamarillo" has become the standard commercial designation for *S. betaceum* in Europe, which includes fruits with a dark-purple and red skin that have purple pulp and fruits with a yellow and orange skin with yellow pulp (The Climbing Fig, 2020). However, in the domestic market of Colombia, the name "Tamarillo" is used exclusively for fruits with a purple skin and pulp.

S. quitoense is cultivated most commonly in Colombia, Ecuador, and Peru at altitudes between 800 and 2,000 m a.s.l. (Bohs, 2015; Gallo *et al*., 2018). In Colombia, the number of lulo crops has grown because domestic demand has increased (Arias and Rendon, 2015). Two varieties of *S. quitoense* commercially cultivated are *S. quitoense* var. *septentrionale* with spines and *S. quitoense* var *quitoense* without spines (Samuels, 2015). *S. quitoense* is considered one of the species with high gastronomic innovation in the Andean biodiversity (Corzo-Barragan *et al.,* 2016) and is known as the "golden fruit of the Andes" (Ramirez *et al*., 2018). There is an interspecific hybrid between *S. quitoense* and *S. hirtum* known as *S. quitoense* cv. "La Selva", which exhibited greater genetic variability than *S. quitoense* (Fory *et al*. 2010).

S. quitoense is propagated by seeds to obtain more vigorous plants, but *S. quitoense* "La Selva" has to be propagated asexually by cuttings or *in vitro* micropropagation to conserve the hybrid characteristics (Franco *et al*., 2002). The propagation of these *Solanum* fruit crops depends mainly on seeds. Micro propagation of hypocotyls, which is an efficient option for massive propagation of *S. betaceum*, starts with seed germination (Criollo *et al*., 2016). However, seeds frequently exhibit dormancy as an evolutive response of the species to prevent germination until the appropriate time (Shut *et al*., 2016).

Seed dormancy is found in several cultivated species of *Solanum*. For instance, seed germination of the common tomato (*Solanum lycopersicum* L. = *Lycopersicon esculentum* Mill.) is restricted by seed tissues, such as the embryo by controlling the water potential, the endosperm by controlling cell wall hydrolysis, and the testa by preventing radicle protrusion (Hilhorst, 1997). In tomatoes, cell cycle activity before germination is required, accompanied by nuclear DNA replication, but is blocked in dormant and gibberellin-deficient tomato seeds (Groot *et al*., 1997). The weed species *Solanum nigrum* and *S. physalifolium* exhibit variation in the level of dormancy, enabling populations to escape from weed control or extreme climatic conditions (Taab and Andersson, 2009).

Germination in Solanaceae species is frequently light sensitive. In tobacco (*Nicotiana tabacum),* there is light dependence during seed germination (Koo *et al*., 2015), but *Solanum elaeagnifolium* has similar germination in dark or light conditions (Stanton *et al*., 2012).

Gibberellic acid promotes germination successfully in potato (*Solanum tuberosum*) seeds when it is applied at high concentrations of 2,000 mg L^{-1} and pre-applied for 24 h (Spicer and Dionne, 1961). In *S. betaceum*, alternating temperatures of 28/24°C for 12/12 h with applications of GA_3 (1,000 mg L^{-1}) promoted seed germination, but with an application of KNO_3 (0.2%), the seed germination of *S. betaceum* and *S. quitoense* decreased (Cárdenas *et al*., 2004).

Breaking seed dormancy is particularly important for agricultural production because seed germination is the initial phase that ensures the establishment of seedlings and, finally, the crop yield. Thus, this research answered the questions: 1) Is it possible to promote seed germination of *S. betaceum* and *S. quitoense* by applying chemical treatments and incubating at constant or alternate temperatures? 2) Are there differences in seed dormancy between cultivars of *S. betaceum* and *S. quitoense*?

MATERIALS AND METHODS

Plant material

Mature S. *betaceum* and *S. quitoense* fruits were purchased from local markets in Cali, Colombia. The storage condition of the seeds are provided in Tab. 1. The seed extraction and conditioning were done following the protocol to extract seeds from fleshy fruits (Torres-González, 2018). All seeds were dehydrated, and some seed lots were stored in cold conditions to prevent loss of viability before testing (Hong and Ellis, 1996). The moisture content test of the seed lots was determined before the germination tests according to the high-constant-temperature-oven method, namely 1 h drying at 130°C (ISTA, 2016). Two samples of 1-2 g each of the entire seeds were dried in Pyrex Petri dishes (85×15 mm) in an oven (Thermocenter, SalvisLab, Switzerland) and weighed on an analytical balance (Metler Toledo International Inc., Switzerland) to the nearest 0.01 mg before and after drying. The moisture content was calculated as percentage wet basis (ISTA, 2016).

Germination tests

Each germination test consisted of four replicates of 50 seeds. No fungicides were added to the substrate to avoid extra chemical factors affecting germination. Scarification for the seeds of these species was not needed.

Constant illumination and alternating temperature incubators were used for the germination tests. The thermoperiod was set for 16/8 h at alternating temperatures. In each case, the first temperature listed for each alternating-temperature regime was provided for 16 h of each daily cycle; *e.g.* 15/25 indicates 15°C applied for 16 h d^{-1} and 25°C applied for 8 h d^{-1}. The temperature regimes were based on the mean, minimum and maximum temperatures of the locations where the crops were grown, according to Vargas-Figueroa and Torres-González (2018). The photoperiod was provided for 16 h per day for both constant and alternating temperatures. In the latter case, the lighting period was synchronous with the longer thermoperiod.

Germination was recorded as radicle protrusion (≥ 2 mm length). The tests were carried out on 8.5 x 20 mm Petri dishes with two filter papers (Whatman

Table 1. Storage time, temperature and moisture of *Solanum betaceum* and *S. quitoense* seeds.

Cultivar	Seed lot	Time (months)	Temperature (ºC)	Moisture(%)
S. betaceum cv. Tamarillo	A	7	20	4.0
S. betaceum cv. Tamarillo	B	0	-	4.0
S. betaceum	C	7	20	4.0
S. betaceum	D	0	-	4.0
S. quitoense	A	2	20	4.0
S. quitoense cv. La Selva	B	0	-	4.0

Ltd., grade 181) moistened with 4.5 mL of deionized water. One Petri dish was used for each replicate, and four dishes were placed in a loosely folded plastic bag. The duration of the test was 21 d. The germination tests were checked three times a week to record the progress of germination during tests.

Several chemical pre-treatments were applied for 24 h at 20ºC before beginning germination tests; 30 mL of solution was used to soak 100-200 seeds.

Experiment design

For each species, the seed lots included the varieties cultivated and commercialized in Colombia (Tab. 1). The temperature regimes were 1) constant temperature, 15ºC; 2) constant temperature, 25ºC; 3) alternating temperatures, 15/25ºC; 4) alternating temperatures, 25/15ºC, 5) alternating temperatures, 20/30 ºC; and 6) alternating temperatures 20/35ºC. The controls and treatments were: 1) dry seeds, as dry control; 2) deionized water, as pre-applied control; 3) gibberellic acid (GA_3), 2,000 mg L^{-1}; and 4) potassium nitrate (KNO_3), 1%.

The experiments had a factorial design that differed among the species. Thus, for S. *betaceum* the factorial was 4×6×4, and, for *S. quitoense,* it was 2×6×4, indicating the number of seed lots, temperature regimes and pre-treatments applied, respectively.

Statistical analysis

The rate of germination was calculated with the formula used by Torres-González (2018): $R = \sum (n/(d*n))$, where *n* is the number of seeds germinated on day *d,* and, *d* is the number of days from the beginning of the germination test.

For normality of variances for the analysis of variance, the germination percent was transformed to angles using the arcsine function for the formula: angles (radians) = Arcsin√ (% germination/100). The angles were transformed from radians to degrees. The analysis of variance (ANOVA) was used to compare the variation between factors. An analysis of normality and homogeneity of variance of the transformed data was performed. The SAS software was used for these analyses (SAS, 2020). The α level to determine the significance in the F test was 0.05. When no interaction between factors was found, *a posteriori* Tukey test was applied ($P \leq 0.05$).

RESULTS

Solanum betaceum Cav. (tree tomato)

Depending upon the treatment combination, the germination ranged from 13 to 99% (Fig. 1, $P<0.05$). Seed lots A and B of cultivar Tamarillo had higher germination than the common cultivar (lots C and D). In the majority of treatments for both cultivars, the recently extracted seeds (lots B and D) germinated better than the seeds stored for seven months (lots A and C). The alternating temperatures 25/15ºC gave the greatest germination for all treatments over the other temperature regimes. The interaction between the effect of alternating temperature (25/15ºC) and application of either gibberellic acid or potassium nitrate for seed lot B showed that the two different pre-treatments not only had similar effects but also had a similar effect to that of temperature alternations, while there were no additive effects from pre-treatment and alternation (Fig. 2).

In consequence, high germination was obtained with gibberellic acid and potassium nitrate pre-treatment at constant temperature (97 and 95%, respectively) or in the water control at an alternating temperature of 25/15ºC (95%). The lowest germination occurred for dry seeds at a constant temperature of 15°C for all seed lots (13-40%), demonstrating that soaking in water at this temperature was beneficial (38-80%), (Fig. 1).

The rate of germination was affected by all factors with significant interactions between them ($P \leq 0.05$). The highest rate of germination was at 25 and 25/15°C with pre-treatment in gibberellic acid

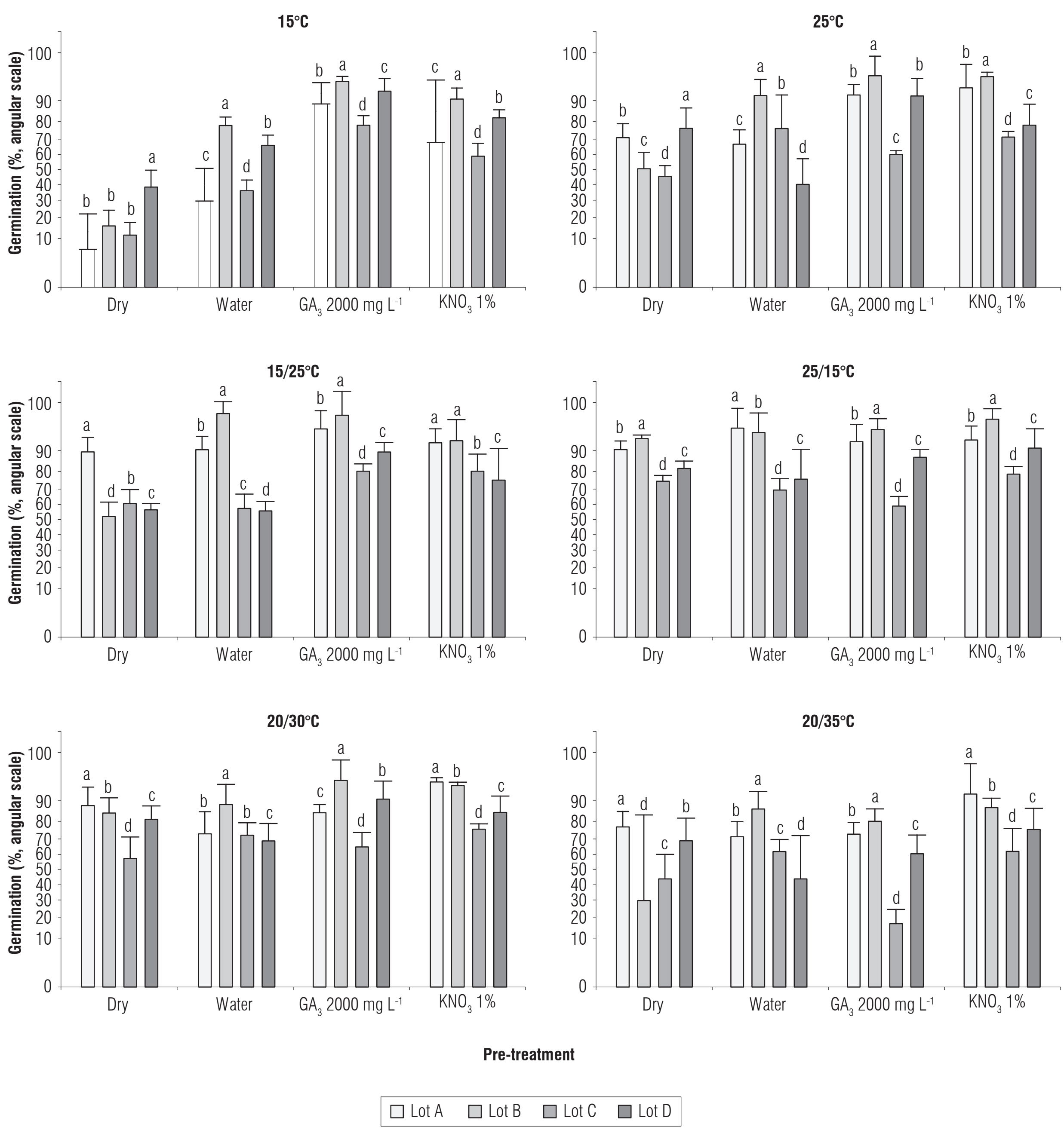

Figure 1. Effect of constant or alternating temperature and several pre-treatments on the germination of four seed lots of *Solanum betaceum* Cav. Lot A: cv. Tamarillo, 7 months in storage, lot B: cv. Tamarillo, 0 months in storage, lot C: common tree tomato, 7 months in storage, lot D: common tree tomato, 0 months in storage. Means with different letters indicate significant statistical difference within each pre-treatment according to the Tukey test ($P \leq 0.05$; $n = 4$). The vertical bars indicate ± standard error.

(Fig. 3). In these regimes, lots A, B and D showed similar rapid rates of germination, with lot C being appreciably slower.

Solanum quitoense (lulo)

The germination was affected by all factors, their second-order interaction and by the first-order interaction between temperature and pre-treatment ($P \leq 0.05$). Generally, lot A (*i.e.* common cultivar) had greater germination in the tested temperatures and treatments than lot B (cv. La Selva), (Fig. 4). The alternating temperature regime 25/15°C gave a higher germination for all treatments, even for the dry and water controls. However, gibberellic acid promoted

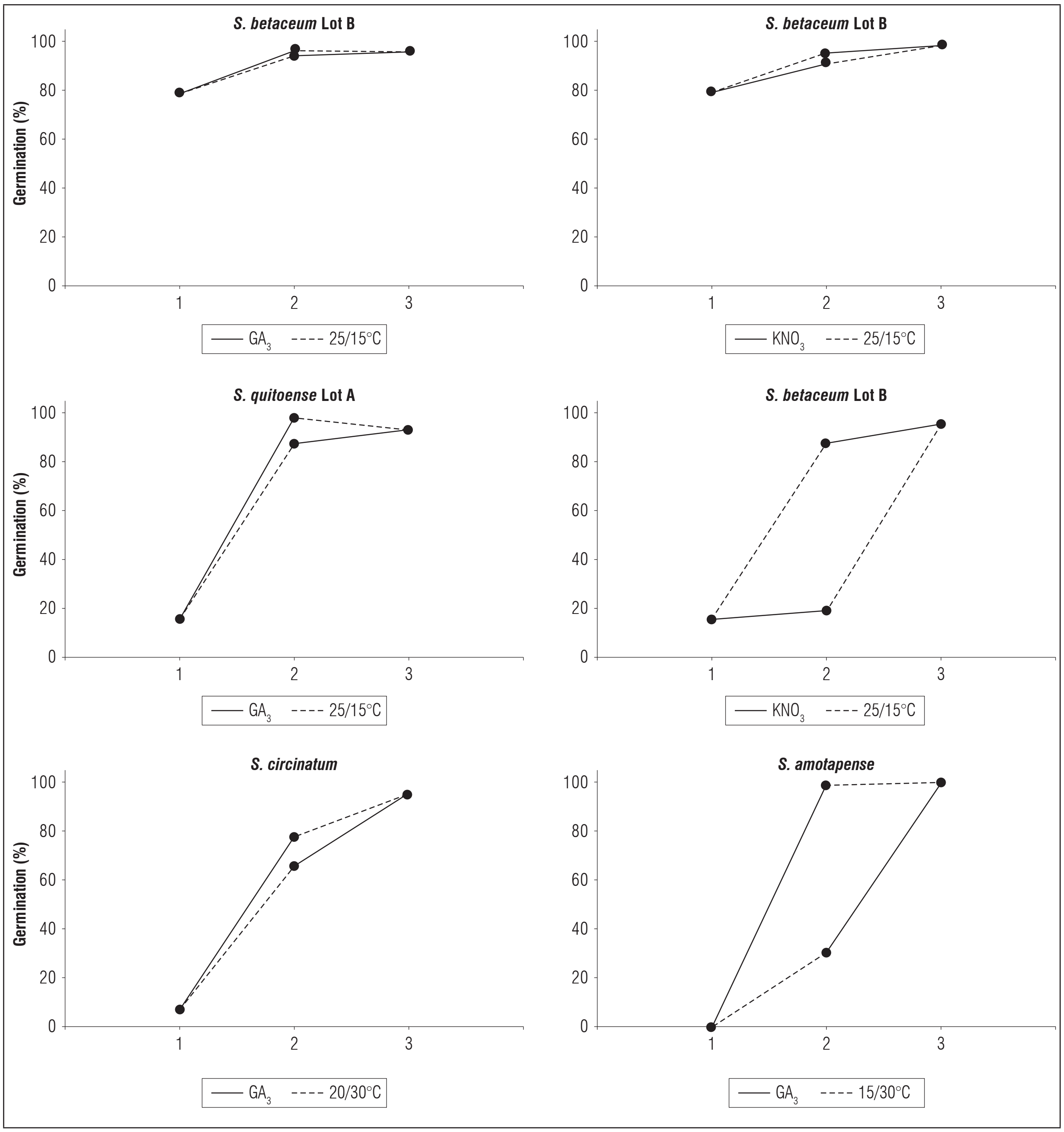

Figure 2. Interaction between factors promoting germination of *Solanum betaceum* **Cav. Lot B (control: 15ºC, water) and** *S. quitoense* **Lam. Lot A (control: 15ºC, water). 0: no factors applied, 1: one factor applied, 2: two factors applied.**

germination substantially at temperatures with less promotion of seed germination (*e.g.* 91-100% at 25°C). The interaction between gibberellic acid and alternating temperature (25/15°C) was, therefore, negative because either factor promoted close to full germination (Fig. 2). In contrast, the effects of alternating temperature (25/15°C) and pre-treatment with potassium nitrate were additives (Fig. 2).

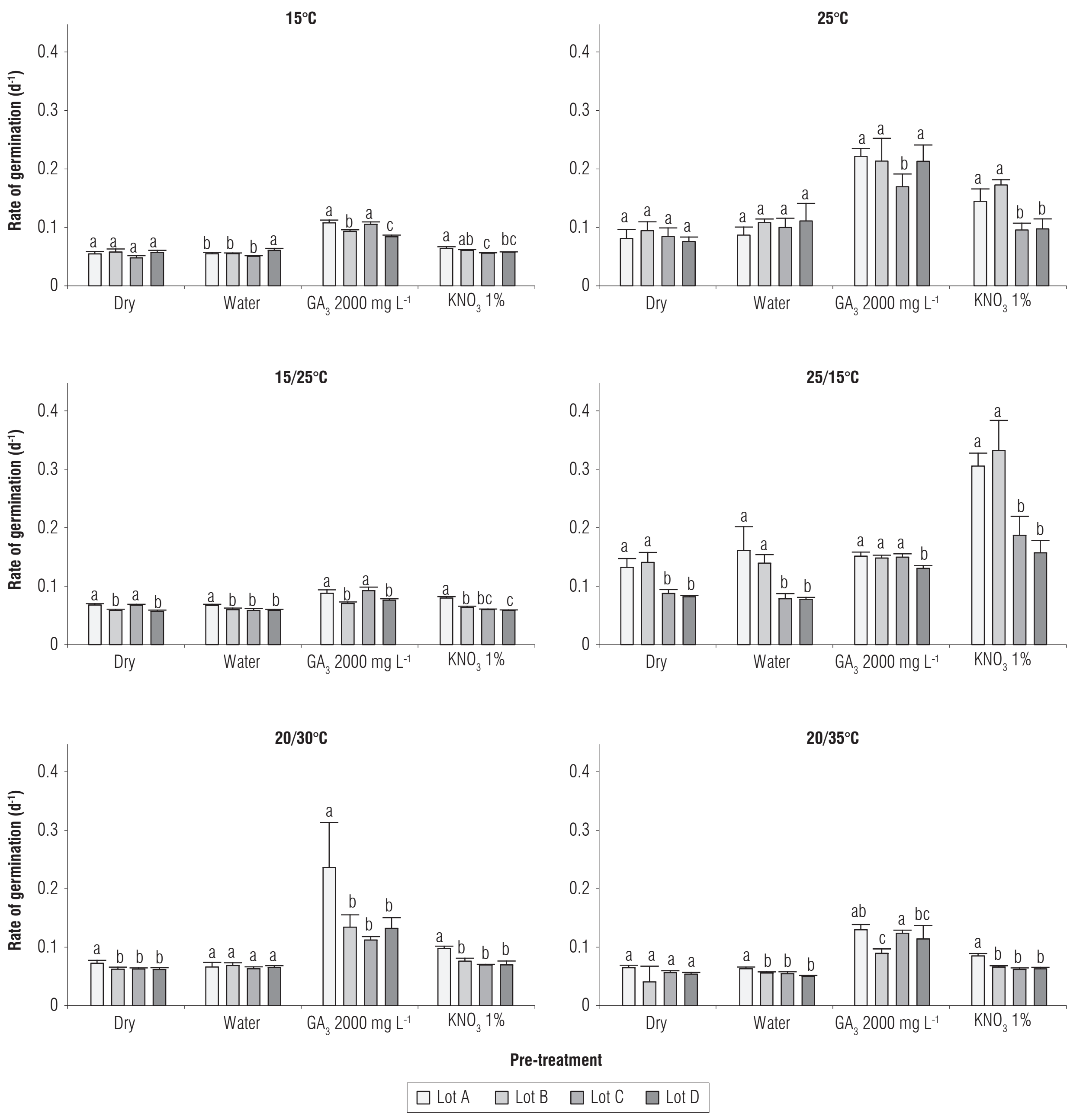

Figure 3. **Effect of constant or alternating temperature and pre-treatment on the rate of germination of four seed lots of *Solanum betaceum* Cav. Lot A: cv. Tamarillo, 7 months in storage, lot B: cv. Tamarillo, 0 months in storage, lot C: common tree tomato, 7 months in storage, lot D: common tree tomato, 0 months in storage. Means with different letters indicate significant statistical difference within each pre-treatment according to the Tukey test ($P \leq 0.05$; $n = 4$). The vertical bars indicate ± standard error.**

The rate of germination was significantly affected by all factors and most of their interactions ($P \leq 0.05$). The highest rate of germination was found for the common variety (lot A) at 25/15°C (16/8h) with pre-treatment in GA_3 (Fig. 5). In all temperature regimes, pre-treatment with GA_3 provided the most rapid germination.

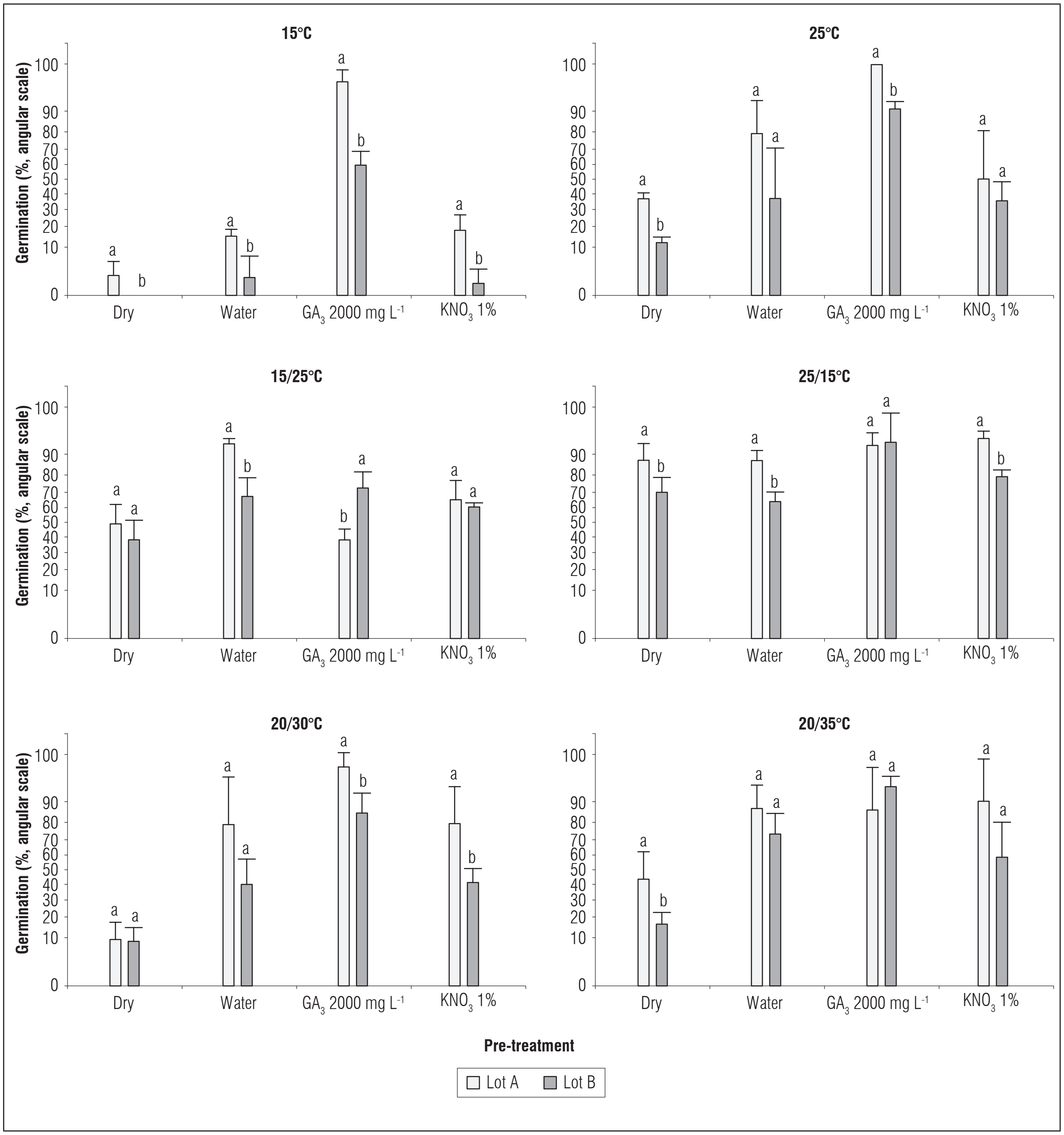

Figure 4. Effect of constant or alternating temperature and several pre-treatments on the germination of two seed lots of *Solanum quitoense* Lam. (21 d duration), Lot A: common lulo, 2 months in storage, Lot B: cv. La Selva, 0 months in storage. Means with different letters indicate significant statistical difference within each pre-treatment according to the Tukey test ($P \leq 0.05$; $n = 4$). The vertical bars indicate ± standard error.

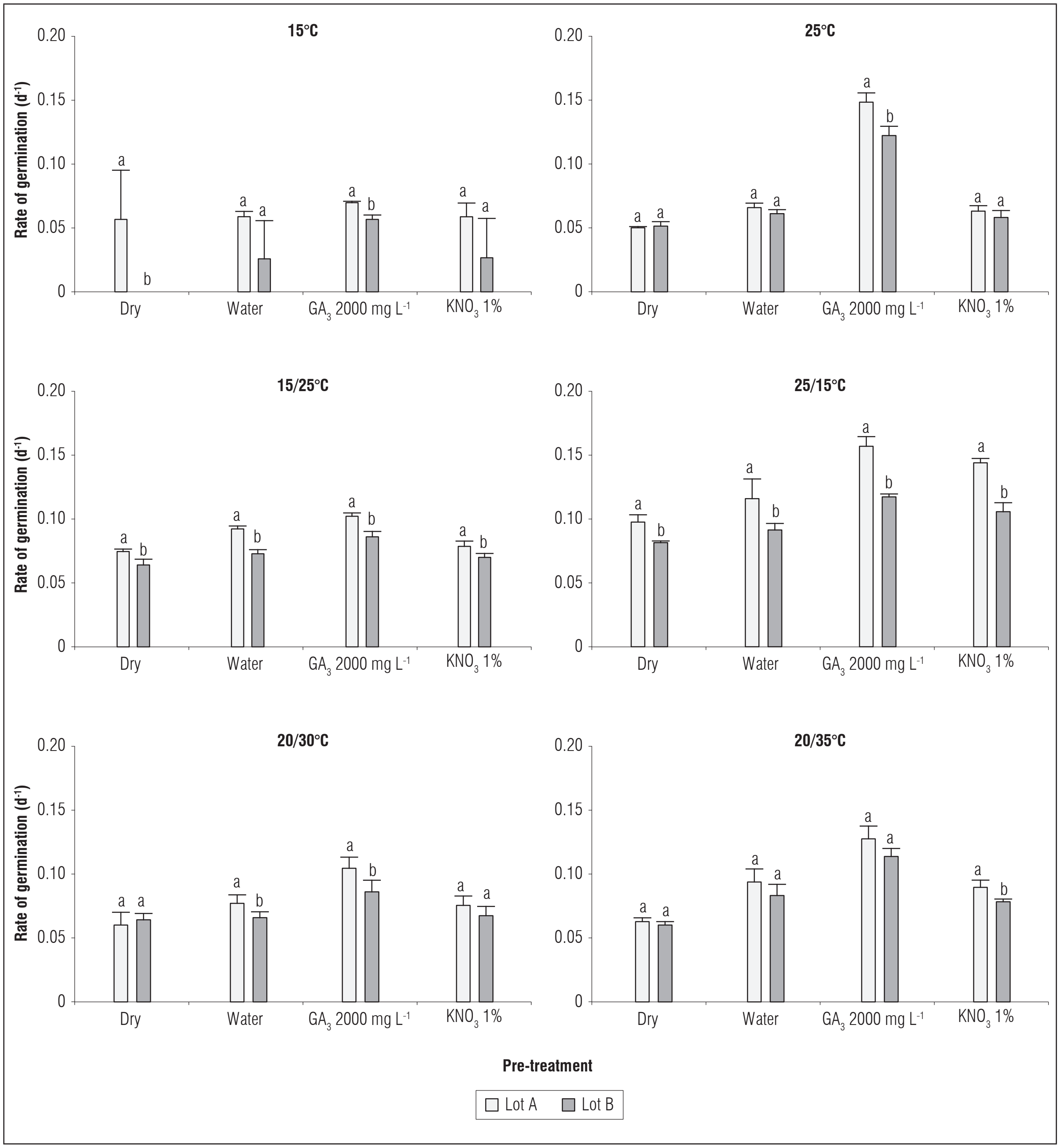

Figure 5. Effect of constant or alternating temperature and several pre-treatments on the rate of germination of two seed lots of *Solanum quitoense* Lam. Lot A: common lulo, 2 months in storage, Lot B: cv. La Selva, 0 months in storage. Means with different letters indicate significant statistical difference within each pre-treatment according to the Tukey test ($P \leq 0.05$; $n=4$). The vertical bars indicate ± standard error.

DISCUSSION

The germination varied considerably among the seed lots, the different temperature regimes, and the different pre-treatments in both species of *Solanum*. The seeds of *S. betaceum* and *S. quitoese* were dormant, and less dormancy was found in the cultivars Tamarillo of *S. betaceum* and common lulo (*S. quitoense*).

Dormancy was broken successfully for the *S. betaceum* and *S. quitoense* seeds. In both species, the best temperature to promote seed germination was the alternating temperature (25/15°C) for all treatments and seed lots. At constant temperatures, the promotion of germination was lower for the dry and water controls, meanwhile, gibberellic acid had a substantial, positive effect. However, the action of alternate temperature and gibberellic acid at the same time did not provide additional benefits to germination. Also, for both species, the gibberellic acid positively affected the rate of germination at all temperatures, particularly at 25/15 and 25°C.

One way to release seed dormancy in other *Solanum* species is alternating temperatures; e.g. *S. elaeagnifolium* (Trione and Cony, 1990), and *S. physalifolium* (Monte and Tarquis, 1997) with amplitudes exceeding 5 °C. Alternating temperatures occur in the natural tropical Andean ecosystem where *S. betaceum* and *S. quitoense* crops are cultivated. Thus, choosing the minimum, mean and maximum temperatures of the Andean ecosystem for the germination test (Vargas-Figueroa and Torres-González, 2018) was a clue to obtaining successful germination for this species.

On the other hand, potassium nitrate (1%) promoted seed germination of the *S. betaceum* seeds at both constant and alternating temperatures. However, potassium nitrate promoted seed germination in *S. quitoense* only when the alternating temperature was applied, and, at the constant temperature, there was no promotion by potassium nitrate at all. These results agree with Yogeesha *et al*. (2006), who broke seed dormancy in two cultivars of *Solanum melongena* L. with gibberellic acid; however, potassium nitrate only improved germination to some extent. In this sense, these results agree with the results of Wei *et al.* (2010), who promoted germination in *S. lycopersicum* with gibberellic acid in higher percent (>98%) than with potassium nitrate (>70%). Also, the application of gibberellic acid to the *S. lycopersicum* seeds produces seedlings with higher vigor (Balaguera-López *et al*., 2009).

These results confirm the study of Cárdenas *et al*. (2004) on *S. betaceum* and *S. quitoense*, where dormancy was broken by an alternating temperature regime of 28/24 °C (12/12 h). They found a similar benefit for these species from GA_3 (1,000 mg L^{-1}), but, in the present study, a slight increase in the germination was obtained for *S. betaceum* with GA_3 at a higher concentration (2,000 mg L^{-1}). However, these results contradict their results of declining germination with the application of KNO_3 (2%), perhaps because a lower concentration was used (1%). The positive response of the *Solanum* species studied here, and in other research, to gibberellins and nitrate in breaking seed dormancy shows the presence of a shallow seed dormancy, which is a result of gene expression (Finch-Savage and Footitt, 2017).

There was a difference in the promotion of germination between the cultivars of both species. *S. betaceum* cv. Tamarillo achieved higher germination than the common cultivar. However, the rate of germination was very similar for both cultivars. On the other hand, the common cultivar of *S. quitoense* had higher germination and rate of germination than cv. La Selva. The most commercial cultivars of these fruits seem to provide the highest germination results. This is because *S. quitoense* cv. La Selva is a cross between the cultivated species and a wild species (Fory, 2010) and an inter-specific hybrid that is much less cultivated. On another hand, *S. betaceum* cv. Tamarillo is a very high-yielding variety that produces large bright red fruits, and, of all varieties of this crop, it is one of the most rewarding and cultivated (The Climbing Fig, 2020).

CONCLUSIONS

S. betaceum and *S. quitoese* exhibited shallow seed dormancy, and less dormancy was found in the more commercialized cultivars, such as *S. betaceum* cv. Tamarillo and *S. quitoense* (*i.e.* common cultivar). For both species, the most recently harvested seeds had more promotion of germination than the seeds stored for several months at the low seed moisture content (4%) and low storage temperature (20°C). Despite this, the storage conditions did not have a detriment on the seed germination of the tomate tree and lulo.

The seed dormancy of *S. betaceum* and *S. quitoense* was broken successfully by applying gibberellic acid (2,000 mg L^{-1}) or alternating temperatures, particularly 25/15°C. The application of one or another treatment was enough because both treatments at the same time did not have an additional benefit for promoting seed germination. Meanwhile, the application of gibberellic acid increased the rate of germination more than potassium nitrate, at all temperatures. However, potassium nitrate (1%) promoted the seed germination of *S. betaceum* seeds at both constant and alternating temperatures and of

S. quitoense seeds only when the alternating temperature was applied.

The suggestion is for farmers to apply any of these treatments that work well at breaking seed dormancy in *S. betaceum* and *S. quitoense;* the most convenient option could be selected depending upon budget and other resources.

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Conflict of interest. The manuscript was prepared by the author, who declares that she has no conflicts of interest that put at risk the validity of the results presented here.

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Effect of irrigation regime on the production of volatiles that affect the aroma of the pear variety Triumph of Vienna (*Pyrus communis* L.)

Efecto del régimen de riego en la producción de volátiles que incide en el aroma de la pera variedad Triunfo de Viena (*Pyrus communis* L.)

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Fruit of pear (*Pyrus communis* L.) var. Triumph of Vienna.

Photo: J.E. Vélez

ABSTRACT

Water is a major component of plants that directly and indirectly affects physiological processes. One of the consequences of a hydric deficit in the pear fruit is modification of the aroma. No information exists on the effect of a water deficit on the sensory profile and volatile composition of this species. The objective was to determine the production of volatiles in the harvest and post-harvest of pear var. Triumph of Vienna (*Pyrus communis* L.) with regulated deficit irrigation (RDI). The irrigation treatments consisted of the application of water regimes that were 100 (Control), 74 and 48% of the ETc during the rapid fruit growth period. The rest of the season plants-were irrigated at 100%ETc. In the deficit treatments, there were no significant reductions with respect to the control in the quality of the fruits, obtaining a water savings in 74 and 48%ETc of 26 and 40%, respectively. The esters were the volatile compounds that contribute greatly to aroma, which increased steadily during the climacteric phase. Under the limited water conditions, watering with regulated, deficit doses obtained production that was similar to that of well-watered crops, provided that it was carried out in the phenological stage of low sensitivity and that the tolerance limits of stress were not exceeded.

Additional key words: post-harvest; solid phase micro extraction; volatile compounds; water deficit; ripening; esters.

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RESUMEN

El agua es el componente mayoritario de la planta que afecta directa e indirectamente los procesos fisiológicos. Uno de los efectos del déficit hídrico en el fruto de la pera es la modificación del aroma y no existe información sobre el efecto del déficit hídrico en el perfil sensorial y composición de volátiles de esta especie. El objetivo fue determinar la producción de volátiles en la cosecha y poscosecha del peral var. Triunfo de Viena (*Pyrus communis* L.) con riego deficitario regulado (RDR). Los tratamientos de riego consistieron en la aplicación de láminas de agua correspondiente al 100 (Control) 74 y 48% de la ETc, durante el periodo de crecimiento rápido del fruto, el resto de la temporada se regaron al 100% de la ETc. En los tratamientos deficitarios no hubo reducciones significativas respecto al control en la calidad de la fruta, obteniéndose un ahorro de agua en 74 y 48%ETc de 26 y 40%, respectivamente. Los ésteres fueron los compuestos volátiles con mayor contribución al aroma que aumentaron de forma constante durante la fase del climaterio. En condiciones limitantes de agua, regar con dosis deficitarias controladas permite obtener producciones similares a las de un cultivo bien regado, siempre que se realice en el estado fenológico de baja sensibilidad y los límites tolerables de estrés no se superen.

Additional key words: poscosecha; micro extracción en fase sólida; compuestos volátiles; déficit de agua; maduración; ésteres.

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INTRODUCTION

The pear tree in Colombia in recent years has had an increase in yield, 15.8 t ha^{-1} in 2018. It ranks second in terms of importance, after the peach (Agronet, 2019). This increase resulted from an increase in the consumption of fresh and processed fruits because of the recognition of nutritional and medicinal properties (Miranda *et al*., 2013).

Water consumption in agriculture represents about 87% of the global total, and demand is increasing. The depletion of water resources, the high costs of water and energy, the increase in demand, the decrease in international prices of fruit and the globalization of markets require improvements in the efficiency of crop production and irrigation (UNESCO, 2015).

Moderate water deficits during filling and maturation may be benefit internal changes in fruit quality, mainly by increasing the content of soluble solids and acids (Morandi *et al*., 2014; Galindo *et al*., 2017; Griñan *et al*., 2019). The pear is a climacteric fruit that produces greater amounts of ethylene and intense aroma during ripening that affect sensory quality and, consequently, consumer satisfaction, with a complex mixture of many volatile compounds that includes terpenes, alcohols, aldehydes, esters, acids, ketones and hydrocarbons, whose concentrations determine organoleptic attributes and consumer preference (Li *et al*., 2012).

This is the first study aimed at determining the effect on the production of volatiles in the harvest and post-harvest periods of the pear Triumph of Vienna cultivar (*Pyrus communis* L.) of regulated deficit irrigation (RDI) during the rapid growth stage of the fruit in 2014, taking into account the water status of the soil and the plants.

MATERIAL AND METHODS

This experiment was carried out in 2014 in Hacienda San Benito of Sesquile, Cundinamarca, Colombia. The plot had an area of 0.32 ha, with 172 pear trees of the Triumph of Vienna cultivar (*Pyrus communis* L.), planted in 1998 at 4 x 4 m. The soil has a loamy texture (IGAC, 2010). The average temperature was 12°C. The total precipitation in the period from November 2013 to April 2014 was 465.4 mm. The annual average evapotranspiration ETo, calculated with the Thornthwaite method, was 650 mm. The average daily ETc, determined with the Penman-Monteith equation (Allen *et al*., 1998; Cleves *et al*., 2016) with crop coefficient Kc= 0.8, was 2.16 mm d^{-1}, and the relative humidity (RH) was 78.2%.

The drip irrigation system used six emitters per tree, 8 L h^{-1}. The experiment design was randomized complete blocks, taking into account the slope of the land and the distribution of the trees in the plot, with three treatments and four repetitions per treatment (12 plots). The experiment plot was formed by 4 or 5 contiguous rows of three, four or five trees (with a total of 12, 15, 16 and 20 interior trees per plot).

The irrigation regime was determined by considering the crop evapotranspiration (ETc). During the cultivation cycle, all treatments were irrigated at 100% of the ETc, and, from January 1 to February 28, 2014, during the rapid growth phase, the following treatments were applied: control of 100%ETc irrigated all year and deficit treatments (74%ETc and 48%ETc) irrigated at 74 and 48% of the control, respectively. The volume of water applied to each treatment was regulated by varying the irrigation time, maintaining the same frequency, every 2 d. The water was measured with 13 mm volumetric meters, Zenner®, installed in each plot.

From a random sample of each repetition on May 9, 2014 to harvest, May 21 (12 days after harvesting DAH) and (24 DAH) June 2, two complete fruits were obtained to determine the volatiles using the SPME solid phase micro extraction methodology (Stashenko and Martínez, 2011; Li *et al*., 2012; Cano-Lamadrid *et al*., 2018) with a 1,160 mL storage vial.

The head space calculation for each of the tests was determined as the volume of the vial minus the volume of the fruit (Tab. 1). The standardization of the method was carried out by means of a time of exposure of the fiber to the volatiles for 30 min at an approximate ambient temperature between 22 and 26°C. The fiber was Supelco brand, three phases (divinil benzene, carboxen and polydimethylsiloxane) with an equilibrium time of 1 h. The internal standard used 2-octanol, 5.0 μL.

The fiber was conditioned with thermal desorption for 5 min in a gas chromatograph (GC), Agilent Technologies 7890A injection port coupled to a 5975C mass spectrometer (MS) (Santa Clara, Ca), at a temperature of 250°C for 30 min. The operating conditions used an Agilent 19091S-433 high resolution gas chromatography column (30 m x 250 mm x 0.25 μm stationary phase film). Helium (He) gas was used as the carrier with a column flow of 1 mL min^{-1}, with a running time of 51 min and at a 1:50 splitless mode ratio. The temperature of the injector and the interface was 250°C. For the acquisition of the mass spectrometer data, Chem Station software was used, and the mass spectra were compared with those stored in the NIST library to identify the compounds.

With the SAS/STAT program (SAS Institute, 2010), the statistical analysis was carried out using analysis of variance and Tukey test, $P\leq 0.05$, to compare the treatments and the control with the 'glm' procedure.

Table 1. Weight and volume of the fruit and head space in the vial for each treatment and repetition at harvest, 12 and 24 DAH in 2014.

Treatment	May 9 (harvest)			May 21 (12 DAH)			June 2 (24 DAH)		
	Weight (g)	Volume (cc)	Head space (cc)	Weight (g)	Volume (cc)	Head space (cc)	Weight (g)	Volume (cc)	Head space (cc)
100%ETCR1	280.26	276.02	883.98	173.28	179.19	980.81	285.98	281.20	878.80
100%ETCR2	229.00	229.62	930.38	174.49	180.28	979.72	244.14	243.32	916.68
100%ETCR3	212.46	214.65	945.35	178.31	183.74	976.26	277.68	273.68	886.32
100%ETCR4	274.36	270.68	889.32	213.03	215.17	944.83	299.68	293.60	866.40
74%ETCR1	267.64	265.27	894.73	220.09	221.14	938.86	327.72	321.02	838.98
74%ETCR2	207.37	209.34	950.66	161.58	166.85	993.15	271.79	269.12	890.88
74%ETCR3	212.32	213.93	946.07	197.70	200.37	959.63	290.64	286.61	873.39
74%ETCR4	226.00	226.63	933.37	181.54	185.37	974.63	249.60	248.53	911.47
48%ETCR1	253.16	248.43	911.57	213.40	214.10	945.90	243.91	240.44	919.56
48%ETCR2	202.97	205.09	954.91	226.77	225.64	934.36	294.67	284.27	875.73
48%ETCR3	179.78	185.07	974.93	138.14	149.12	1010.88	288.20	278.68	881.32
48%ETCR4	181.02	186.14	973.86	136.32	147.55	1012.45	255.44	250.40	909.60

RESULTS AND DISCUSSION

The chromatogram standardization method of the volatile fraction (Fig. 1) corresponded to one of the repetitions of the 100%ETc treatment at harvest, where the more abundant peaks were identified in the retention times (tr), which were: (1) methyl acetate, tr = 2.2 min (5.20, 2.26 and 4.19%); (2) butyl acetate, tr = 5.7 min (12.29, 12.52 and 14.46%); (3) ethyl hexanoate, tr = 8.8 min (3.95, 4.49 and 7.13%) and (4) octanol, tr = 16.38 min (18.18, 24.43 and 18.22%). The determinations were made at harvest and 12 and 24 DAH for each treatment and repetition, whose mass spectra were compared with those stored in the NIST library.

Twenty-three (23) volatile compounds were found, including 2-octanol, used as an internal standard, equal to the number of volatiles identified by Li *et al*. (2014) in the pear 'Pingxiangli' in different stages of maturation. The sensory descriptors correspond to SAFC (2011), which have also been reported in pear and other fruit trees by Altisent *et al*. (2011), Verzera *et al*. (2011) and Griñan *et al*. (2019).

The total concentration in the percentage of area of the volatile was 53.72, 56.69 and 60.92% at the time of harvest for 100%ETc, 74%ETc and 48%ETc, respectively, with no significant statistical differences although the deficient treatment 48%ETC was higher (Tab. 2).

The 12 DAH concentration increased to 87.26; 89.70 and 86.08%, respectively, with no difference between treatments. While, the 24 DAH concentration decreased, with a difference between treatments 100 and 74%ETc, with a total concentration of 85.62; 66.86 and 79.56% for 100%ETc, 74%ETc and 48%ETc, respectively (Tab. 2).

The dominant compounds in percentage of area for 12 DAH were ethyl acetate, propyl acetate, butyl acetate, hexyl acetate, octanol and α-farnese, similar to the compounds found by Zlatic *et al*. (2016) in the 'Bartlett' pear and by Bhavadharani *et al*. (2019) in the pear fruit (*Pyrus communis)*.

According to the percentages of areas corresponding to the chromatograms and the weight of the fruits, the content of the volatile compounds, which contribute to the aroma of the pear cultivar Triumph of Vienna, was calculated, with a total concentration in 100%ETc, 74%ETc and 48%ETc, without differences for the harvest at 38.14; 23.65 and 43.38 mg kg^{-1} L^{-1};

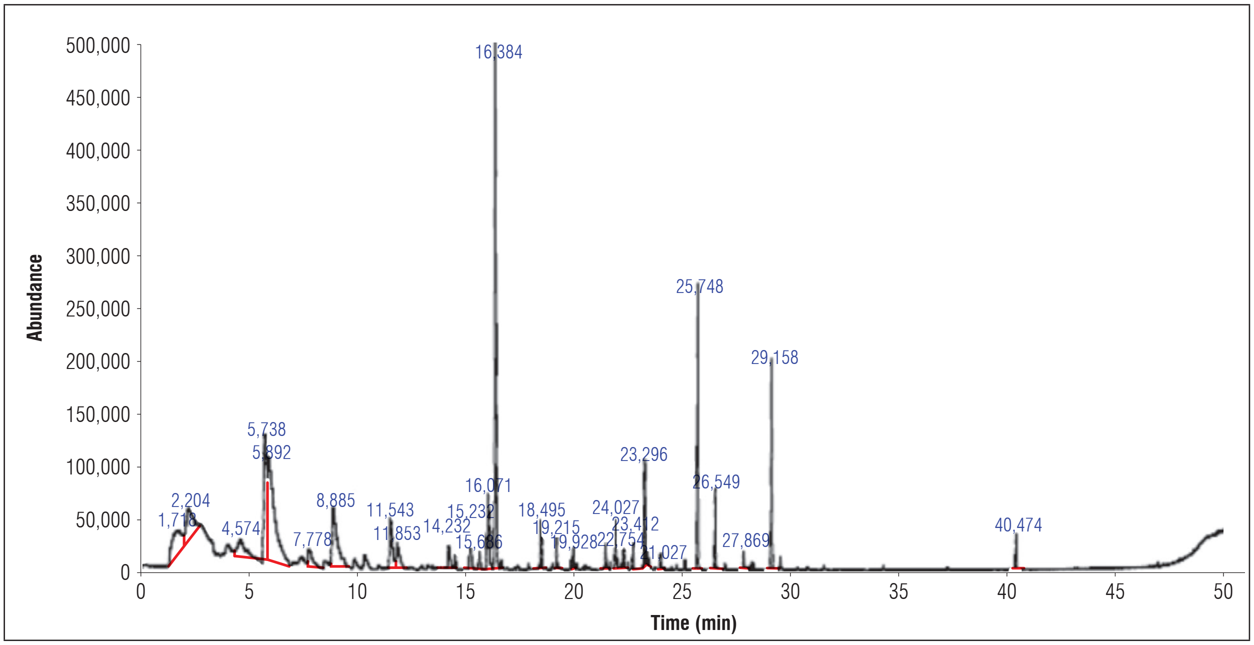

Figure 1. Chromatogram of the qualitative analysis of the volatile compounds constituting the characteristic aroma of the pear variety Triumph of Vienna in 100%ETc treatment at harvest in 2014, with four of the peaks identified: (1) methyl acetate, tr = 2.2 min; (2) butyl acetate, tr = 5.7 min; (3) ethyl hexanoate, tr = 8.8 min and (4) octanol, tr = 16.38 min.

12 DAH increased to 237.49, 219.96 and 296.60, mg $kg^{-1} L^{-1}$; and 24 DAH decreased to 227.31, 151.67 and 153.41 mg $kg^{-1} L^{-1}$ (Tab. 3). The production of volatile compounds increased during maturation and slowed down at 24 DAH, possibly because of a restricted supply of substrates for esterification, similar to that found by Bangerth *et al*. (2012) in apples. This behavior is typical of climacteric fruits, which produce a greater quantity of compounds that are characteristic of intense aromas during ripening, coinciding with a high production of ethylene and high respiration, which produce several physiological changes.

The content of volatiles in mg $kg^{-1} L^{-1}$ in general did not differ between the treatments, except: at harvest between 74%ETc and 48%ETc for methyl hexanoate; 12 DAH between 74%ETc and 48%ETc for ethanal; 100%ETc and 48%ETc for butyl acetate and 100%ETc with (74%ETc and 48%ETc) for 2-methylbutyl acetate (Tab. 3).

The total volatile content increased in the sampling point, with statistical differences in the 100%ETc treatment, from 38.14 to 237.49 mg $kg^{-1} L^{-1}$ between the harvest and 12 DAH, respectively, and without differences between 12 and 24 DAH; the 74%ETc treatment had differences, with values of 219.96, 151.67 and 23.65 mg $kg^{-1} L^{-1}$, 12, 24 DAH and harvest, respectively; in 48%ETc, 12 DHA showed differences from the other sampling points (Tab. 4).

Table 2. Percentage of area of the volatile compounds in the pear Triumph of Vienna cultivar in 2014.

Volatile (% area)	Time (min)	May 9 (harvest)			May 21 (12 DAH)			June 2 (24 DAH)		
		100%ETc	74%ETc	48%ETc	100%ETc	74%ETc	48%ETc	100%ETc	74%ETc	48%ETc
Pentanal	1.69	2.70 a	0.00 a	2.15 ab	0.00 a	0.00 a	0.00 a	0.00 a	0.00 a	0.00 a
Ethanal	1.70	1.70 a	2.34 a	4.93 bc	0.53 a	1.68 ab	0.00 a	1.10 a	1.10 a	1.37 ab
methyl acetate	2.20	5.20 a	2.26 a	4.19 abc	1.75 ab	2.35 abc	2.15 ab	2.03 a	1.14 a	1.46 ab
Ethyl acetate	2.73	0.00 a	0.00 a	0.00 a	24.25 e	15.30 e	15.32 d	13.08 bc	5.70 ab	9.15 b
Ethyl Propanoate	3.53	0.00 a	0.00 a	0.00 a	0.00 a	0.73 a	0.00 a	0.48 a	0.58 a	0.00 a
Propyl acetate	3.80	0.00 a	0.00 a	0.00 a	8.45 c	6.32 cd	6.28 c	1.34 a	1.03 a	1.24 a
Ethyl butanoate	4.97	0.00 a	0.00 a	0.00 a	4.12 abc	3.37 abc	3.08 ab	3.39 a	1.93 a	2.77 ab
Butyl acetate	5.70	12.29 b	12.52 b	14.46 d	17.11 d	24.04 f	21.86 e	24.07 d	23.60 c	31.05 c
2-methylbutyl acetate	7.01	0.00 a	0.00 a	0.00 a	1.17 a	2.44 abc	2.02 ab	0.63 a	0.73 a	0.59 a
3-methylbutyl acetate	7.02	0.00 a	0.00 a	0.00 a	0.00 a	0.23 a	0.43 a	0.70 a	0.64 a	0.00 a
Pentyl acetate	8.40	0.00 a	0.00 a	0.00 a	0.44 a	0.46 a	0.16 a	0.42 a	0.41 a	1.20 ab
Heptyl acetate	8.49	0.00 a	0.00 a	0.00 a	0.19 a	0.24 a	0.16 a	0.00 a	0.43 a	0.00 a
methyl hexanoate	8.80	3.95 a	4.49 a	7.13 c	1.46 a	1.77 ab	1.72 ab	2.95 a	2.52 a	2.36 ab
Ethyl hexanoate-ethyl ester	10.30	0.00 a	0.41 a	0.00 a	4.26 abc	4.07 abcd	3.72 b	6.49 ab	2.80 a	6.50 ab
Hexyl acetate	11.50	0.94 a	1.20 a	0.81 ab	7.05 bc	7.33 d	8.02 c	6.38 ab	5.71 ab	5.99 ab
Octanone	11.80	2.93 a	3.52 a	2.69 ab	0.21 a	0.21 a	0.00 a	0.31 a	0.34 a	0.55 a
Hexanol	14.20	0.43 a	0.54 a	0.27 a	0.50 a	0.69 a	0.44 a	0.68 a	0.72 a	0.96 ab
hexyl butanoate	16.07	2.65 a	1.90 a	2.36 ab	0.35 a	0.38 a	0.94 ab	2.18 a	1.58	1.57 ab
Octanol	16.38	18.18 c	24.43 c	18.22 d	4.37 abc	4.69 bcd	3.80 b	3.44 a	3.92 a	5.81 ab
ethyl octanoate	16.66	0.00 a	0.00 a	0.00 a	0.49 a	0.46 a	0.51 a	0.22 a	0.23 a	0.41 a
ethyl hexanoate	18.40	2.14 a	2.70 a	1.93 ab	0.42 a	0.40 a	0.37 a	0.30 a	0.43 a	0.43 a
ethyl decanoate	22.75	0.00 a	0.00 a	0.15 a	0.58 a	0.37 a	0.62 a	0.21 a	0.16 a	0.10 a
α farnese	25.70	0.62 a	0.39 a	1.64 ab	9.60 c	12.19 e	14.50 d	15.22 c	11.22 b	6.09 ab
Total		53.72 a	56.69 a	60.92 a	87.26 a	89.70 a	86.08 a	85.62 b	66.86 a	79.56 ab

Means with different letters in the same row indicate significant statistical differences within each day, between treatment, according to the Tukey test ($P \leq 0.05$; $n=4$).

The total content of the 48%ETc deficit treatment at harvest and during the initial storage process was greater, with significant differences (43.38 to 296.60 mg kg^{-1} L^{-1}). Table 4 shows the differences in the volatile content.

100%ETc showed different statistics between harvest and 12 DAH for: propyl acetate; butyl acetate; 2-methylbutyl acetate; hexyl acetate; octanone; hexanol; ethyl octanoate and ethyl decanoate; between harvest and 24 DAH for: butyl acetate; 2-methylbutyl acetate; methyl hexanoate; hexyl acetate; octanone; hexanol; ethyl hexanoate and α-farnese; and between 12 and 24 DAH for: propyl acetate and methyl hexanoate.

74%ETc had differences between harvest and 12 DAH for: methyl acetate; ethyl acetate; propyl acetate; ethyl butanoate; butyl acetate; 2-methylbutyl acetate; methyl hexanoate; hexyl acetate; hexanol; octanol; ethyl octanoate; ethyl decanoate and α farnese; between harvest and 24 DAH for: butyl acetate; methyl hexanoate; hexyl acetate; hexanol and α farnese; and between 12 and 24 DAH for: methyl acetate; ethyl acetate; propyl acetate; 2-methylbutyl acetate; octanol and ethyl decanoate.

48%ETc had differences between harvest and 12 DAH for: ethyl acetate; propyl acetate; ethyl butanoate; butyl acetate; 2-methylbutyl acetate; hexyl acetate; octanone; ethyl octanoate and α-farnese;

Table 3. Content of volatiles of the treatments at harvest, 12 DAH and 24 DAH of the pear cultivar Triumph of Vienna.

Volatile (mg kg^{-1} L^{-1})	Time (min)	May 9 (harvest)			May 21 (12 DAH)			June 2 (24 DAH)		
		100%ETc	74%ETc	48%ETc	100%ETc	74%ETc	48%ETc	100%ETc	74%ETc	48%ETc
Pentanal	1.69	1.84 a	0.00 a	1.56 a	0.00 a	0.00 a	0.00 a	0.00 a	0.00 a	0.00 a
Ethanal	1.70	1.54 a	1.27 a	2.84 a	1.07 ab	3.99 b	0.00 a	2.83 a	2.27 a	2.07 a
Methyl acetate	2.20	4.28 a	0.89 a	3.37 a	4.56 a	5.67 a	7.09 a	4.83 a	2.51 a	2.80 a
Ethyl acetate	2.73	0.00 a	0.00 a	0.00 a	69.00 a	38.80 a	54.73 a	41.62 a	12.80 a	24.88 a
Ethyl propanoate	3.53	0.00 a	0.00 a	0.00 a	0.00 a	1.87 a	0.00 a	1.56 a	1.36 a	0.00 a
Propyl acetate	3.80	0.00 a	0.00 a	0.00 a	23.47 a	15.66 a	21.46 a	4.34 a	2.04 a	3.29 a
Ethyl butanoate	4.97	0.00 a	0.00 a	0.00 a	11.38 a	8.70 a	11.59 a	10.83 a	4.42 a	7.51 a
Butyl acetate	5.70	9.62 a	5.34 a	10.50 a	45.66 a	57.31 ab	70.31 b	58.85 a	57.21 a	48.59 a
2-methylbutyl acetate	7.01	0.00 a	0.00 a	0.00 a	2.92 a	5.94 b	7.07 b	1.53 a	1.43 a	1.10 a
3-methylbutyl acetate	7.02	0.00 a	0.00 a	0.00 a	0.00 a	0.70 a	1.10 a	1.70 a	1.26 a	0.00 a
Pentyl acetate	8.40	0.00 a	0.00 a	0.00 a	1.08 a	1.07 a	0.67 a	1.18 a	0.80 a	2.06 a
Heptyl acetate	8.49	0.00 a	0.00 a	0.00 a	0.50 a	0.49 a	0.73 a	0.00 a	1.24 a	0.00 a
Methyl hexanoate	8.80	2.64 ab	1.93 a	5.12 b	3.92 a	4.35 a	5.90 a	7.54 a	5.63 a	4.32 a
Ethyl hexanoate-ethyl ester	10.30	0.00 a	0.22 a	0.00 a	11.66 a	10.61 a	13.82 a	20.61 a	6.52 a	17.66 a
Hexyl acetate	11.50	0.85 a	0.56 a	0.60 a	19.20 a	18.51 a	27.48 a	17.68 a	13.26 a	13.44 a
Octanone	11.80	1.83 a	1.41 a	1.90 a	0.42 a	0.46 a	0.00 a	0.61 a	0.59 a	0.56 a
Hexanol	14.20	0.39 a	0.25 a	0.20 a	1.32 a	1.69 a	1.44 a	1.70 a	1.59 a	1.57 a
Hexyl butanoate	16.07	2.03 a	0.80 a	1.72 a	0.97 a	0.94 a	3.35 a	4.57 a	2.77 a	2.01 a
Octanol	16.38	11.24 a	9.73 a	12.93 a	11.51 a	11.28 a	12.35 a	8.39 a	8.26 a	8.50 a
Ethyl octanoate	16.66	0.00 a	0.00 a	0.00 a	1.29 a	1.21 a	1.92 a	0.67 a	0.52 a	0.95 a
Ethyl hexanoate	18.40	1.33 a	1.08 a	1.33 a	1.09 a	0.96 a	1.21 a	0.70 a	0.90 a	0.67 a
Ethyl decanoate	22.75	0.00 a	0.00 a	0.11 a	1.40 a	0.94 a	2.30 a	0.65 a	0.36 a	0.27 a
α farnese	25.70	0.56 a	0.16 a	1.21 a	25.07 a	28.81 a	52.08 a	34.92 a	23.90 a	11.15 a
Total		38.14 a	23.65 a	43.38 a	237.49 a	219.96 a	296.60 a	227.31 a	151.67 a	153.41 a

Means with different letters in the same row indicate significant statistical differences within each sampling point, between treatment, according to the Tukey test ($P \leq 0.05$; $n = 4$).

Table 4. Content of volatiles corresponding sampling point in 100%ETc, 74%ETc and 48%ETc of the pear cultivar Triumph of Vienna.

Volatile (mg kg^{-1} L^{-1})	Time (min)	100%ETc			74%ETc			48%ETc		
		May 9 (harvest)	May 21 (12 DAH)	June 2 (24 DAH)	May 9 (harvest)	May 21 (12 DAH)	June 2 (24 DAH)	May 9 (harvest)	May 21 (12 DAH)	June 2 (24 DAH)
Pentanal	1.69	1.84 a	0.00 a	0.00 a	0.00 a	0.00 a	0.00 a	1.56 a	0.00 a	0.00 a
Ethanal	1.70	1.54 a	1.07 a	2.83 a	1.27 a	3.99 a	2.27 a	2.84 a	0.00 a	2.07 a
Methyl acetate	2.20	4.28 a	4.56 a	4.83 a	0.89 a	5.67 b	2.51 a	3.37 a	7.09 a	2.80 a
Ethyl acetate	2.73	0.00 a	69.00 a	41.62 a	0.00 a	38.80 b	12.80 a	0.00 a	54.73 b	24.88 ab
Ethyl propanoate	3.53	0.00 a	0.00 a	1.56 a	0.00 a	1.87 a	1.36 a	0.00 a	0.00 a	0.00 a
Propyl acetate	3.80	0.00 a	23.47 b	4.34 a	0.00 a	15.66 b	2.04 a	0.00 a	21.46 b	3.29 a
Ethyl butanoate	4.97	0.00 a	11.38 a	10.83 a	0.00 a	8.70 b	4.42 ab	0.00 a	11.59 b	7.51 ab
Butyl acetate	5.70	9.62 a	45.66 b	58.85 b	5.34 a	57.31 b	57.21 b	10.50 a	70.31 b	48.59 b
2-methylbutyl acetate	7.01	0.00 a	2.92 b	1.53 b	0.00 a	5.94 b	1.43 a	0.00 a	7.07 b	1.10 a
3-methylbutyl acetate	7.02	0.00 a	0.00 a	1.70 a	0.00 a	0.70 a	1.26 a	0.00 a	1.10 a	0.00 a
Pentyl acetate	8.40	0.00 a	1.08 a	1.18 a	0.00 a	1.07 a	0.80 a	0.00 a	0.67 ab	2.06 b
Heptyl acetate	8.49	0.00 a	0.50 a	0.00 a	0.00 a	0.49 a	1.24 a	0.00 a	0.73 a	0.00 a
Methyl hexanoate	8.80	2.64 a	3.92 a	7.54 b	1.93 a	4.35 b	5.63 b	5.12 a	5.90 a	4.32 a
Ethyl hexanoate. ethyl ester	10.30	0.00 a	11.66 a	20.61 a	0.22 a	10.61 a	6.52 a	0.00 a	13.82 a	17.66 a
Hexyl acetate	11.50	0.85 a	19.20 b	17.68 b	0.56 a	18.51 b	13.26 b	0.60 a	27.48 b	13.44 a
Octanone	11.80	1.83 b	0.42 a	0.61 a	1.41 a	0.46 a	0.59 a	1.90 b	0.00 a	0.56 a
Hexanol	14.20	0.39 a	1.32 b	1.70 b	0.25 a	1.69 b	1.59 b	0.20 a	1.44 a	1.57 a
Hexyl butanoate	16.07	2.03 a	0.97 a	4.57 a	0.80 a	0.94 a	2.77 a	1.72 a	3.35 a	2.01 a
Octanol	16.38	11.24 a	11.51 a	8.39 a	9.73 a	11.28 b	8.26 a	12.93 b	12.35 ab	8.50 a
Ethyl octanoate	16.66	0.00 a	1.29 b	0.67 ab	0.00 a	1.21 b	0.52 ab	0.00 a	1.92 b	0.95 ab
Ethyl hexanoate	18.40	1.33 b	1.09 ab	0.70 a	1.08 a	0.96 a	0.90 a	1.33 b	1.21 b	0.67 a
Ethyl decanoate	22.75	0.00 a	1.40 b	0.65 ab	0.00 a	0.94 b	0.36 a	0.11 a	2.30 a	0.27 a
α farnese	25.7	0.56 a	25.07 ab	34.92 b	0.16 a	28.81 b	23.90 b	1.21 a	52.08 b	11.15 a
		38.14 a	237.49 b	227.31 b	23.65 a	219.96 c	151.67 b	43.38 a	296.60 b	153.41 a

Means with different letters in the same row indicate significant statistical differences in and between sampling points according to the Tukey test ($P \leq 0.05$; $n=4$).

between harvest and 24 DAH for: butyl acetate; pentyl acetate; octanone; octanol; ethyl hexanoate; and between 12 and 24 DAH for: propyl acetate; 2-methylbutyl acetate; hexyl acetate; ethyl hexanoate and α-farnese.

Once the volatile compounds were identified and quantified, they were separated into 5 groups or chemical families: 1) aldehydes (pentanal and ethanal), 2) esters (methyl acetate; ethyl acetate: ethyl propanoate, propyl acetate, ethyl butanoate, butyl acetate, 2-methylbutyl acetate, 3-methylbutyl acetate, pentyl acetate, heptyl acetate, methyl hexanoate, ethyl hexanoate, hexyl acetate, hexyl butanoate, ethyl octanoate and ethyl decanoate), 3) acetones (octanone). 4) alcohols (hexanol and octanol) and 5) terpenes (α farnese).

In the treatments, it was found that the compounds with the highest proportion that contributed to aroma included esters, mainly methyl, ethyl and butyl; alcohols such as octanol; and terpenes such as α farnese and ethanal, similar to that found by Griñan *et al*. (2019) in quince (*Cydonia oblonga* Mill.) (Tab. 5). These compounds have also been identified in fresh fruits, juices and pear liqueurs by Zlatic *et al*. (2016) in Bartlett, Sevilla *et al*. (2011) in pear juice Conference, Zhou *et al*. (2015) in 'Nanguoli', Li *et al*. (2014) in 'Pingxiangli' and 'Ruanerli', Li *et al*. (2012) in 'Ruanerli', 'Kurle Xiangli', 'Bartlett', 'Youhongli',

Table 5. Content and percentages chemical groups of the volatiles (mg kg^{-1} L^{-1}) for the treatments 100%ETc, 74%ETc and 48%ETc of the pear cultivar Triumph of Vienna.

Volatile	May 9 (harvest)					
	100%ETc	Error	74%ETc	Error	48%ETc	Error
Aldehydes	3.38	1.96	1.27	1.27	4.40	1.61
% aldehydes	8.00 aAB	5.66	4.10 aAB	4.10	11.97 aB	5.33
Esters	19.42	11.50	9.75	3.27	21.31	5.35
% esters	41.05 aA	10.55	36.08 aA	12.50	46.60 aAB	6.26
Acetones	1.83	0.06	1.41	0.14	1.90	0.33
% acetones	6.22 aB	1.48	6.79 aB	1.77	4,.58 aB	0.79
Alcohols	12.95	1.80	11.05	0.58	14.56	1.61
% alcohols	44.00 aB	12.35	52.45 aB	11.87	34.68 aB	3.69
Terpenes	0.56	0.56	0.16	0.16	1.21	1.10
% terpenes	0.73aA	0.73	0.57 aA	0.57	2.17 aAB	1.89
Total	38.14 a	15.89	23.65 a	5.41	43.38 a	10.00
% total	100		100		100	
May 21 (12 DAH)						
Aldehydes	1.07	1.07	3.99	1.36	0.00	0.00
% aldehydes	0.60 abA	0.60	1.88 bA	0.64	0.00 aA	0.00
Esters	195.60	27.29	171.84	22.57	227.21	37.18
% esters	81.34 aC	3.72	77.45 aC	4.35	76.97 aC	2.29
Acetones	0.42	0.42	0.46	0.46	0.00	0.00
% acetones	0.24 aA	0.24	0.23 aA	0.23	0.00 aA	0.00
Alcohols	15.33	0.88	14.87	0.97	17.30	1.97
% alcohols	6.72 aA	0.99	6.86 aA	0.50	6.08 aA	0.55
Terpenes	25.07	4.77	28.81	5.90	52.08	13.18
% terpenes	11.11 aABC	2.38	13.57 aABC	3.38	16.95 aBC	2.55
Total	237.49 a	34.43	219.96 a	31.26	296.59 a	52.34
% total	100		100		100	
June 2 (24 DAH)						
Aldehydes	2.83	1.22	2.27	0.92	2.07	1.20
% aldehydes	1.31 aA	0.49	1.61 aA	0.54	1.84 aA	1.34
Esters	177.51	46.40	113.79	30.50	128.61	44.24
% esters	74.36 aC	9.53	69.61 aBC	11.46	79.99 aC	6.15
Acetones	0.61	0.36	0.59	0.34	0.56	0.34
% acetones	0.37 aA	0.23	0.59 aA	0.39	0.77 aA	0.46
Alcohols	11.44	0.22	11.12	0.72	11.01	0.71
% alcohols	5.46 aA	0.93	8.37 aA	2.03	9.56 aA	2.72
Terpenes	34.92	14.25	23.90	9.42	11.15	4.47
% terpenes	18.50 aC	8.37	19.82 aC	9.32	7.84 aABC	3.95
Total	227.31 a	35.43	151.68 a	26.18	153.40 a	46.79
% total	100		100		100	

Means with different lowercase letters in the row of sampling point and different capital letters between the sampling point of the treatments indicate different statistics according to the Tukey test ($P \leq 0.05$) ($n = 4$).

'Daxiangshui', 'Xiaoxiangshui', 'Nanguoli', 'Pingxiangli', 'Dongguoli', 'Hongxiangsu' and 'Zaobaimi', Cano-Lamadrid *et al*. (2018a) in Pomegranates, Cano-Lamadrid *et al*. (2018b) in citrus and Andreu-Coll *et al*. (2020) in Prickly pear fruit.

With the imposition of the deficit, the water potential at dawn (Ψa) and the midday stem water potential (Ψt), as expected, tended to decrease: the smallest regime (48%ETc) had Ψa values of -0.29 MPa and Ψt values of -0.80 and -0.99MPa in 2014 and 2015, respectively. 74%ETc had similar values of Ψa (-0.26 MPa) and Ψt (-0.78 and -0.96 MPa) in 2014 and 2015, respectively, while, in the control, the Ψa was -0.30 MPa, and the Ψt was -0.70 and -1.03 MPa in 2014 and 2015, respectively.

The different water regimes resulted in changes in the concentrations of the principle compounds and chemical families of volatiles, with no differences at harvest, 12 DAH or 24 DAH, except the percentage of aldehydes at 12 DAH (Tab. 5).

The low total concentrations of the main chemical families form the aroma of fresh and processed fruits (Sevilla *et al*., 2011), which, in the cultivar Triumph of Vienna, is characteristically sweet and fruity with an herbaceous smell resulting from the dominance of esters, which had the greatest contribution to aroma for the volatile compounds in all treatments, which increased steadily during the climacteric phase and increased in concentration with maturity, while the alcohols decreased as did the aldehydes although the latter did so significantly in 48%ETc (Wang *et al*., 2011; Altisent *et al*., 2011).

As the fruit began to mature, the content of esters increased drastically in all the treatments, which resulted in an increase in aroma, similar to that found in pears by Li *et al*. (2013). The percentages of esters reached their maximum in deficit treatment 48%ETc during maturation, coinciding with the increase in the production of ethylene. At harvest, the predominant compounds were esters, alcohols and aldehydes, which decreased after 12 DAH, with an increase in the percentage of terpenes (Li *et al*., 2014; Sevilla *et al*., 2011). At 24 DAH, the predominants were esters, terpenes and alcohols (Tab. 5). α-farnese was detected in *P. communis* by Yanine *et al*. (2013), similar to that found at 24 DAH for terpenes. The esters were the dominant volatile in deficit treatment 48%ETc: at harvest (46.0%); 12 DAH (76.97%) and 24 DAH (79.99%), while, at harvest, 100%ETc and 74%ETc presented a higher percentage of alcohols (Tab. 5).

CONCLUSION

The production of volatile compounds in all treatments increased during maturation and slowed down at 24 DAH, possibly resulting from a restricted supply of substrates for esterification. This behavior is typical of climacteric fruits, which produce a greater quantity of compounds with characteristic intense aromas during ripening, coinciding with a high production of ethylene and high respiration, which produce several physiological changes because of a higher concentration of sugars but a lack of precursors at the end of the maturation process.

In the pear cultivar Triumph of Vienna, the volatile compounds with the highest proportion that contributed to aroma were esters, mainly methyl, ethyl and butyl; alcohols such as octanol; terpenes such as α-farnese and ethanal.

The different water regimes resulted in changes in the concentrations of the principle volatile compounds without differences between harvest, 12 DAH and 24 DAH. There were also no differences in the percentages of volatile content between the chemical groups at harvest, 12 DAH and 24 DAH, except at 12 DAH between treatments 74%ETC and 48%ETC for the percentage of aldehydes.

Under limited water conditions, watering with regulated deficit doses can provide production that is similar to that of a well-watered crop, provided that it is carried out in the phenological state of low sensitivity and the tolerance limits of stress are not exceeded.

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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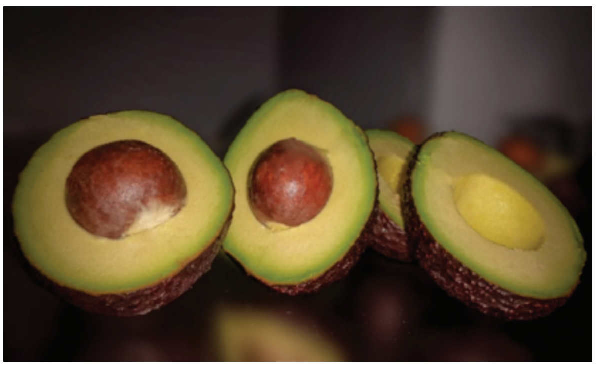
Review of the concept of quality in Hass avocado and the pre-harvest and harvest factors that determine it under tropical conditions

Revisión del concepto de calidad en aguacate Hass y los factores pre-cosecha y cosecha que la determinan bajo condiciones tropicales



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The main challenge of the avocado chain in Colombia is to produce quality.

Photo: J.C. Henao-Rojas

ABSTRACT

Avocado is one of the most desirable fruits worldwide because of its flavor and nutritional contributions. Currently, Colombia is a very dynamic country where the cultivated area and volumes of exported fruits have been increasing year after year. This situation poses a challenge for the country, which must produce fruits with excellent parameters of ripening, external and internal quality. This study used a series of elements associated with quality in avocado and its application in tropical conditions, which were determined based on reports from the world scientific literature reported in journals, books, manuals and conferences. In addition, information obtained from commercial esays on plantations and the experiences of packing and marketing companies in Colombia was used. The approach presented here is associated with a modern concept of quality in the food industry and how it can be applied to the avocado value chain; preharvest aspects that determine quality, management practices conducive to improving quality, and harvest parameters and their relationship with quality were also used. This study generated alternatives for increasing the added value in the avocado production system based on quality parameters and identified delays in the supply chain of this important agro-industry under tropical conditions.

Additional keywords: consumers; supply chain; harvest index; sustainable agriculture.

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RESUMEN

El aguacate es una de las frutas más deseables en todo el mundo debido a su sabor y aportes nutricionales. Colombia es un país muy dinámico, donde el área plantada y los volúmenes de fruta exportada han ido creciendo año tras año. Esta situación plantea un desafío para el país, que debe producir en función de la calidad, logrando posicionar una fruta con excelentes parámetros de maduración, calidad externa e interna. En este trabajo, se presentan una serie de elementos asociados con la calidad del aguacate y su aplicación a las condiciones tropicales, que se determinaron con base en reportes de la literatura científica mundial como revistas, libros, manuales y conferencias. Además, utilizamos información obtenida a través de ensayos comerciales de plantaciones y experiencias de empresas de empaque y comercialización en Colombia. El enfoque presentado aquí está asociado con un concepto moderno de calidad en la industria alimentaria y cómo se puede aplicar a la cadena de valor del aguacate, también presentamos aspectos de cosecha y poscosecha que determinan la calidad y prácticas de manejo conducentes a mejorar la calidad. Este trabajo genera alternativas para aumentar el valor agregado en el sistema de producción de aguacate con base en parametros de calidad e identifica los retrasos de la cadena de valor de esta importante agroinustria bajo condiciones tropicales.

Palabras clave adiconales: consumidores; cadena de suministro; índice de cosecha; agricultura sostenible.

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INTRODUCTION

Avocado, especially the Hass variety, has grown significantly in planted areas in Colombia, leading to increases in production and exported volumes (Ramírez-Gil *et al*., 2018). This growth is supported by a higher domestic demand and mainly by the boom in exports, especially to the European Union. Thus, the country went from exporting 3 t in 2005 to 30,008 t in 2018 (MADR, 2018). There is currently great expectation for the possibility of exporting fruit to other countries such as Japan, The United Arab Emirates, The United States, Canada, and China, among others.

The avocado fruit is very popular and highly consumed in many countries given its high nutritional content of vitamins A, B, C, potassium, phosphorus, magnesium, iron, unsaturated fatty acids, and antioxidants (Araújo *et al*., 2018). In addition, it has been demonstrated that the consumption of this fruit reduces the risk of cardiovascular diseases, hyperlipidemia, and visceral adipose tissue and contributes to weight control and healthy aging (Duarte *et al*., 2016).

Fruit and vegetable quality is expressed by different indicators, which cover a large number of organoleptic parameters (e.g. visual appearance, shape, size, color, texture, taste, aroma, etc.), nutritional characteristics (e.g. minerals, proteins, fiber, cofactors, carbohydrates, fatty acids, vitamins, enzymes, coenzymes, etc.), functional properties (e.g. anti-cancer properties and removal of saturated fats, among others), origin (e.g. the way it is produced, place of origin, certificates, etc.), and defects (e.g. mechanical, physical, etc.). Quality is defined, to a large extent, by the final consumer, who sets criteria based on personal preferences and expectations. However, it has a direct relationship with all the actors in the value chain (e.g. producers, packers, transporters, and traders).

The Hass avocado production system does not currently have a validated technological model for the Colombian tropics, a situation that increases uncertainty in production. Fruit production must ensure delivery of an excellent quality product to the market that meets the international standards set by importing countries, taking into account agronomic aspects such as cultivation, harvest, post-harvest, logistics, storage, and marketing. For this reason, the objective of Colombia, as an avocado producer, should be the production of fruit of the highest quality in order to position the country and the Colombian avocado brand in markets worldwide.

Thus, it is important to determine the basic quality criteria for Colombian avocado, which should be defined from the consensus among producers, packers, traders, and consumers. Having specific quality standards may help chain stakeholders to perceive these benefits in monetary terms as they seek to increase profitability and sustainability. In addition, consumers can obtain all the benefits in terms of organoleptic, nutritional and functional quality that this fruit can provide.

This manuscript aimed to introduce a concept of quality and define the basic pre-harvest and harvest parameters that determine Hass avocado quality. This review was carried out based on the information reported by different sources such as scientific papers, proceedings from seminars, congresses, and technology transfer events. In addition, data provided by producer farms and the experience of Colombian technical assistants and traders were considered, also previous validation of the information and analysis of the results. The approach presented here was carried out in chapters in which the following topics: (i) quality concepts and application to the avocado industry and its production systems, (ii) pre-harvest aspects that determine quality and management practices conducive to improving quality, and (iii) harvest parameters and their relationship with quality.

GENERAL PARAMETERS ASSOCIATED WITH QUALITY IN THE FOOD INDUSTRY AND THEIR RELATIONSHIP WITH THE AVOCADO FRUIT

Definition of quality

In the case of the avocado market, the quality requirements for exported fruits are completely different from the ones for fruit for domestic consumption. Similarly, differentiated properties are required for the production of avocado pulps for the pharmaceutical industry (content of polyunsaturated fatty acids, vitamin E, avocatin B, terpenes, phytosterols, and antioxidant activity, etc.) (Sarkar *et al*., 2017). There are different characteristics that make a specific market interested in a particular concept of quality. This idea was developed by the International Organization for Standardization, which created the currently accepted definition of quality as "the ability of a product or service to meet the declared or implied needs of the consumer through its properties or characteristics" (Singels *et al*., 2001). Accordingly, in every production chain, suppliers, intermediaries, and traders must know their market (needs and expectations) in detail in order to generate differentiated qualities for specific customers (Orjuela-Castro *et al*., 2017), a rare action in Colombian production economies.

Concept of multidimensional quality

The concept of multifunctional or multidimensional quality has been a trend since the end of the 20th century. In this theory, the perception of quality should be the result of the interrelation of several components, which can be measured, classified and weighted according to the level of importance given by end customers and some intermediaries of the value chain (Prieto *et al*., 2008; Liverani *et al*., 2019).

The components of the multidimensional quality "function" can be classified into two large groups. Firstly, the incorruptible and basic elements of the product or those aspects that are decisive for the product to be accepted. Secondly, and equally important, are the so-called "surprise" aspects. These are characteristics that, although final customers do not expect to find them *a priori*, once they are perceived they are appreciated and considered as added value. In the case of avocado, this type of quality criterion would include the nutritional and sensory characteristics that stand out in fruits. Such characteristics are a better trans/cis ratio of fatty acids (Serpa *et al*., 2014) or some unusual aroma and flavor tones in ripe fruits (Obenland *et al*., 2012). Accordingly, for the "Colombian avocado" product, a scalable and specific quality function could be created for the interests of customers, which should include the usage needs and imaginary consumption of target markets.

Quality perception in Hass avocado

The quality perception characteristics for Hass avocado can be separated into three types: physical, chemical and microbiological. For the physical quality characteristics in Hass avocado, there are a number of factors that could be associated with these parameters. These parameters include polar and equatorial diameters, sphericity index, and peel, pulp and seed percentages, etc. However, the most used criteria in exports and marketing are size and weight, which are currently considered the main quality factors offered by trading companies worldwide (Tab. 1). There is also a series of visual specifications related to physical quality that are currently the basic parameters of fruit selection (Tab. 2).

Table 1. Hass avocado calibers and their categorization depending on the country of consumption for 10 kg packaging boxes[1].

Europe	Canada	USA	Colombia
Caliber 14	>258 g	40	Extra
Caliber 16	227-258 g	48	Extra
Caliber 18	227-203 g	48-60	First
Caliber 20	203-184 g	60	First
Caliber 22	184-165 g	60	First
Caliber 24	<165 g	70	First-Second

[1]Specifications taken from the criteria used by avocado producing, sorting, exporting and distributing companies operating in Colombia.

On the other hand, chemical factors are associated with the metabolic richness of Hass avocado fruits. Among these factors, the lipid profile and triterpene and vitamin E contents are some of the most important in this fruit (Tan *et al*., 2017). This is important given the new trends of healthy consumption and the reports on the role of unsaturated fatty acids in the decrease of cardiovascular pathologies (Botero and Morales, 2018). Finally, microbiological quality is related to a product's health at the consumption stage. The presence of fungi, molds or yeasts in fruits is an excluding factor for end customers and is one of the most heavily sanctioned by international markets (Tab. 2).

According to Prieto *et al*. (2008), other ways to classify Hass avocado quality may depend on hygienic, sanitary, bromatological, health, organoleptic and ethical or emotional characteristics. Hygienic-sanitary quality refers to the absence in the food matrix of components that can cause any harm or put consumers' health at risk. These factors can be biotic (pathogens such as bacteria, parasites, viruses, prions, toxins, and allergens) and abiotic (pesticide residues, pesticides, and contaminants, etc.). In the case of the Hass avocado production system, this is considered an exclusion factor for the export market since fruits cannot be used for this purpose if they have agrochemical residues.

Bromatological quality refers to the nutritional quality that a foodstuff provides in terms of macronutrients (carbohydrates, proteins, fats, fiber, and ashes). This quality is one of the most historically appreciated by consumers in previous decades. However, modern markets tend to request more specificity and migrate to health-focused quality (Prieto *et al*., 2008).

Nowadays, quality focused on health is restricted to the knowledge of any substance that is bioavailable for consumers and that may have some beneficial effect on aspects associated with the prevention and protection of any human pathology. In the case of Hass avocado, its monounsaturated and polyunsaturated fatty acid contents are particularly relevant for consumers given the multiple reports of their effect on chronic non-transmissible pathologies such as metabolic syndrome (Fulgoni *et al*., 2013), the reduction of dyslipidemia severity in patients with hypercholesterolemia (Lottenberg *et al*., 2002), or even the

Table 2. Visual quality characteristics and classification in Hass avocado for Colombian traders[1].

Category	Characteristic of mandatory compliance	Tolerance
Extra	Fruits at proper ripeness stage, intact and visibly healthy, free of insects, with a fresh appearance and firm consistency; free of abnormal external moisture, odors, flavors and foreign materials; free of damage in the epidermis by temperature; fruits with pedicel (< 5 cm) and with minimum values of pesticide residues.	Superficial spots from lenticellosis and slight damage caused by thrips.
I	Fruits at proper ripeness stage, intact and visibly healthy, free of insects, with a fresh appearance and firm consistency; free of abnormal external moisture, odors, flavors and foreign materials; free of damage to the epidermis by temperature; fruits with pedicel (< 5 cm) and with minimum values of pesticide residues.	Superficial scratches from lenticellosis and superficial scars caused by insects. These must not exceed 10% of the epidermis. Fruit deformations are accepted but should not compromise the pulp.
II	Fruits at proper ripeness stage, intact and visibly healthy, free of insects, with a fresh appearance and firm consistency; free of abnormal external moisture, odors, flavors and foreign materials; free of damage to the epidermis by temperature; fruits with pedicel (< 5 cm) and with minimum values of pesticide residues.	Defects in shape and color, external damage caused by lenticellosis and insects. These should not exceed 10% of the epidermis.
Industrial	Fruit without pesticide residues that do not exceed the allowed limits.	Fruit can have multiple defects but they cannot compromise the pulp.

[1] Adapted from Icontec (2018).

activation of the immune system and contribution to the treatment of diseases as relevant as cancer or HIV (Bouic, 2002).

Hass avocado from Colombia has the potential to capture new international consumers in terms of quantity and quality of fatty acids since fruits produced in the country's soil and climate conditions present comparative levels of these fatty acids to those reported worldwide (Tab 3).

On the other hand, organoleptic quality is related to the consumers' sensory perception when ingesting a product in terms of smell, taste, texture and visual aspects. In the case of hass avocado, fully ripe fruits with a dark purple epidermis color, slightly yellowish green pulp, a soft, creamy and even oily texture on the palate accompanied by nuances of nutty, fruity and herbal aromas and slightly sweet and not so bitter flavors are highly appreciated by international markets (Arpaia *et al*., 2018).

Ethical quality aspects are mainly related to the social influences of a productive system on a specific population. For example, a specific plant material cultivated by vulnerable populations such as single mothers who are heads of households or victims of the armed conflict with its respective traceability and certification could be a differentiating factor that generates added value for international altruistic markets (Gutjar *et al*., 2015).

Finally, the emotional aspects are related to the sensations that the customer perceives when buying or consuming a particular product. This aspect is mainly transcendental in the evolution of the avocado imaginary of consumption since its appearances on social networks such as Facebook, Instagram, and Twitter have increased in recent years. In addition, in other *digital marketing* platforms, avocados are being perceived as a symbol of "healthy life", social status or are even being related to graphic euphemisms of sexual connotation in the growing trend of "*food porn*" (McDonnell, 2016). The sensations at the time of buying, ingesting, photographing, publishing or displaying this product on the *social web* have generated a trend among the *millennial* population, which has resulted in the consolidation of aesthetic characteristics of fruits to be acquired. These characteristics will produce pleasure in people when sharing this fruit in their social environment, entertainment platforms and other networks (Ellstrand, 2018).

Quality requirements depending on the target market

Avocado fruit quality emphasizes the requirements of a given market, which is based on certain criteria such as (i) phytosanitary requirements, (ii) food safety, (iii) market accessibility, (iv) required quantities, and (v) value, etc. This implies a definition of quality in terms of trade and negotiation by the countries involved in commercial transitions. However, some aspects related to consumers and the characteristics of the product they demand have been forgotten. This has caused final consumers not to feel identified with

Table 3. Lipid profile of Hass avocado pulp reported in producer countries[1].

Fatty acid		Country (% fatty acid-base)				
		Australia	Mexico	New Zeland	United States	Colombia
Palmitic acid	C16:0	25.63	22.59	20.61	22.24	16.81
Palmitoleic Acid	C16:1	7.29	11.63	10.31	13.14	7.85
Stearic Acid	C18:0	0.45	0.24	0.3	0.93	0.14
Oleic acid	C18:1	42.59	49.19	50.97	47.69	61.45
Linoleic acid	C18:2	20.87	14.72	16.1	14.47	4.37
α Linoleic Acid	C18:3	3.19	1.63	1.72	1.54	1.64
Sum of saturated fatty acids		26.07	22.83	20.91	23.16	16.96
Sum of monosaturated fatty acids		49.88	60.83	61.28	60.83	69.30
Sum of polyunsaturated fatty acids		24.06	16.35	17.81	16.01	13.94
Saturated/unsaturated ratio		0.35	0.29	0.26	0.30	0.20

[1]Adapted from Tan *et al*. (2017) and Henao-Rojas *et al*. (2019).

the product they are accessing since their imaginary of consumption is unknown to the value chain.

As described above, avocado consumption, especially the Hass variety, has been increasing since it has adequate flavor and functional properties because of its nutritional quality. That is why consumers will be the ones who largely define the concept of quality according to their preferences, which can be objective (nutritional content, shape, color, size, and texture, among others) or subjective (product origin, type of production system and its relationship with the environment, and form of commercialization, etc.) (Migliore *et al*., 2015, 2018; Bader *et al*., 2016).

In this regard, the most used criteria by consumers to select fruits at stores are associated with visual factors such as: (i) fruit weight (commercial size), (ii) shape (oval or pyriform), (iii) skin color (green, purple, black), (iv) skin texture (smooth or rough), and (v) absence of defects (for example, sunburn). On the other hand, the factors that are evaluated at the time of consumption are related to the internal quality of the fruit: (i) taste, (ii) pulp texture, (iii) pulp color, and (iv) seed size (Storey *et al*., 1973; Migliore *et al*., 2018). Additionally, it is reported that purchases are associated with fruit consumption habits, neophilia attitudes and various intrinsic and extrinsic quality attributes (attributes of particular and cultural beliefs) (Migliore *et al*., 2018).

In the case of Colombia, the particular quality requirements associated with intrinsic factors of avocado fruits according to export destinations or internal consumer preferences are not well known. In addition, there are no studies at the consumer level that show customer likes and quality criteria that could be implemented in order to improve fruit production, harvest, and post-harvest processes. In this regard, there are some parameters that were previously stated (Tab. 1 and 2) in the section related to harvest parameters or criteria, which refer to calibers and quality categorization (Icontec, 2018).

Marketing companies have a series of quality criteria based on characteristics such as chemical product residues, harvest time or ripening, visual defects caused by fruit deformities, mechanical damage or insects, and visible presence of pathologies (Ramírez-Gil *et al*., 2020). In addition, they carry out a rigorous process to define fruit quality and caliber (Tab. 1 and 2). It is important to state that fruits are rejected or accepted for export and/or national market according to damage, their origin, and affected area.

PRE-HARVEST ASPECTS THAT DETERMINE THE QUALITY OF HASS AVOCADO AND MANAGEMENT PRACTICES CONDUCIVE TO IMPROVING QUALITY

Avocado fruit quality is achieved in the crop as a result of the interaction among the genetics of the variety, environmental factors, the relationship between genotype and environment, the agronomic management of the crop and harvest criteria, among others (Ochoa, 2012; Schaffer *et al*., 2013; Ferreyra *et al*., 2016; Salazar-García *et al*., 2016b). Thus, fruit quality must start from a series of agronomic practices implemented at pre-harvest and harvest and must be complemented with adequate post-harvest work since, at this stage, fruit quality can only be maintained and not improved.

Environmental and edaphic variables and/or pre-harvest agronomic criteria that determine avocado fruit quality are highly variable and still little studied in each of the producer regions of the world. There is currently a trend to identify the specific characteristics of some areas in which better quality fruits are produced (Ferreyra *et al*., 2016).

The factors that affect avocado fruit quality include: environmental variables such as temperature, precipitation, solar brightness, relative humidity, and elevation, among others; edaphic properties associated with nutrient content (e.g. calcium, nitrogen and boron), texture, land slope, drainage networks, and nutrient ratios (e.g. N/Ca, K/Ca); the genetic material used as rootstock; crop management practices that include fertilization, planting density, pruning, irrigation, incidence of pests and diseases (e.g. avocado wilt complex), hormonal relationships (e.g. gibberellins), and leaf area index, among others (Ochoa, 2012; Bernal, 2016; Ferreyra *et al*., 2016; Salazar-García *et al.*, 2016a; Ramírez-Gil *et al*., 2017; Ramírez-Gil, 2018b; Rivera *et al*., 2017; Novoa *et al*., 2018).

In Colombia, not much progress has been made in this regard and only a few studies have focused on the effect of altitude on the quantity and quality of fatty acids. As a result, it has been determined that, at a higher elevation, the concentration of fatty acids increases (Carvalho *et al*., 2015). In this regard, it is possible to increase fruit size percentage under these environmental conditions, which will also improve the extra quality percentages, pulp yield, and nutrient contents, among others (Bernal, 2016). However, limitations associated with lower yields and longer production cycles under these environmental conditions have to be considered (Bernal, 2016).

On the other hand, some studies have characterized exportable fruits in terms of physical and chemical variables, but have not made any contributions in terms of defining criteria to improve quality (Astudillo and Rodríguez, 2018). In this sense, some authors have determined how avocados that are produced under certain conditions in Colombia have good quality standards in terms of physical, chemical and nutritional variables (Henao-Rojas and Rodríguez, 2016; Rodriguez *et al*., 2018). However, it is reported that according to a scale from 1 to 10 by European importers, Colombian fruits obtained an average value of 5.9 based on three criteria: (i) ripening process (5.5), (ii) external quality (5.3), and (iii) internal quality (6.1). These values are much lower than the ones obtained by direct competitors such as Israel (6.8), Peru (7.1), South Africa (7.2) and Chile (7.4) (Analdex, 2017). This situation may be due to the great production system variability and the absence of a technological package for the country's conditions. For this reason, there is a need to unify the production criteria to improve fruit quality and thus increase producers' profitability levels.

It is important to understand that avocado fruit quality is the sum of the entire production process, which involves plant material suppliers, fruit producers, packers, distributors and consumers (Ochoa, 2012). That is why quality is a commitment of the entire value chain; in each link, all activities should be performed in the best way to ensure that Colombian fruit is positioned worldwide as a value brand and that the country is recognized for quality. This will allow Colombian fruits to continue conquering new market niches.

In general terms, there are a number of agronomic practices (Fig. 1) that producers can implement in their production systems with the objective of improving fruit quality. In this regard, a series of strategies has been evaluated in commercial plots under

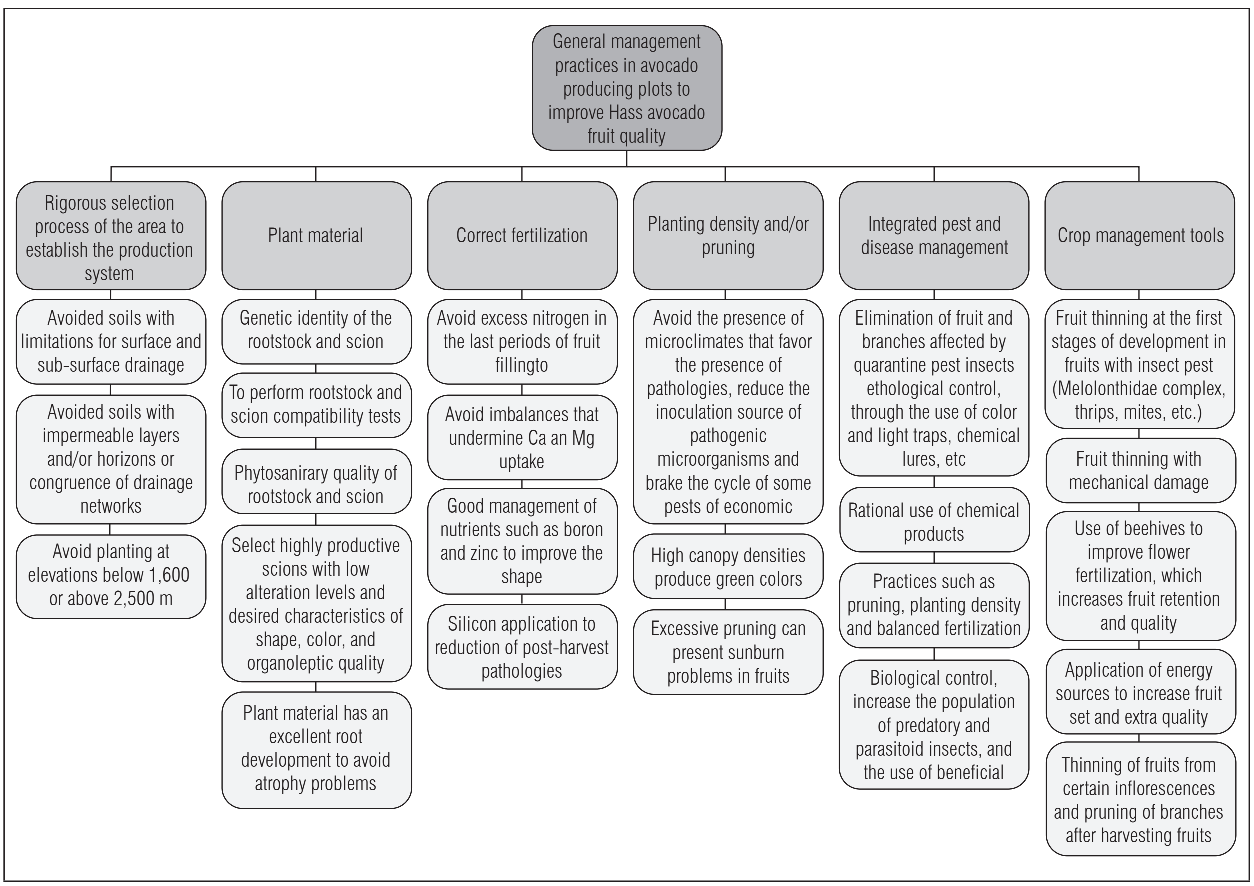

Figure 1. Basic pre-harvest agronomic practices that improve Hass avocado quality under conditions of the Colombian tropics.

Colombian tropical conditions and reported by different scientific research with good results (see: Willingham *et al*., 2006; Everett *et al*., 2007; Crowley *et al*., 1996; Dann and Le, 2017; Kaluwa *et al*., 2010; Gazit, 1976; Ramírez-Gil, 2017; Carvalho *et al*., 2015; Henao-Rojas *et al*., 2019; Malhi *et al*., 2010; Bernal, 2016; Ramírez-Gil *et al*., 2018; Ramírez-Gil, 2013, 2018a; Ramírez-Gil and Morales-Osorio, 2018). As a result, more innocuous fruits with better calibers, less susceptibility to physiological disorders and postharvest pathologies, with outstanding sensory characteristics (color, shape, firmness, taste, etc.), higher nutrient levels, and longer shelf life have been obtained.

HARVEST-ASSOCIATED ASPECTS THAT DETERMINE HASS AVOCADO QUALITY

One of the global challenges the avocado industry has to face is offering the consumer a product with homogeneous ripening (Rivera *et al*., 2017). Avocado is a climacteric fruit that reaches consumption ripening several days after harvest (Hershkovitz *et al*., 2009). Ripening in this fruit is a highly complex process associated with multiple biochemical changes and hormonal relationships, which include increases in ethylene and respiration, loss of firmness, and development of sensory variables (taste and color), among others (Ochoa, 2009). This situation implies the need to identify harvest criteria with great influence on the avocado ripening processes and its quality in order to prevent the harvest of immature or overripe fruits and reduce post-harvest losses.

The first element for a correct harvest is the definition of harvest criteria. This decision should not only be based on a single indicator but on several criteria. This situation implies the need to use different harvest criteria designed among producers, packers, exporters, and traders, which must obey the target market and the desired shelf-life for the fruit. At present, there is a series of harvest indicators that are classified into destructive and non-destructive methods, which must be determined and calibrated per cultivar and at each producer region.

The mesocarp's oil content is considered to be the best avocado ripeness indicator (Lee, 1981; Ochoa, 2012). The minimum value for harvest should be 8% (Lee *et al*., 1983), but its determination is complex and expensive as it represents a high risk for operators, given that toxic organic solvents are used in the process (Lee *et al*., 1983; Ochoa, 2012). For this reason, dry matter has become the world's most widely used crop index since it is a simple, fast, and safe method and is highly correlated with the fatty acid content (Lee *et al*., 1983; Ochoa, 2012; Carvalho *et al*., 2014). On the other hand, there is currently a growing trend for the application of non-destructive techniques using a portable spectrometer. These techniques offer rapid results that are highly correlated with dry matter and mesocarp oils (Ncama *et al*., 2018).

In this regard, some advances have been made in Colombia, such as those reported by Carvalho *et al*. (2014) and Rodriguez *et al*. (2018), who determined that avocados must have a minimum of 23% dry matter at the time of harvest. This concept has become a misused criterion since it was the only parameter to determine the optimum harvest time without considering aspects such as shelf life, target market, and the differences of this variable according to environmental, agronomic and genotype parameters. This is why this indicator must be calibrated for the different avocado producing regions in Colombia and should be based on the importing market. The most commonly used crop indicators and their reference values for Colombia are described below.

Non-destructive methods use changes in fruit color and shape. Avocado fruit color changes depend on the ripening stage; fruits at early stages of development have a bright green color that later becomes opaque or dark green, and, at advanced ripening stages, they show a purple color that evolves to black. For fruit color, Ochoa (2012) reported a scale with values from 0 to 6, which was calibrated based on fruit firmness and ripening process. Color 0 (opaque olive green) is equivalent to a hard firmness (> 25 lbs of pressure, harvest time), whereas a level 5 color (deep purple) is equivalent to fruit with soft firmness (5 lbs of pressure, ripe for consumption). Color can also be calibrated based on the ripeness level, and its measurement is taken with the use of tristimulus colorimetry (Henao-Rojas and Rodríguez, 2016). Additionally, in most varieties, the portion of the pedicel closest to the fruit turns yellowish and swollen, which is a good ripeness indicator. The skin structure must be rough with notorious lenticels, the seed must be separated from the pulp, and the pulp must have a green-yellowish color and creamy texture with transparent and removable tegument (Ochoa, 2012). In some situations, the size can also be a harvest indicator (Ochoa, 2012; Rodriguez *et al*., 2018).

Firmness is a harvest criterion that is rarely used under field conditions; however, when used, it consists in quantifying the fruit hardness level (pounds of pressure). This parameter has been correlated with the ripening process in countries such as Mexico (Ochoa, 2012). Firmness can be measured by touch, but this technique is very subjective. For this reason, different devices such as the penetrometer or acoustic firmometer are used to determine this variable (Magwaza and Tesfay, 2015; Zhang *et al*., 2018), whose results are expressed in kgf cm^{-2} and then in pounds of pressure (lbs). It is reported that the maximum and minimum values quantified in avocado skin at consumption ripeness for Colombia should be between 0.90 and 1.51 kgf cm^{-2} (Icontec, 2018).

Regarding destructive methods, the fatty acid profile is perhaps the method with the closest relationship with avocado fruit ripening, but it presents multiple disadvantages, such as its cost and the difficulty to obtain oil extracts, as described above (Lee, 1981; Ochoa, 2012). For Colombia, the oil content for harvest must be a minimum of 12% in mass fraction (Icontec, 2018). On the other hand, the most used harvest indicator in Colombia is dry matter, and it is recommended to harvest fruits when this parameter is equal to or higher than 23% (Icontec, 2018). However, Carvalho *et al*. (2015) and Rodriguez *et al*. (2018) reported that the dry matter content for harvest in the Department of Antioquia ranges between 22 and 26% as a base for a minimum oil percentage of 11.2%. Another indicator used is the minimum pulp percentage, which must be a minimum of 58% for consumption ripeness (Icontec, 2018).

Once the harvest criterion is defined, a pesticide residue analysis must be carried out a month before the harvest (Icontec, 2018). This test determines if the presence of pesticide residues is below the maximum limits allowed according to the regulations of the importing country. These criteria are different according to the destination, and the European Union establishes its own criteria just as the US market does.

A correct harvest is carried out after the harvest criteria have been selected and calibrated and the pesticide residue test has been performed. The process is carried out avoiding mechanical damage, which affects the cosmetic appearance of fruits or can be the gateway to pathogens that cause postharvest diseases.

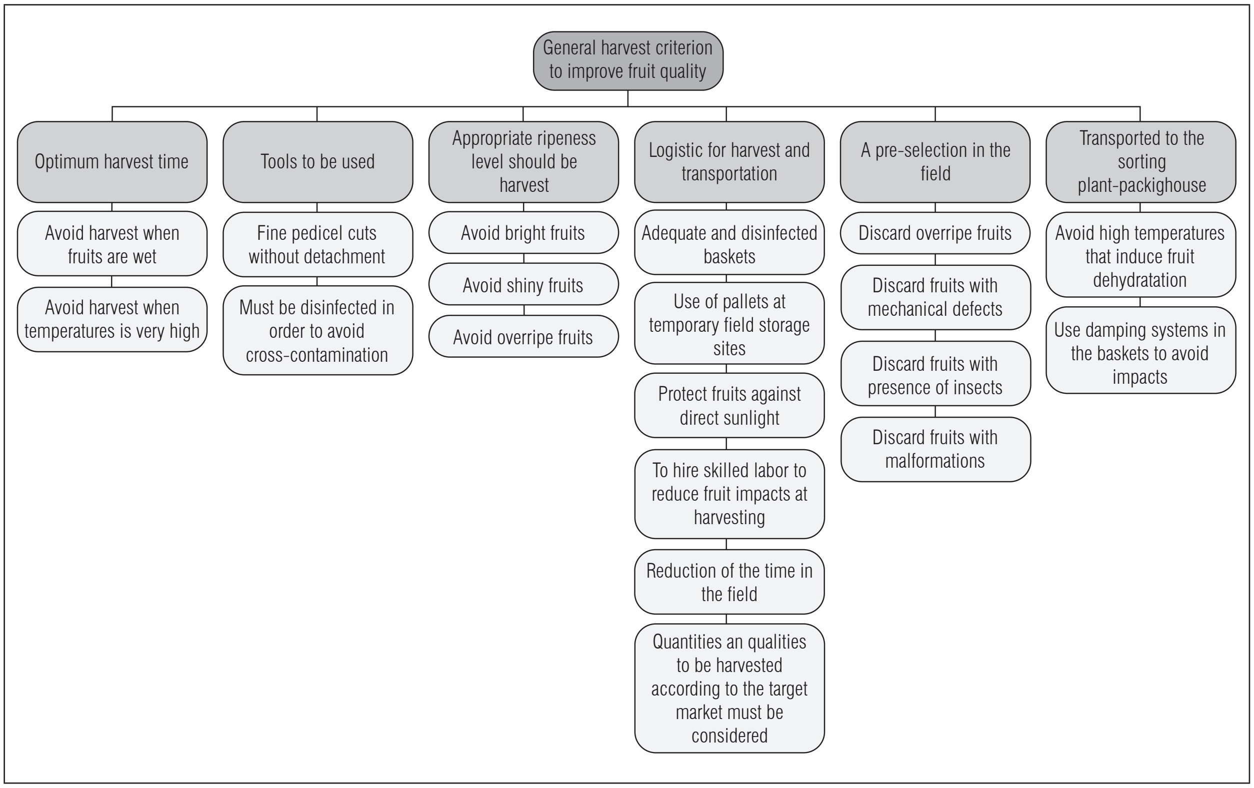

Figure 2. Main harvest practices associated with the preservation of Hass avocado quality.

It is also recommended to handle fruits according to the criteria of the international code of practice general principles of food hygiene (CAC/RCP, 1-1969, Rec. 3-1997). In addition, the microbiological requirements defined in the legislation according to the principles for the establishment and application of microbiological criteria for foods (CAC/GL 21-1997) must be followed (Icontec, 2018).

An adequate harvest involves the following parameters reported in figure 2 (see: Duvenhage, 1993; Ramírez-Gil, 2018b; Perkins *et al*., 2019).

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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Botanical biopesticides: research and development trends, a focus on the Annonaceae family

Biopesticidas de origen botánico: Tendencias en investigación y desarrollo, un enfoque en la familia Annonaceae

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Annonaceae fruit species.

Photos: A.-I. Giraldo-Rivera

ABSTRACT

This document analyzed research and development trends related to chemical, biological and economical biopesticides based on plant extracts, with emphasis on the Annonaceae family. A systematic review of the literature between 1947 and 2018 was done with an advance search equation in a specialized data base. This paper contains the development of plant bio pesticides, their modes of action, the more prominent families according to their bioactivity and the secondary metabolites that exert control over pests that are significant in agriculture and public health. The dynamics of publications, at the national and international levels, were identified, along with the countries that lead research, patents assigned in the last 20 years, market trends and bio pesticide regulation. The reviewed research is relevant to bioprospecting plant extracts with potential insecticidal activity and to the subsequent development of biocides using botanical extracts; the Annonaceae family is promising.

Additional key words: biological control agents; chemical products; metabolites; patents; bioprospecting.

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RESUMEN

El siguiente documento tuvo como objetivo analizar las tendencias de investigación y desarrollo sobre la importancia biológica, química y económica de los biopesticidas a partir de extractos vegetales y de modo particular un enfoque de la familia Annonacea. Se llevó a cabo una revisión sistemática de literatura entre 1947 y 2018 empleando ecuaciones de búsqueda en bases de datos especializadas. Se describe el desarrollo de los biopesticidas de origen vegetal, sus modos de acción, las familias más destacadas por su bioactividad y los metabolitos secundarios que ejercen un control sobre las plagas de importancia agrícola y en salud pública. Según la búsqueda, se identificaron las dinámicas de las publicaciones a nivel nacional e internacional, los países que lideran la investigación, las patentes asignadas en los últimos 20 años, la tendencia del mercado y su reglamentación. Con este proceso de búsqueda y análisis de datos se pudo establecer la pertinencia de la investigación sobre la bioprospección de extractos de plantas con potencial actividad insecticida y posterior desarrollo de biocidas usando extractos de origen botánico resaltando la familia Anonnacea como una de las más promisorias.

Palabras clave adicionales: agentes de control biológico; productos químicos; metabolitos; patents; bioprospección.

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RENAISSANCE: USE OF PRODUCTS OF NATURAL ORIGIN

The use of pesticides with a botanical origin goes back at least two millennia in China, Greece, Egypt, and India. For North America and Europe, the use of these pesticides began one hundred and fifty years before the appearance of synthetic pesticides (organophosphates, organochlorines, pyrethroids and carbamates). Worldwide, the use of natural insecticides has increased because of regulatory actions that control the indiscriminate use of synthetic pesticides that have harmful effects and negative impacts on air, soil and water (Isman, 2006; Benelli *et al.*, 2015; Sänchez *et al*., 2019).

In the last twenty-five years, the tendency for healthy and sustainable agriculture in developed countries has increased the need to find products capable of controlling the pests that affect agriculture (Ehlers, 2011). Global production of biopesticides (3,000 t year^{-1}) is increasing at an accelerated rate. The participation of biopesticides is 2.5% in the total pesticide market. The global trends for the Biocontrol markets show that the percentage of biopesticides has continuously increased since 1997, and this increase is expected to maintain a rate of change of 10% per year (Gupta and Dikshit, 2010).

The use of botanical insecticides is emerging as an important tool for protecting agricultural products and the environment. For this reason, the development of this subject is an interesting topic.

New trend: Biopesticides

Biological pesticides are derived from natural raw materials such as plants, animals, bacteria and minerals. They are used to attack a specific pest, offering an eco-friendly and effective solution for pest control and representing little or no danger to the environment or humans (Gupta and Dikshit, 2010; Pavela, 2014a).

Some benefits offered by biopesticides that make them preferable to conventional synthetic pesticides are: (i) they have a specific mode of action, (ii) they are biodegradable in nature, and (iii) they generate little waste (Pavela, 2014b).

Biopesticides include a lot of potential products that can be classified into three main types, such as: microbial pesticides and other entomopathogens, incorporated plant protectors (PIP) and biochemical pesticides (Sarwar, 2015). Botanical pesticides are classified in the latter and are an important group of natural crop protectors are often slow acting, and incorporate mixtures of biologically active compounds; no resistance develops in pathogens or pests In their basic form, botanical pesticides can be raw preparations of plants, such as powders from flowers, roots, seeds, leaves, stems, and essential oils. The formulas are commonly concentrated or liquid extracts (Isman, 2006; Pavela, 2016).

Botanical pesticides

Natural plant products have gained a lot of importance in recent years with researchers as natural sources of new insecticides (Pavela, 2016). It is estimated that more than 6,500 species of plants have been examined for anti-insect properties. Of these, more than 2,500 species belonging to 235 families have shown a bio pesticide activity (Celis *et al.*, 2008; Walia *et al*., 2012). Table 1 shows families with plants that have different chemical groups for insect control (Tab. 1) (Castillo *et al*., 2010).

Annonaceae is a family of plants widely distributed in tropical and subtropical regions and includes approximately 2,400 species and 108 genera (Castillo *et al.*, 2010). The Annonaceae family contains four economically important genera for fruit growing: Annona, Rollinia, Uvaria and Asimina. In recent decades, chemical studies on the Annonaceae family have intensified because of the discovery of molecules with broad potential in agriculture and medicine (González *et al.*, 2014).

Mechanisms and importance of botanical pesticides

The insecticidal activity of plants is attributed to the presence of secondary metabolites (Castillo *et al.*, 2010). These compounds can have diverse mechanisms of action (Celis *et al.*, 2008; El-Wakeil, 2013; Miresmailli and Isman, 2014); besides insecticide activity, they have an insect static effect, meaning they can act as inhibitors (Eriksson *et al.*, 2008), dissuasive of the oviposition (Dimetry, 2012), repellents (Peterson and Coats, 2001), anti-alimentary (Isman, 2006) and as growth regulators (Celis *et al.*, 2008; Dimetry, 2012).

Some of the most successful natural products with application at the field level are obtained from neem (*A. indica*) (Benelli, 2015), essential oils, capsaicin oil (*Capsicum annuum* L.) (El-Wakeil, 2013) and preparations of *Ryania speciosa* Vahl (Murillo and Salazar, 2011).

Analysis of trends in scientific publications on botanical biopesticides

The analysis of scientific publications on botanical biopesticides was carried out according to the Scopus database. A comparative search was carried out with Web of Science, where a lesser registry of publications was obtained. According to the search parameters, the following search equation was used in Scopus: TITLE-ABS-KEY: ((“botanic*pesticide*”) OR(“biocides*organic*”)OR(“biocides*natural*”) OR(“plaguicide*botanic*”)OR(biopesticides*) OR(“insecticidal*activity*”)OR(antifeed*) OR(deterr*)OR(repell*)OR(acaricidal*) OR(biocida*)OR(“botanic*insecticide*”) OR(“plant*extract*”))AND((annonaceas*) OR(annona*)OR(Annonaceae*)OR(Rutaceae*) OR(Lamiaceae*)OR(Meliaceae*)OR(Asteraceae*) OR(Solanaceae*)OR(Asteraceae*)OR(Malvaceae*) OR(labiate*)OR(“essential*oil*”)).

Table 1. Examples of the most common plants with activity for insect control.

Family	Plants	Active molecules	Activity type
Annonaceae	*Annona muricata* L. *Annona squamosa* L. *Annona cherimola* Miller	Acetogenins, alkaloids, isoquinoline	Synergistic, insecticide, fungicide, citotoxic, antiparasitic
Liliaceae	*Allium sativum* L. *Allium fistulosum* L.	Disulfide, thiosulfinate, thiosulfonate	Insecticide, acaricide, nematicide, herbicide, fungicide, bactericide
Solanaceae	*Nicotiana tabacum* L. *Capsicum frutescens* L.	Alkaloids (atropine, nicotine, solanine)	Toxic, deterrent, non-persistent contact insecticide
Meliaceae	*Azadirachta indica* A.Juss. *Melia azedarach* L.	Limonoids terpenoids	Insecticide
Piperaceae	*Piper nigrum* L.	Lignans	Synergistic, insecticide

Source: modified from Tello *et al*. (2010).

The number of publications on the topic on botanical biopesticides has an exponential trend; the first publication was found in 1947 (Fig. 1), entitled: "Annona species as insecticides", which observed that seed and root extracts of *A. reticulata* and *A. squamosa* showed insecticidal activity when in contact with *Aphis fabae* Scopoli*, Macrosiphoniella sanborni* Takahashi and *Macrosiphum solanifolii* Ashm., and repellent activity against larvae of *Plutella maculipennis* Muller-Rutz (Harper *et al*., 1947). The Annonaceae family has been an important source of compounds with bio pesticidal activity since the 40s.

Until 1990, the dynamics of publications was from 1 to 7 articles per year. This topic has gained momentum since 2001, with more than 100 publications on average per year. For the years 2015 and 2016, a total of 559 and 560 publications were reached, respectively, resulting in the period of greatest production for biopesticides to date.

In general, the proportion of articles focusing on botanical insecticides has increased from 0.94% in 1989 to 8.81% in 2000, and increased to 40.12% in 2010, approximately 5 times greater. However, the last 8 years have seen the highest scientific production, representing 59.81% of the total information. This indicates that the dynamics of publications is in the growth phase and that, currently, this topic is of great interest worldwide.

In the scientometric analysis, it was found that the areas of knowledge with the greatest number of publications are: Pharmacology, Toxicology and Pharmaceutics (25.4%), Biochemistry, Genetics and Molecular Biology (17%), Medicine (16.2%) and Agriculture and Biological Sciences (16%). The largest number of publications has been made in the Journal of Ethnopharmacology. It is reported that most of the botanical biopesticides that are evaluated are extracts of leaves, seeds, roots, and essential oils. The most reported species with insecticidal activity is *A. indica* also known as neem tree. Azadirachtin (Fig. 2), which is a limonoid, has been reported as the main secondary metabolite in its seeds (Isman, 1997; Isman and Grieneisen, 2014; Celestino *et al.*, 2016; Costa *et al.*, 2016).

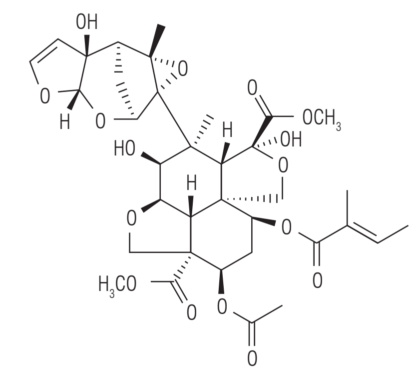

Figure 2. The structure of the azadirachtin active ingredient in botanical insecticides.

Since pest control was one of the more interesting objectives in this review, many publications related to pests of greater economic importance at the agricultural level as well as those related to public health problems were highlighted (Mann and Kaufman, 2012).

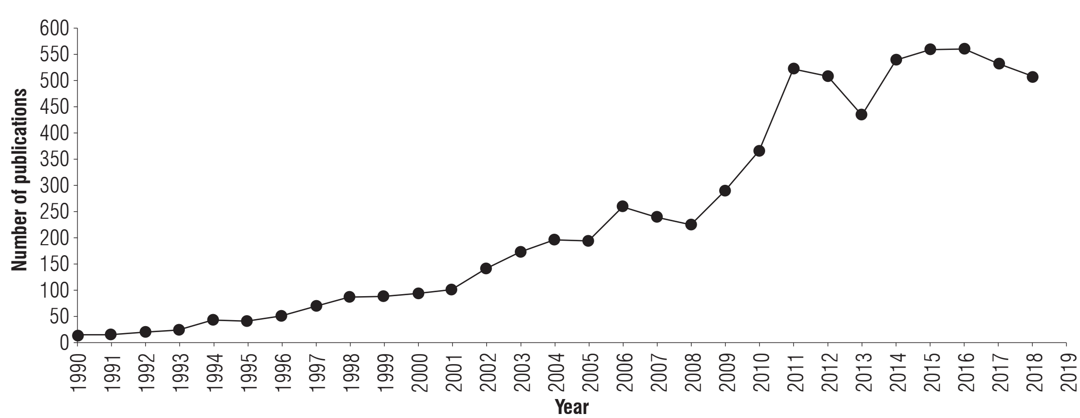

Figure 1. Literature on botanical biopesticides obtained from Scopus using the search equation.

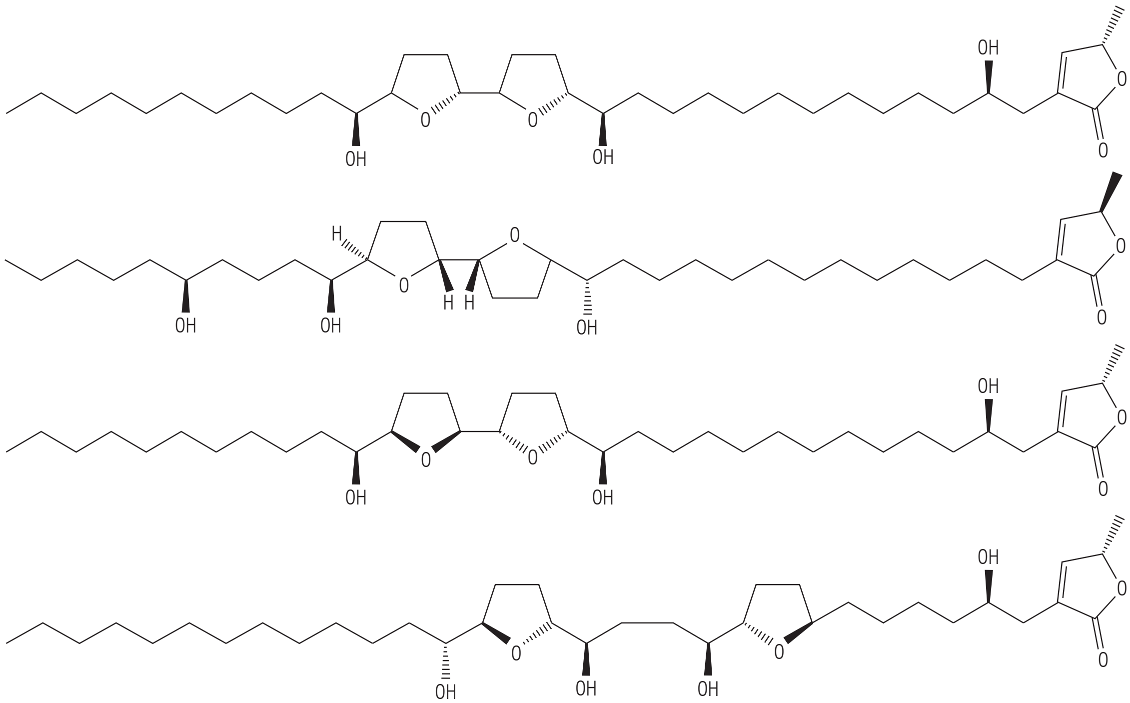

Figure 3. Structures of the acetogenins report (1) Bullatacin, (2) Annonin (3), Rolliniastatin, (4) Gigantecin.

In the first case, publications on controlling insects of the Lepidoptera order were found, especially studies on *Spodoptera littoralis* Boisduval (cotton leaf worm) and *Spodoptera frugiperda* Smith (corn fall armyworm). From the coleoptera order, studies were found on *Tribolium castaneum* Herbst, which highly affects stored products such as wheat, barley, rice, flours and bran, *Sitophilus zeamais* Motschulsky (corn weevil) and *Callosobruchus maculatus* Fabricius (bean weevil) (Ondarza, 2017). For studies on pests associated with public health problems, studies on insects of the order Diptera, *Aedes aegypti* Linnaeus (yellow fever mosquito), *Anopheles stephensi* Liston (malaria vector) and *Culex quinquefasciatus* Say (vector of filariasis) are of great interest (Benelli, 2015; Grzybowski *et al.*, 2012).

With respect to anonaceous, the dynamics of publications increased in recent years by 12.01% in terms of articles. The greatest number of articles was in the period 2011-2012. Since 2004, an average of 60 publications per year has been maintained. The articles on this family showed that they are related to obtaining extracts from leaves and seeds and evaluating insecticidal activity *in vitro*. The most studied species included *A. muricata* and *A. squamosa*. Many publications were focused on pest control, such as *A. aegypti* and *C. quinquefasciatus,* which are of great importance for human health (Benelli, 2015).

Several Annonaceas studies were related to the isolation and characterization of compounds associated with bioactivity. Acetogenins are the main active compounds and are found mainly in the seed. These compounds contain cytotoxic, antitumor, and insecticidal activities, among others. Within this group of compounds, studies on gigantecin, bullatacin, rolliniastatin, and anonin (Fig. 3) stood out because of their great bioactivity (Rojas and Uribe, 2009; Gupta *et al.*, 2011; Alara *et al.*, 2012; González *et al.*, 2014; Kedari and Khan, 2014; Ansante *et al.*, 2015; Ma *et al.*, 2017).

They are also reported as active compounds of annonaceous, isoquinolic alkaloids, and phenolic compounds and, to a lesser extent, cycle peptides, vitamins, carotenoids, amides, carbohydrates, glycosides and volatile compounds (González, 2005; Cortes *et al.*, 2014; Moghadamtousi *et al.*, 2015; Ma *et al.*, 2017; Coria *et al*., 2018). According to the studies, Annonaceae leaves are enriched with different types of isoquinoline alkaloids (Fig. 4), which are attributed similar effects as antidepressants; in some cases, neurotoxic effects are reported. The other compounds are distributed in different parts of the plant.

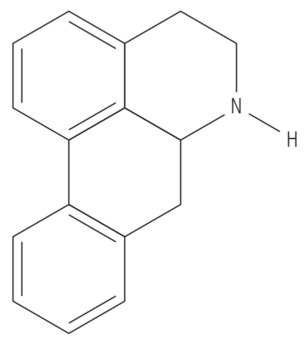

Figure 4. General structure of isoquinoline alkaloids.

Although acetogenins and alkaloids are characterized as the main metabolites with insecticidal activity, the contribution of other types of metabolites such as peptide cycles has recently been considered. The case of Annomuricatin A, B and C isolated from *A. muricata* is particularly reported (Coria *et al.*, 2018). There are also articles that demonstrate the toxicity of fatty acids, which is attributed to their amphibolic properties throught different mechanisms such as penetration, inhalation and contact (Moghadamtousi *et al.*, 2015).

The content of metabolites varies for each species of Annonaceae (Coria *et al.*, 2018; Ma *et al.*, 2017), which is why some species have greater insecticidal activity with respect to others. But the bioactivity of extracts can be more promising for product development than the use of isolated compounds as active ingredients.

In addition, the following research is notable: Insecticidal activity of extracts of raw seeds of different plants against Lepidoptera larvae, where A. *muricata* and *A. squamosa* extracts were the most lethal (Leatemia and Isman, 2004a). According to several studies, acetogenins are the more important metabolites from this family, which present different biological activities (Ahammadsahib *et al*., 1993; Alali *et al*., 1999; Colom *et al.*, 2007; Bajin ba Ndob *et al.*, 2009; Moghadamtousi *et al.*, 2015). The evaluation of extracts from different plant parts for the control of various pests (Leatemia and Isman, 2004a, 2004b; Odalo *et al.*, 2005; Coelho *et al*., 2007; Deomena *et al.*, 2007; Broglio-Micheletti *et al.*, 2009; De Cássia *et al.*, 2010; Barbalho *et al.*, 2012; Roopan *et al.*, 2012; Ribeiro *et al.*, 2014) has established that botanical insecticides based on Annonaceae extracts can be very suitable for the integral management of pests.

Dynamics of publications by country: domination of India

According to the results (Fig. 5), there was a clear trend, where India dominated as the country with the highest number of publications, representing 19.88%, followed by Brazil (10.2%) and China (7.24%). In a more detailed analysis, the increase in publication for these countries started in 2011; for India, there was an average of 173 publications per year. In Brazil, the figures were approximately 88 per year and in China 63 per year.

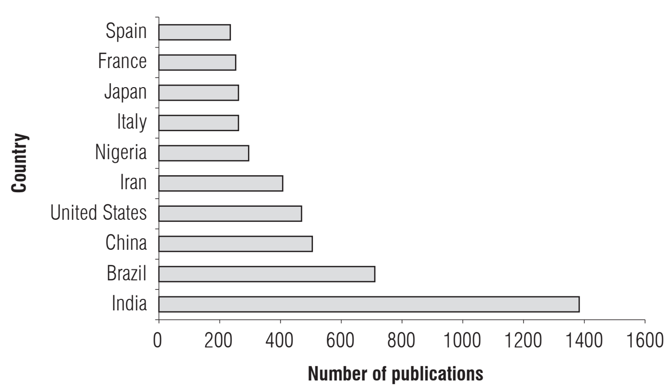

Figure 5. Institutions or affiliations that research biopesticides by country, obtained from Scopus using the equation of search.

On the other hand, the analysis indicated that research in the Americas is based, for the most part, on the development presented by Brazil, the United States and Mexico. However, to a minor extent, there are emerging countries on the subject such as Canada, Colombia and Argentina.

At the national level, Colombia represents 1.02% with 71 publications; it is an emerging country in this area. The dynamic for the analysis of scientific publications on botanical biopesticides from 1992 to 1997 showed little scientific production was developed (Fig. 6). 2018 was the year with the most publications.

Notable there were publications on the families Asteraceae, Piperacea, Solanaceae and Annonaceae. Approximately 40% of the articles evaluated the essential oil of the plants and included chemical characterization by Gas Chromatography coupled to Mass Spectrometry (GC-MS). The studies focused on repellent activity. The other 40% of the articles referred to the use of extracts obtained mainly from seeds and leaves. The focus was on the evaluation of insecticidal activity *in vitro, without* including chemical characterization in most cases. The remaining percentages were related to the isolation of different metabolites in order to potentiate the activity.

The more reported pests included the genera Diptera, Lepidoptera and Coleoptera. The more studied species correspond to: *A. aegypti* (yellow fever mosquito), *T. castaneum* (red flour weevil), *Drosophila melanogaster* Meigen (fruit fly), and *Spodoptera frugiperda* (fall armyworm in maize) (Nerio *et al*., 2010; Ratnadass *et al*., 2012).

15 articles related to research of the Annonaceae family were found in the bibliographic search, and 3 of these were reviews. Among the publications, "Activity insecticidal *in vitro* of the *Annona spraguei* Saff (Annonaceae) in two models' biologics of order Diptero: *D. melanogaster* y *A. aegypti*" was cited (Granados *et al.*, 2001). In the Colombian entomology journal, there were two articles: one of them on the insecticidal effect of *Annona muricata* L. seed extracts and the other on three species of guatteria (Annonaceae) controlling *A. aegypti*, with promising results (Aciole *et al.*, 2011).

Bearing in mind that Colombia is an origin center for Anonaceae (Murillo, 2001) and, according to the bibliographic review at the national level, there are important studies on this family (Giraldo-Rivera and Guerrero-Álvarez, 2018), There is a tendency towards studies on public and agricultural health problems (Muñoz *et al.*, 2013; Ríos *et al*., 2017); research on this family must continue. Therefore, there is an opportunity to obtain and evaluate extracts with bio pesticide potential for the control of pests of economic importance.

Scientific production *vs*. Patenting

To obtain an overview of the patent dynamics on the subject, we consulted the Patent Inspiration and The Lens International Databases. A search equation was established according to the code of the international patent classification (IPC Code): search the IPC path: A HUMAN NECESSITIES; A01; A01N; A23); A61K; C05G; A01N65/00; AO1N65/08. The results of the search yielded a total of 1692 patents.

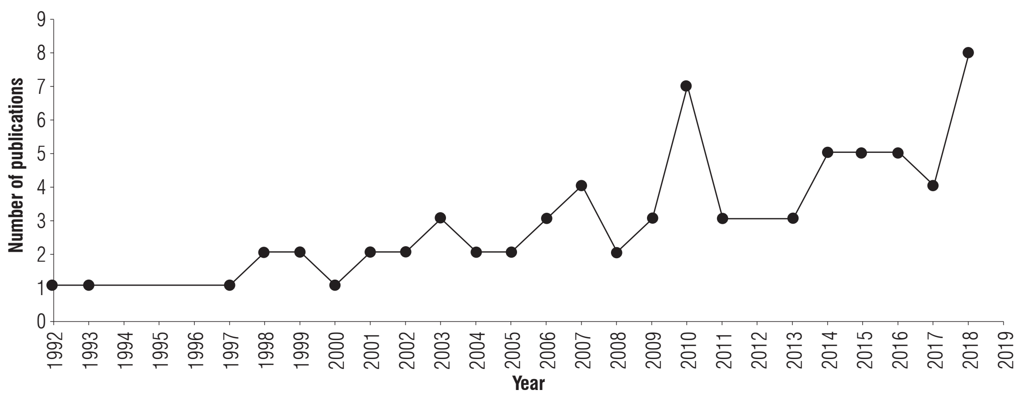

Figure 6. Distribution of the number of publications per year on biopesticides in Colombia.

General patents dynamics: Patents related to biopesticides enjoyed interest in the 80s; however, since 1989, there has been an increase in patents related to this topic. The dynamics of patenting is growing, with the last 30 years having the greatest number of granted patents (Fig. 7), representing 90% of the total patents of the analyzed period.

The United States is among the countries that lead botanical bio pesticide patents, with 261 assigned patents, corresponding to 15.4% of the total number of patents. The United Kingdom has 188 patents, and France is in third place with 166 patents, with percentages of participation of 11.1% and 9.81%, respectively. As for Latin American countries, Mexico has 12 patents, Chile has 8 and Colombia has 7 patents.

The following general conclusions were obtained from the analysis of 50 patents: Biocides from the class Magnoliopsida and from the families Lamiaceae, Myrtaceae, Asteraceae and Rutaceae stand out. The biological control of insects and nematodes is the main use for these developments. The main novelty s is seen in the composition; the greatest advantage is the use of mixtures for their synergistic effect. Patenting botanical biocides protects the methods of obtaining the extracts and the composition of the final mixture.

There are few patents related to extracts of Annonaceae, which are related to the isolation of compounds with bioactivity from species such as *A. reticulata*, *A. squamosa* and *A. muricata*. The use of acetogenins, such as squamocin and annonacin as active ingredients of products for the control of different pathological pests was notable. The combination of Annonaceae extracts with other species of the Piperaceae family was also reported as a strategy for the control of *A. aegypti* (Universidade Federal do Paraná, 2015; Yong, 2015).

Table 2. Annonaceae patents.

Code	Title	Applicant
US6991818	Compunds iso-squamocin obtained from the seed *Annona squamosa* and the composition thereof	Council of Scientific and Industrial Research
CN104663661A	Application of annosquacin B in preparation of pesticide preparations	Univ. Nanjing chinese medicine
BRPI1105786(A2)	Composições fitopraguicidas sinérgicas a partir da combinação de extratos de annonaceae e piperaceae e processos de utilização contra *Aedes aegypti* e outras pragas	Univ. Edf. Parana
CN 103651598A	Pesticide containing *Annona reticulata* L and *Derris elliptica* extracts, and preparation method and application thereof	Chengdu Newsun Crop Science Co Ltd
PH12014000351(A1)	Mosquito larvicide from guayabano (*Annona muricata*)	Nat. Res. Council of the Philippines

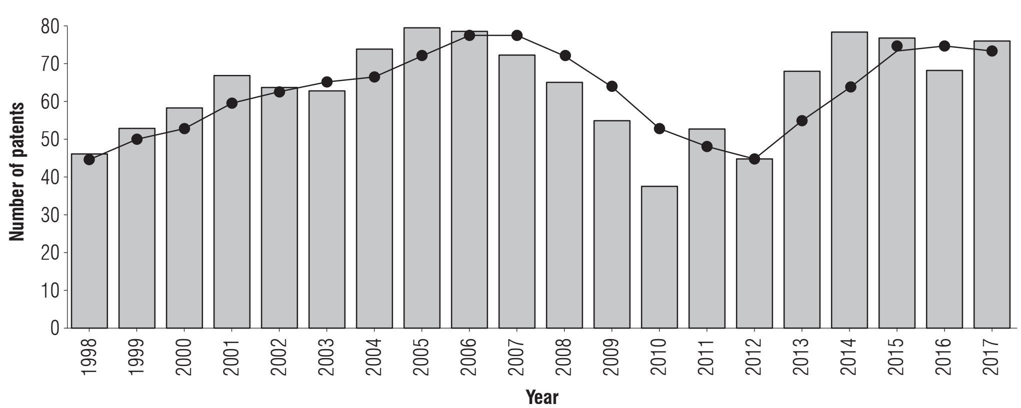

Figure 7. Number of patents per year for botanical biopesticides.

Nowadays, there is a product derived from Annonaceae, which is known as ANOSOM® (Agri Life, India) and has the active ingredient squamocin, obtained of *Annona squamosa* L. Studies have indicated that the mode of action of this compound for insecticidal and fungicidal activity is inhibiting mitochondrial Complex III.

A growing market

Regulatory requirements, such as government policies and environment protection laws in different countries, restrict pest control market agents because regulatory requirements in terms of the environment are becoming stricter and more stringent, resulting from an increase in demand for organic agricultural products.

In Europe, Commission Regulation EU 2017/1432, which amends Regulation (EC) No 1107/2009 (European Commission, 2017), authorized the review of pesticides, and their use was agreed to in European countries. During this evaluation, about 70% of the active materials were banned from the Market. The objective was: "to reduce the risks and impacts of the use of pesticides on human health and the environment while promoting the development and introduction of IPM together with alternative techniques, in order to reduce the dependence on the use of chemical pesticides." Meadows-Smith noted that the global tendency towards low environmental impact supplies, called "Low Chem", presented a huge opportunity for the bio pesticide industry.

The market for biopesticides is projected as the fastest growing market because of growing demand from farmers. According to the report posted by Transparency Market Research, the biopesticides global market was valued at US$ 1.72 billion in 2014. Additionally, driven by the growing concern for sustainability, new regulations and population growth, this industry can reach US$ 4.17 billion by 2023 and 9 billion dollars by 2050. For the use of bio pesticides, 80% are used to protect crops of high economic value such as fruits and vegetables, largely because they do not leave residues, something very appreciated by consumers (ReportsnReports, 2015).

North America is expected to dominate the global bio pesticide market in terms of demand (Markets and Markets, 2017). In 2015, it represented the largest market share, followed by Asia-Pacific and Europe. Increased awareness of the benefits of biopesticides among growers and the increase in population are causing market growth in this region. Central and South America account for 10% of the global market for bio pesticides, and Brazil represents the largest market in Latin America (72.47%), followed by Argentina.

The Asia Pacific market presents a great opportunity for bio pesticides, driven by the mega-economies of China and India. For its part, China, given its extensive area, has directed efforts for the sustained use of bio pesticide with strategies such as providing subsidies to farmers or planting bases for the use of bio pesticides. In India, according to the Ministry of Agriculture, biopesticides account for just 2.89% of the 100,000 metric tons of the pesticides market but could increase to a rate of 2.3% per year (Markets and Markets, 2016).

The main companies identified in the global bio pesticide market were Bayer Crop Science (Germany), BASF SE (Germany) and Monsanto Company (USA). These large global companies invest in new biopesticides development with different active ingredients and have implemented programs of Integrated Pest Management. The most important players in the market have explored new places, promoting new products, cooperations and acquisitions around the world in order to achieve benefits through combined synergies. Since 2011, the bio pesticide market has experienced an increase in demand, mainly in countries such as Spain, Canada, Brazil, China and India (Markets and Markets, 2017).

In Colombia, the registration and control of chemical pesticides for agricultural use is regulated by Decision 804 (Andean Community, 2015). In Resolution 534 (ICA, 2012), provisions were made for the preparation and registration of chemical products for agricultural use. Resolution 698 (ICA, 2011) dictates the provisions for the Registration and Control of Bio-inputs and Vegetable Extracts of Agricultural Use in Colombia.

At a national level and according to the report presented by the Colombian Agricultural Institute (ICA) and the Ministry of Agriculture and Rural Development on Bio-input companies (December 2019), 36 companies are producers and importers, which work in the development of bio-pesticides from plant extracts. The companies that stand out at the national level include Eco flora Agro, ADN verde, Agro Biológicos del Ajo, Orgánicos de Colombia and Green seal company, which develop plant extracts for effective and sustainable crop protection (ICA, 2019).

CONCLUSIONS

This review for botanical biopesticides o established that there is a potential interest in research and in the subsequent development of biocides using extracts of botanical origin, where the Annonaceae family is promising.

Although secondary metabolites with biocidal activity have been identified, it is necessary to delve further into composition studies on the promissory extracts because the examined documents indicate that a chemical characterization of the extracts is lacking.

The evaluation of synergistic mixtures and field tests should be encouraged for new product development for pest control that will affect crops and human health.

The analysis of the market trend of botanical biopesticides indicated that it is growing and that there is great opportunity for Latin America and for Colombia because of its great diversity of plants.

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The phyllosphere microbiome and its potential application in horticultural crops. A review

El microbioma de la filosfera y su aplicación potencial en la horticultura. Una breve revisión

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Phyllosphere, host, plant surface.

Photo: S.E. Barrera

ABSTRACT

Microorganisms are essential for life on Earth. They are found in different environments and conditions, such as pH, temperature, pressure, and humidity, etc. In natural and agricultural ecosystems, nutrient cycling and plant protection are important roles played by microorganisms associated with plant species. However, the mechanisms to colonize those environments are not fully understood. This mini-review describes bacterial communities associated with the phyllosphere and an agricultural approach for potential applications. In the context of foodborne illnesses and losses in agricultural production, important issues have arisen because of pathogen attacks. On the other hand, the use of beneficial microorganisms in agriculture is an alternative for improving plant growth, health and production. In this sense, growth promoting bacteria and biocontrol agents isolated from the phyllosphere of several plant species have been less exploited than those from the soil or rhizosphere. However, the treatment of some plant diseases, reduction in pathogen incidence and nitrogen fixation in natural and agricultural systems are successful examples. In the context of food safety, a better understanding of how the indigenous phyllosphere microbiota enable plants to protect themselves against pathogens and to acquire nutrients is expected to prove its importance in the agricultural field. Microbial sources can be managed to reduce the use of chemical products and could be used as an alternative of agronomical applications for improving agroecosystem productivity.

Additional key words: epiphytic community; plant health and growth; ecosystem productivity; biocontrol.

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RESUMEN

Los microorganismos son esenciales para la vida en la tierra. Ellos se encuentran colonizando diferentes ambientes y en diferentes condiciones de pH, temperatura, humedad, etc. En ecosistemas naturales y agrícolas, el ciclado de nutrientes y la protección de la planta, son funciones importantes desempeñadas por los microorganismos asociados a las especies vegetales. Sin embargo, los mecanismos para colonizar esos ambientes no son completamente entendidos. En esta corta revisión se describen las comunidades bacterianas asociadas a la filosfera, con un enfoque agrícola de sus aplicaciones potenciales en esta área, relacionadas con nutrición y control biológico. En el contexto de alimentos contaminados y pérdidas en la producción agrícola, han surgido graves problemas debido al ataque de patógenos. Por otra parte, es claro que el uso de microorganismos benéficos en la agricultura es considerado como alternativa para mejorar el crecimiento, la producción y la salud de la planta. En este sentido, bacterias promotoras de crecimiento vegetal y agentes de biocontrol, aislados de la filosfera de diferentes especies vegetales han sido menos explotados que los microorganismos de la rizosfera. No obstante, el tratamiento de enfermedades, reducción de la incidencia de patógenos y la fijación de nitrógeno, en sistemas naturales y agrícolas, son ejemplos exitosos. En el contexto de seguridad alimentaria, se espera entender mejor cómo la microbiota nativa de la filosfera ayuda a la planta a protegerse contra patógenos y a la adquisición de nutrientes, para demostrar su importancia en el área agrícola. Esto indica que, fuentes microbianas pueden ser usadas para reducir el uso de productos químicos y aplicarlas como una alternativa agronómica para mejorar la productividad de los agroecosistemas.

Palabras clave adicionales: comunidad epifítica; crecimiento y salud vegetal; productividad del ecosistema; biocontrol.

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INTRODUCTION

Microorganisms are found in various environments, such as water, air, soil, plant surfaces, animals, food, the human body and buildings (Prussin and Marr, 2015; Rosenberg and Zilber-Rosenberg, 2016). Some of them live in symbiosis with plants or animals, while others have a free-living lifestyle (Dutta and Paul, 2012). Symbiotic associations between plants and soil microorganisms such as arbuscular mycorrhizal fungi (Igiehon and Babalola, 2017), nitrogen-bacteria in legumes (Mus *et al.,* 2016) or the water fern *Azolla* and the cyanobacterium *Anabaena azollae* (Bhuvaneshwari and Singh, 2015) have been amply studied because of the positive outcomes of these associations in natural and agricultural systems. However, microorganisms associated with aerial plant surfaces could have positive effects, as do soil microorganisms, and could be used as sources of inoculum (Andreote *et al.,* 2014).

The phyllosphere is the aerial portion of plants, mainly the leaf surface, which is an environment widely inhabited by bacteria that form biofilms or larger aggregates (Lindow and Brandl, 2003; Baldoto and Olivares, 2008). Biofilm formations, extracellular polymeric substances (EPS) and enzyme production protect the epiphytic microbial community from a stressful environment (Remus-Emsermann and Vorholt, 2014; Müller *et al.,* 2016a). Despite the phyllosphere being low in nutrients, plants release an adequate concentration to support large microbial communities (Mercier and Lindow, 2000), and microbial communities develop mechanisms to acquire nutrients (Delmmote *et al.,* 2009; Bulgarelli *et al.,* 2013).

A core bacterial microbiome composed of Proteobacteria, Actinobacteria, Bacteroidetes and Firmicutes phyla has been found in different plant species, in both forests and agricultural ecosystems (Redford *et al.,* 2010; Vorholt, 2012; Rastogi *et al.,* 2012; Bulgarelli *et al.,* 2013; Kembel *et al.,* 2014; Lambais *et al.,* 2014; Laforest-Lapoint *et al.,* 2016, Müller *et al.,* 2016b). However, the bacterial abundance depends on several factors, such as plant species, geographical distance and environmental conditions (Remus-Emsermann and Vorholt, 2014; Copeland *et al.,* 2015; Laforest-Lapoint *et al.,* 2016). This suggests that some small bacterial groups are highly efficient at colonizing and surviving in the phyllosphere (Griffin and Carson, 2015). At the same time, bacterial communities share a core of proteins on different plant hosts, suggesting similar mechanisms for adaptation and survival on different plant species (Remus-Emsermann and Vorholt, 2014; Lambais *et al.,* 2017).

In the context of food safety, a better understanding of how the natural microbiota enables plants to protect themselves against pathogens and/or to acquire nutrients will be valuable in agricultural production (Rastogi *et al.,* 2013). This suggests that microbial sources can be managed as an alternative of agronomical applications to improve the productivity of the agricultural ecosystem (Peñuelas and Terradas, 2014). Likewise, agroecosystems are subject to intense chemical management; therefore, the microbial diversity associated with leaf surfaces can have variations across space, time, season and environmental conditions (Rastogi *et al.,* 2013).

Nowadays, we have to deal with difficult challenges, such as the concern for foodborne illnesses and agricultural production losses from pathogen attacks. In Colombia, a few studies have been done to identify microbial communities associated with the phyllosphere (Toloza and Lizarazo, 2014). These studies have focused mainly on the characterization of pathogenic microorganisms that inhabit the phyllosphere (Restrepo *et al.,* 2000; Marín *et al*., 2003), meanwhile other studies have focused on biological control (Salazar *et al.,* 2006; Medina *et al.,* 2009; Cruz-Martín *et al.,* 2016) and taxonomical profiles of bacterial communities (Ruíz-Pérez *et al.,* 2016). Although there is increasing evidence that beneficial bacteria may stimulate plant growth and health (Vogel *et al*., 2016), microbial community dynamics at the community level and their interactions with the plant host are still unknown (Schlechter *et al*., 2019). This mini-review provides an agricultural approach on the potential applications of microbial communities associated with the phyllosphere in horticulture crops through microbial bioprospecting. First, phyllospere generalities are stated, such as habitat for bacteria and fungi. Second, abiotic and biotic factors affecting the microbial community associated with the phyllosphere are also described. Subsequently, references are made about potential applications in agriculture, focusing on nutrition and biological control. Patent processes found in the Patentscope database from studies on the phyllosphere microbiome are shown. This information shows examples of bioprospecting bacteria with biotechnological potential. Finally, several examples of studies carried out in Colombia on horticultural plants are presented. The information was accessed with keywords such as: phyllosphere, microbiome, agriculture, bioprospecting, biotechnology, microbial communities associated with the phyllosphere, in Google Scholar and Scopus.

THE PHYLLOSPHERE IS A MICROBIAL HABITAT

The phyllosphere is the portion found in upper and lower leaf surfaces (Fig. 1), covering an area of $640 \cdot 10^8$ km^2 on Earth (Lindow and Brandl, 2003; Peñuelas and Terradas, 2014). As well as bacteria, this environment is colonized by other microorganisms such as archaea, filamentous fungi, lichens, bryophytes, yeast and protozoa, all living in limited water and nutrients conditions (Vorholt, 2012; Rastogi *et al*., 2013; Müller and Ruppel, 2014).

The leaf surface is an oligotrophic environment that obligates microorganisms to compete for nutrients and space (Delmotte *et al*., 2009; Bringel and Couée, 2015). Carter *et al*. (2012) suggested that competition for nutrients, essentially carbon and nitrogen, is the first mechanism of interaction among microorganisms that colonize a phyllosphere. Leaf nitrogen content is correlated with the phyllosphere community structure in several plants (Kembel *et al.,* 2014; Kembel and Mueller, 2014; Laforest-Lapoint *et al.,* 2016), while carbohydrates produced by photosynthesis are exudated on the leaf surface along with methanol, volatile organic compounds (Vacher *et al.,* 2016), amino acids, organic acids, inorganic compounds, and various salts (Mercier and Lindow, 2000). Leaf chemical composition and morphology affect the distribution of microorganisms across leaves (Remus-Emsermann and Vorhold*,* 2014). For instance, the leaf cuticle is a substrate composed of polymeric and soluble lipids that is difficult to metabolize by microorganisms, but could be *involved* in phyllosphere colonization (Morris, 2002). Chemical, structural and physiological properties related to a spectrum running from quick to slow return on investments of nutrients and dry mass in leaves, "The leaf economics spectrum" (Wright *et al.,* 2004) could explain the variation in microbial community structures, according to acquisitive or retentive resource strategy (Friesen *et al.,* 2011).

Undoubtedly, bacteria are the most abundant microbial group in the phyllosphere followed by fungi (Lindow and Brandl, 2003). The density of bacteria is from 10^6 to 10^7 cells per square centimeter of leaf tissue (Vorholt, 2012; Rastogi *et al.,* 2013). Despite bacteria playing essential roles in nutrient cycling (Vacher *et al.,* 2016), plant protection against pollutants and pesticides (Müller and Ruppel, 2014) and improving plant development (Delmotte *et al.,* 2009), little is known about bacterial diversity and

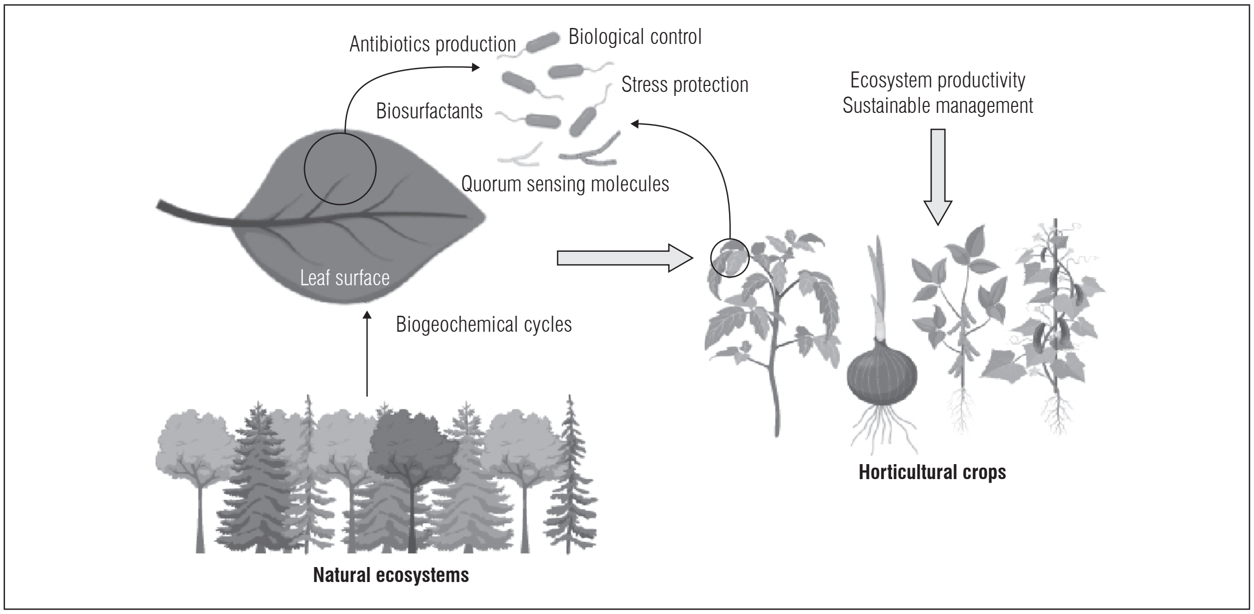

Figure 1. Scheme of the leaf surface colonized by several bacteria and fungi genera detected in the phyllosphere, along with ecological functions and applications.

biogeography in this environment since most of the bacteria detected in the phyllosphere have not yet been described (Lambais *et al.,* 2014). Filamentous fungi and yeast communities associated with the phyllosphere are also important components involved in nutrient cycling (Vacher *et al.,* 2016) and plant protection (Arnold *et al.,* 2003). Jumpponen and Jones (2009) suggested that plant exudates are decomposed by epiphytic fungi; however, the fungi colonization in phyllosphere has not been well characterized (Kembel and Mueller, 2014).

The assemblage of microbial communities on the phyllosphere of distinct plant species could be modulated by the interaction of several environmental factors (Andreote *et al.,* 2014). Similarly, interaction between microorganisms and their hosts drives the microbiota assembly (Müller *et al.,* 2016a). On the other hand, colonizing microorganisms of the phyllosphere not only can come from the air but also can come from an early recruitment on seeds, soil or other plants (Knief *et al.,* 2010; Copeland *et al.,* 2015; Lemanceau *et al.,* 2017).

The microbial community in the phyllosphere presents high richness (Kim *et al.,* 2012; Kembel *et al.,* 2014) but low diversity when compared with the rhizosphere (Delmotte *et al.,* 2009; Knief *et al.,* 2012). The classes Alphaproteobacteria and Gammaproteoacteria are the most abundant groups detected in several plant species (Fürnkranz *et al.*, 2008; Kim *et al.,* 2012; Lambais *et al.,* 2006, 2014, 2017). In tropical rainforests, *Sphingomonas* (Alphaproteobacteria) and *Pseudomonas* (Gammaproteobacteria) have been reported as the dominant bacterial genera (Lambais *et al.,* 2006, 2014; Bodenhausen *et al.,* 2013). In several crops such as rice, bean, cucumber, soybean, lettuce maize clover and *Arabidopsis*, *Sphingomonas, Methylobacterium* (Alphaproteobacteria) and *Pseudomonas* (Gammaproteobacteria) are the most abundant genera (Delmotte *et al.,* 2009; Knief *et al.,* 2012; Rastogi *et al.,* 2013; Müller *et al.,* 2016b). The dominance of *Sphingomonas* spp. and *Methylobacterium* spp. can be explained by the presence of carbon resources that they consume (Remus-Emsermann and Vorholt, 2014), while *Pseudomonas* spp. can be explained by its mobility towards nutrient sites on the leaf, with significant advantages over immobile bacteria (Vorholt, 2012). The phototrophic lifestyle, as part of their metabolic requirements, offers bacteria an ecological advantage to survive in the phyllosphere. For instance, the presence of anoxygenic phototrophic bacteria and rhodopsins in clover, *Arabidopsis* and Tamarisk phyllosphere (Atamna-Ismaeel *et al.,* 2012a, 2012b; Finkel *et al.,* 2016).

With respect to fungi phyllosphere community, it has been linked to pathogens, saprotrophs and lichenized fungi (Jumpponen and Jones, 2009). Filamentous fungi and yeast, such as Ascomycota and Basidiomycota, are reported as dominant groups in the phyllosphere (Cordier, 2012). For example, *Hypocrea, Aureobasidium* and *Cryptococcus* genera were reported as dominant in the tomato phyllosphere (Ottesen *et al.,* 2013).

FACTORS AFFECTING BACTERIAL COMMUNITY IN PHYLLOSPHERE

The phyllosphere is a hostile and complex environment influenced by abiotic and biotic factors, including plant metabolism, UV radiation (Vorholt 2012), temperature (Delmotte *et al.,* 2009), carbohydrate levels (Hunter *et al.,* 2010), elevated CO_2 (Ren *et al.,* 2014), plant traits, seasonal leaf changes, position (Copeland *et al.,* 2015; Laforest-Lapoint *et al.,* 2016) and the chemistry of waxy cuticle covering the leaf (Bodenhausen *et al.,* 2014; Remus-Emsermann and Vorholt, 2014). However, how these factors affect the microbial communities in the phyllosphere is not fully understood.

Temperature is greatly variable on leaf surface over time and space (Chelle, 2005), even in the same plant or leaf (Vacher *et al.,* 2016). Changes in phyllosphere communities are related to rainfall events (Copeland *et al.,* 2015). Water films cover leaf surfaces, causing chemical reactions between water and compounds or molecules deposited on the leaves (Vacher *et al.,* 2016), which modify the water pH and affect the nutrient availability for microorganisms (Morris, 2002).

Leaves are exposed to large amounts of sunlight, causing DNA-damage (Remus-Emsermann and Vorholt, 2014); therefore, adaptation to and protection from stressful conditions from UV radiation are related to the detection of DNA protection proteins (Delmotte *et al.,* 2009). Furthermore, microbial communities in the phyllosphere can be highly variable depending on the season (Jackson and Denney, 2011; Williams *et al.,* 2013), while other plants have similar bacterial communities all year round (Redford and Fierer, 2009). Changes in plant metabolism caused by abiotic factors indirect affect the phyllosphere microbiome as well (Turner *et al.,* 2013).

Plant genotype may also drive the assembling of bacterial communities in the phyllosphere (Redford *et al*., 2010; Rastogi *et al*., 2013). Possible mechanisms used by plants to assemble their microbial community have not yet been elucidated, but interaction plant genotype-microbial community (Kim *et al.,* 2012) and identifications of essential genes in the plant and the microorganisms are important factors to manipulate the leaf microbiota and to improve plant protection (Müller and Ruppel, 2014). The selection of different bacterial consortia by plants (Lambais *et al.,* 2006) occurs by compounds exudated on the leaf surfaces (Yadav *et al*., 2011).

For instance, Beattie and Lindow (1999) reported indol-acetic acid and extracellular polysaccharides as compounds produced by phyllosphere bacteria to modify the environment, improving nutrient availability and increasing bacterial community survival on the leaf surface.

Changes in the composition of bacterial communities associated with the phyllosphere might occur because of geographical distance (Bokulich *et al.,* 2014). Redford *et al*. (2010) reported on bacterial communities that were highly similar in plants phylogenetically, suggesting evolutive history between plants and bacteria. However, different plant species from a single location can assemble bacterial communities that are highly similar, as influenced by local conditions (Whipps *et al.,* 2008; Finkel *et al.,* 2011). Furthermore, differences have also been observed in bacterial communities associated with individuals of the same plant species but geographically distant, which indicates that differences could arise from distinct climatic conditions or different leaf traits (Redford and Fierer, 2009; Finkel *et al.,* 2012). The composition and abundance of the microbial community associated with leaf surfaces are not enough to understand the driving factors affecting the phyllosphere microbiome because of leaf surface heterogeneity (Remus-Emsermann and Schlechter, 2018). Therefore, spatial scale analysis could be used to explain how ecological microbial interactions could contribute to the identification of key organisms associated with plant health and function (Berry and Widder, 2014; Poudel *et al.,* 2016).

POTENTIAL AGRICULTURAL APPLICATIONS

Communication (volatile organic compounds), protection (antibiosis), nutrient cycling (N, C), and plant growth (phytohormones) are essential ecosystem services provided by the phyllosphere microbial communities (Tab. 1) (Morris, 2002; Vacher *et al.,* 2016). Manipulating the foliar microbiome to reduce the use of synthetic pesticides and inorganic fertilizers is a beneficial activity that promotes plant growth and health (Adesemoye and Kloepper, 2009).

Table 1. Ecosystem functions of microorganisms in the phyllosphere.

Microbial function	Plant benefits	Ecosystem service	Source
Nitrogen fixation	Foliar nitrogen content Plant growth	Productivity, nutrient acquisition	Fürnkranz *et al.* (2008)
Phytohormones production	Plant growth	Biomass production	Almethyeb *et al*. (2013)
Pathogen suppression	Protection, plant health	Productivity	Wei *et al.* (2016)
Antimicrobial activity	Protection, plant health	Productivity	Bulgarelli *et al.* (2013)
Induced systemic resistance	Protection, plant health	Productivity	Bulgarelli *et al.* (2013)
Phylloremediation	Detoxification	Atmospheric depollution	Bringel and Couée (2015)
Probiotic agents	Reduction chemical products	Sustainable agriculture	Berlec (2012)

Possible nutritional inputs through the phyllosphere

Microbial communities mediate the nutrient exchanges occurring between the phyllosphere and the atmospheric interface (Abril *et al.,* 2005). In tropical forests and agriculture or silviculture systems, foliar diazotrophs contribute to nitrogen fixation from the atmosphere (Abril *et al.,* 2005; Fürnkranz *et al.,* 2008). Proteogenomic analyses of several phyllosphere microbial communities have revealed species that assimilate simple carbohydrates, amino acids and ammonium exudated by plants (Turner *et al.,* 2013). On the other hand, ammonifiers and cellulose-degrading bacteria has been reported in the phyllosphere of woody plants (Abril *et al.,* 2005), and nitrifiers were found in soybean leaves (Arias *et al.,* 1999). A complex metabolic feedback between plants and phyllosphere communities may be occurring (Bringel and Couée, 2015) when the enzymatic activity of the microorganisms in the phyllosphere act as plant metabolites (Huang *et al.,* 2014).

Nitrogen fixation is one of the most studied functions of the foliar microbiota (Abril *et al.,* 2005; Daza *et al*., 2015). *Klebsiella* spp. and *Beijerinckia* spp. are common free-living nitrogen-fixing bacteria found in phyllosphere microbial communities (Morris, 2002). Furthermore, *Beijerinckia* spp. strains have increased rice yield, when compared between a *Beijerinckia* spp.-inoculated field and one with conventional fertilizers (Morris, 2002). Similarly, *Enterobacter radicincitans* has been isolated from the phyllosphere of wheat (Ruppel *et al.,* 2006) and promoted plant growth through nitrogen fixation and phytohormone production when inoculated in the soil (Almethyeb *et al*., 2013). On the other hand, *Klebsiella* spp. and various cyanobacteria were found in the phyllosphere of plants from a tropical forest in Costa Rica, related to high N_2 fixation rates (Fürnkranz *et al.,* 2008).

With respect to Actinobacteria class, *Arthrobacter* has been found to inhabit leaf surfaces (Rastogi *et al.,* 2012). This bacteria genus can degrade aromatic hydrocarbons and pesticides (Scheublin and Leveau, 2013) and presents a high resistance to desiccation (Labeda *et al.,* 1976). Thus, it could be a good choice for decreasing contamination by pesticides application (Turnbull *et al.,* 2001).

Biological control

The use of biological control agents isolated from phyllosphere has been less exploited than those isolated from the soil or roots. However, there are biocontrol agents that have been successful in the treatment of some diseases associated with the phyllosphere (Fernando *et al.,* 2007).

Interactions between phyllosphere bacteria could trigger changes in leaf transcriptome, suggesting molecular recognition by plants (Lemanceau *et al.,* 2017). In this respect, the presence of the pathogen *Xanthomonas campestri* pv. *vitians* in lettuce is positively correlated to the presence of *Alkanindiges,* also reported in lettuce phyllosphere (Hunter *et al.,* 2010), and negatively correlated with *Bacillus, Erwinia* and *Pantoea,* which act as antagonists (Rastogi *et al.,* 2012). *Pseudomonas syringae* is an important pathogen found in the phyllosphere and can be controlled with *Sphingomonas melonis* because of the expression of pathogenesis-related proteins and antimicrobial proteins (Innerebner *et al.,* 2011). Meanwhile, *Pseudomonas fluorescens* A506 reduces fire blight disease in pear and suppresses *Erwinia amylovora* growth in the phyllosphere through competition for nutrients and space (Wilson and Lindow, 1993).

Furthermore, antagonistic activity from bacteria can reduce fungal pathogenicity in the phyllosphere (Griffin and Carson, 2015). For example, *Pseudomonas* spp. and *Bacillus* spp. can produce compounds, inducing systemic resistance responses in several plant species (Vorholt, 2012) and dramatically reducing fungal infection (Ceballos *et al.,* 2012). *Bacillus* spp. are the most used biological control agents in agriculture. They have a broad spectrum of antagonistic activity (Huang *et al.,* 2012), and several strains have been used as biocontrol agents in cacao (Melnick *et al.,* 2008; Villamil *et al.,* 2015), sugar beet (Collins *et al.,* 2003), citrus (Huang *et al.,* 2012), strawberry (Wei *et al.,* 2016), cotton, rice and amaranth leaves (Wang *et al.,* 2014). The ability to form endospore, produce secondary metabolites, proteins (Zhang *et al.,* 2008), and antibiotics (Raaijmakers *et al.,* 2002) and induce systemic resistance (Lahlali *et al.,* 2013) has made *Bacillius* a widely used biocontrol agent for phyllosphere pathogens (Wei *et al.,* 2016).

Recently, the use of native microorganisms as biocontrol agents has attracted special interest because of their special attributes (Kumar and Gopal, 2015; Cruz-Martín *et al*., 2016). They are adapted and established to local abiotic conditions or hosts and play a protecting role in the host plant against foreign pathogen microorganisms (Kumar and Gopal, 2015). For instance, representatives of the Bacillaceae family isolated from *Mussa* spp. phyllosphere showed antifungical activity against black Sigatoka disease (*Mycosphaerella fijiensis*) (Poveda *et al.,* 2010; Cruz-Martín *et al.,* 2016). Also, in *Mussa* spp., Salazar *et al*. (2006) found chitinolytic and glucanolytic activity against *M. fijiensis* by native bacteria.

On the other hand, changes in nutrient allocation towards leaf surfaces can manipulate the phyllosphere microbial community as a protection mechanism against pathogens (Manching *et al.,* 2014). In crops, specific bacterial communities colonizing the phyllosphere have an important role protecting plants against pathogens (Williams *et al.,* 2013; Manching *et al.,* 2014). For instance, rice (Ren *et al.,* 2014) and lettuce (Williams *et al.,* 2013) seem to benefit from the Enterobacteriaceae group, increasing biomass production through pathogen suppression (Pusey *et al.,* 2011) and nitrogen fixation (Feng *et al.,* 2003).

Several studies on the phyllosphere microbiome have revealed a large number of novelties, which could be important for maintaining agriculture sustainability (Gupta and Bhargava, 2018), and more institutions are using their resources for the search for new products based on microorganisms. Patent processes found in the Patentscope database attached to WIPO-World Intellectual Property confirm that products based on microorganisms isolated from the phyllosphere can be used in agriculture.

a) Use of lactic acid bacteria associated with the phyllosphere as biocontrol agents to reduce the growth of pathogenic bacteria (McGarvey *et al.,* 2017),
b) Microbial consortium for agricultural use and formulation (Suárez, *et al.,* 2019),
c) *Enterobacter* sp. 3bh19 for preventing downy mildew. Application in cucumber phyllosphere (Luo *et al.,* 2017),
d) Bacteria isolated from the phyllosphere promoting plant growth (Bai *et al.,* 2014),
e) Novel *Methylobacterium* sp. CBMB 27 having an effect of promoting plant growth (Sa *et al.,* 2012),
f) Bacteria degrading pyrethroid insecticide and method for preparing fungicide (Bai *et al.,* 2008b),
h) Screening method for thermophilic bacteria isolated from the phyllosphere (Bai *et al.,* 2008a).

STUDIES OF PHYLLOSPHERE COMMUNITIES IN COLOMBIA

Pathogenic microorganisms inhabit the phyllosphere of two important crops in Colombia: *Xanthomonas axonopodis* pv. *Manihotis* and *M. fijiensis,* are causal agents of bacterial blight in cassava (*Manihot esculenta*) (Restrepo *et al.,* 2000) and black sigatoka in banana (*Musa* spp.) (Marín *et al*., 2003), respectively.

On the other hand, several examples of biological controls are known in Colombia. In Cundinamarca, *Candida kunwinensis* and *Rhodotorula colostri* were isolated from blackberry crops (*Rubus glaucus*), which presented antagonist activity against *Botritys cinerea* (Medina *et al.,* 2009). In another study in Uraba, bacterial strains with antagonist activity against *M. fijiensis* were isolated from leaves of *Mussa* spp. They were identified as *Bacillus subtibilis* and *B. amyloliquefaciens* and decreased *M. fijiensis* infection by more than 90% (Ceballos *et al.,* 2012).

Taxonomical profiles of the phyllosphere in crops such as the cape gooseberry (*Physalis peruviana*) have been identified. Leaf surfaces of cape gooseberry crops in Boyacá showed a high abundance of bacteria *Pseudomonas* spp. and *Enterobacter* spp., followed the yeast *Rhodotorula* and fungi *Capnodium, Cladosporium* and *Penicillium* (Toloza and Lizarazo, 2014). Furthermore, in natural ecosystems such as Andean high-mountain, the phyllosphere microbial community associated with *Espeletia* plants in the Los Nevados Natural National Park was accessed to compare important microbial contributions to geo-biological processes, as well as the potential in terms of bioprospecting for microbial processes such as bio-remediation, nutrient acquisition, and antimicrobial compound production (Ruíz-Pérez *et al.,* 2016).

CONCLUSIONS AND FUTURE PERSPECTIVES

Most of the studies on phyllosphere have focused on plant protection or antagonistic activity. A few have characterized nitrogen-fixing communities or plant growth-promoting bacteria in natural or agricultural systems. The phyllosphere is an environment that can harbor microorganisms linked to ecosystem functions, especially those involved with carbon and nitrogen cycles that are closely related to plant nutrition. Research on foliar nutrition by microorganisms could expand our understanding of microbial mechanisms as a strategy to be applied in agriculture and used in the inoculation of soil microorganisms.

Characterizing the taxonomic and functional profiles of the microbial community, in addition to evaluating ecological interactions, identifies core groups and functions that can occur in different plant species in natural environments. In turn, the information obtained from studies on natural environments allows these profiles to be explored in plant species of horticultural interest in order to increase plant productivity.

Biotechnology offers great potential for applications of beneficial microorganisms in agriculture in order to increase plant productivity. Furthermore, it will help in understanding how the microbial community in the phyllosphere affects plant growth and health in agroecosystems. Bioprospecting the use of microorganisms associated with plants, in this case, the microbial community associated with the phyllosphere, is important as an alternative for fertilization and protection in horticultural crops (Fig. 2).

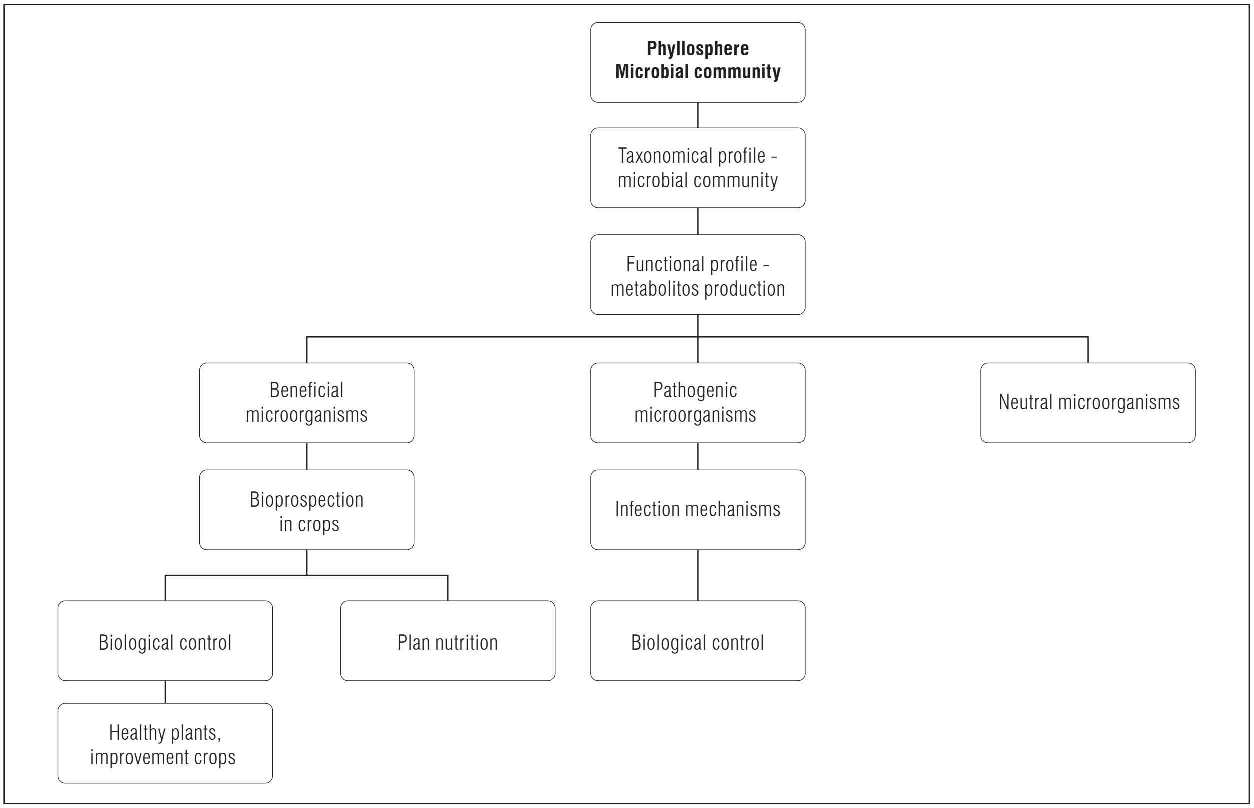

Figure 2. Fluxogram showing the more important characteristics in phyllosphere studies.

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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Plant density and growth regulator applications in a tomato crop for industrial processing

Densidad de plantas y aplicación del regulador de crecimiento al cultivo de tomate para procesamiento industrial

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Tomato cultivation for industrial processing.

Photo: N.C. Ferreira

ABSTRACT

Growth regulators are used in agriculture to reduce vegetative growth, promote flowering and fruiting processes, and regulate production alternations. In this study, the effects of the use of paclobutrazol (PBZ) and different plant arrangements on the development and productive yield tomatoes grown for industrial use were evaluated. This experiment was carried out in the experimental area of the Universidade Estadual de Goiás, in a randomized block design with a 2×5 factorial arrangement (presence of PBZ and absence of PBZ in seedlings × planting spacing), with four replications and ten plants per plot. The paclobutrazol (growth regulator) applications were carried out in seedlings 15 days after emergence by applying 3.5 mL of the solution per tray at a concentration of 42.5 mg L^{-1} using a commercial product containing 25% of the active ingredient for the solution. The spacings were 0.15, 0.20, 0.25, 0.30, and 0.35 m between plants and 1.4 m between rows, corresponding to populations of 47,619; 35,714; 28,714; 23,809 and 20,408 plants/ha, respectively. The initial growth and development of the seedlings, characteristics related to productivity, and the physical and chemical quality of fruits harvested in each experiment were evaluated. It was observed that the application of paclobutrazol influenced the phylotechnical characteristics and technological quality of tomato fruits grown for industrial processing. The planting spacings between 0.21 and 0.27 m had better crop development and productivity.

Additional keywords: *Solanum lycopersicum* L.; plant management; paclobutrazol; spacing.

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RESUMEN

Los reguladores del crecimiento se utilizan en la agricultura para reducir el crecimiento vegetativo, promover los procesos de floración y fructificación, además de regular la alternancia de producción. En este estudio, los efectos del uso de paclobutrazol (PBZ) y diferentes arreglos de plantas en el desarrollo y el rendimiento productivo de los tomates para la industria. El experimento se realizó en el área experimental de la Universidade Estadual de Goiás, en un diseño en bloques aleatorizados, en un arreglo factorial de 2×5 (presencia de PBZ y ausencia de PBZ en plántulas × distancia de siembra), con cuatro repeticiones y diez plantas por parcela. La aplicación de paclobutrazol (regulador del crecimiento) se realizó en plántulas 15 días después de la emergencia aplicando 3,5 mL de la solución por bandeja a la concentración de 42,5 mg L^{-1} utilizando el producto comercial que contiene el 25% del ingrediente activo para la solución. Los espacios disponibles fueron de 0,15; 0,20; 0,25; 0,30 y 0,35 m entre plantas y 1,4 m entre hileras, correspondientes a poblaciones de 47.619, 35.714, 28.714, 23.809 y 20.408 plantas/ha en cada espacio. Se evaluó el crecimiento inicial y el desarrollo de las plántulas, las características relacionadas con la productividad y la calidad física y química de los frutos cosechados en cada experimento. Se observó que la aplicación de paclobutrazol influyó en las características filotécnicas y la calidad tecnológica de los frutos de tomate para el procesamiento industrial. En cuanto a la densidad de siembra, el espacio de siembra entre 0,21 a 0,27 m mostró un mejor desarrollo del cultivo y productividad.

Palabras clave adicionales: *Solanum lycopersicum* L.; manejo de plantas; paclobutrazol; espaciamiento.

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INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is an important source of vitamins, fiber and minerals (Naika *et al.,* 2006). The fruits are a significant source of nutrients rich in phytochemicals, which are naturally produced by plants to protect them from viruses, bacteria and fungi. When ingested, they may have anticancer properties. Globally, the tomato crop is notable because of its economic, social importance, and versatility. It is consumed raw or processed as juice, sauce, pasta, and dehydrated (Fontes and Silva, 2005).

In Brazil, tomato crops occupy 65,200 ha. Production in Goiás totaled 1,290,134 t in 2019, with an area of 13,700 cultivated hectares, corresponding to 21% of the country's cultivated area (IBGE, 2020). The State of Goiás is the largest producer of industrial tomatoes in Brazil.

Industrial use requires special fruits produced in low-altitude crops without complex cultural treatments, which reduces production costs. The fruits must have a high resistance to bulk transport, a uniform, intense red color, high soluble solids content, and high citric acid content. Tomato processing industries depend on the quality of the raw material to obtain final product quality (Schwarz *et al*., 2013).

Proper crop management is necessary for productivity gains and fruit quality with low production costs. Spacing is one of the main techniques in tomato cultivation. The effects of spacing can be observed on light exposure, increasing photosynthetic efficiency. Dense plantings provide a greater overlap and shading of leaves, and consequently a greater competition for light. This causes more energy expenditure (Mueller and Wamser, 2009). Spacing interferes with the crop cycle, making it difficult to control weeds and diseases and reducing fruit quality (Carvalho and Tessarioli Neto, 2005).

Plants produce plant hormones in certain parts and transport them to other parts where specific physiological responses are produced. Some hormones act in the same part where they are synthesized. Hormones are chemical messengers that are able to indicate the developmental status of cells, tissues, or organs. They are produced in various plant parts and are active at very small quantities (Taiz and Zeiger, 2013; Evert *et al*., 2017). The classes of plant hormones that receive the most attention are abscisic acid, auxins, cytokinins, ethylene, and gibberellins. The nomenclature "growth regulators" includes the natural or synthetic form of hormonal substances or substances that interfere with biosynthesis or hormonal action, which, when applied to plants, influence growth and development (Evert *et al*., 2017).

Growth regulators are used in agriculture to reduce vegetative growth, promote flowering and fruiting, and regulate the alternation of production (Silva and Fay, 2003). However, the degree of response of regulators varies according to species, cultivar, method of application, and concentration. These compounds include paclobutrazol, which generally provides increased productivity. However, some adverse effects, such as flowering inhibition and reduction of fruiting, have also been reported in the literature. Moreover, such effects vary according to factors such as mode, dose and timing of application, cultivar, climatic conditions, and crop culture (Silva and Faria Junior, 2011).

The use of growth regulators may be an option to obtain more compact plants, which allow for spatial arrangements that can improve yields and reduce the number of disbudding operations. Silva and Faria Junior (2011), working with growth regulators in a tomato crop, observed a decrease in the emergence of lateral buds, as well as a reduction in plant height.

The system of plant conduction may also interfere with results. Plant density, as well as apical pruning height in undetermined growth cultivars, significantly affects the yield and fruit quality of tomato crops. This study aimed to evaluate the effects of using a growth regulator and different spacings on tomato crops grown for industrial processing.

MATERIAL AND METHODS

Characterization of the experimental area

This project was developed at the experimental area of the State University of Goiás, campus Ipameri, located in the municipality of Ipameri, GO. The geographic coordinates are 17°43'04'' S and 48°08'43'' W, and the altitude is 794 m.

The climate of the region, according to the Köppen-Geiger classification (Cardoso *et al*., 2014), is tropical climate (Aw), consisting of a dry season in winter. The soil of the experimental area is classified as dystrophic Red-Yellow Latosol (Santos *et al*., 2013). The chemical attributes and particle size analysis were determined before the installation of the experiment according to the methodology proposed by Ribeiro *et al.* (1999). The chemical attributes at the 0.0-0.20 m layer were 14 mg dm^{-3} of P (resin), 26 g m^{-3} of organic matter, pH 5.4 ($CaCl_2$), K, Ca, Mg, H+Al = 3.2, 19.0, 13.0, and 29.0 $mmol_c$ dm^{-3}, respectively, and 55% base saturation. The physical attributes were clay: 390 g, silt: 97 g, and sand: 513 g.

Characterization of the experiment

This experiment used completely randomized blocks in a 2×5 factorial arrangement (presence of PBZ and absence of PBZ in seedlings x planting spacing), with four replications and ten plants per plot. The hybrid was N901, with a cycle between 115 and 120 d, used for industrial processing purposes. The color is intense red. It has high soluble solids contents, average mass of 80 g, and resistance to various pests and diseases.

The evaluated spacings were 0.15, 0.20, 0.25, 0.30, and 0.35 m between plants and 1.4 m between rows, corresponding to populations of 47,619; 35,714; 28,714; 23,809 and 20,408 plants/ha, respectively. The plots consisted of four rows, 5 m long each. The two central lines were considered as the useful area, excluding 0.50 m from the ends of each line.

The production of seedlings was carried out in a commercial nursery with infrastructure, support, and specialized technicians. Polypropylene trays containing 450 cells and commercial organic-mineral substrate were used. The crop management and treatments were performed as recommended for the crop. The seedlings were transplanted 35 d after sowing.

The application of paclobutrazol (growth regulator) was carried out in seedlings 15 d after emergence by applying 3.5 mL of the solution per tray at a concentration of 42.5 mg L^{-1} using a commercial product containing 25% of the active ingredient for the solution.

Phytotechnical evaluations

The following were evaluated: *Chlorophyll*: indirect reading of chlorophyll content of sunflower leaves using the SPAD index obtained with a portable chlorophyll meter (clorofiLOG, modelo CFL 1030, Falker, Brazil); *Final height*: average of five plants per plot at harvest time using measuring tape; *Final diameter*: average of five plants per plot at the time of harvest using a caliper; *Plant height growth rate*: mean growth rates for plant height were obtained following Benincasa (1988); *Stem diameter growth rate*: mean growth rates for stem diameter were obtained following Benincasa (1988); *Number of flowers*: number of flowers of all useful plants of the plot in each treatment; *Height of the first inflorescence*: the height of the first inflorescence was measured using a graduated ruler; *Fruit length*: average length of fruits of five plants/plot was measured using a graduated ruler; *Fruit diameter*: average diameter of fruits of five plants/plot measured with a caliper; and *Productivity*: average productivity, in kg m^{-2} per plot, for each treatment, weighed with the aid of digital scale.

Technological evaluation of fruits

The technological quality evaluations were carried out according to the Analytical Standards of the Adolfo Lutz Institute (1985). The following characteristics were evaluated:

Total titratable acidity (*TTA*): determined with titration using a NaOH solution (0.05N) in 10 mL of pure juice obtained after liquefying at least three fully mature fruits; and *Total soluble solids (TSS):* determined by transferring a drop of the fruit juice to the prism of an refractometer (Insthutherm, model RTA-50) followed by a reading. The reading was corrected by the temperature conversion table and expressed in ºBrix (Chacón-Padilla and Monge-Pérez, 2017). *TSS/TTA ratio (Maturation index - MI)* was obtained with the ratio between soluble solids content and titratable total acidity; *pH:* was determined, directly in the ground pulp, using a digital benchtop pHmeter (Akso, model MP511); and *Fruit texture:* measured on the sides of the fruit using a penetrometer (Insthutherm, model PTR-100).

Statistical analysis

The data were subjected to analysis of variance (F test), and the means of qualitative (presence of PBZ and absence of PBZ) factor were compared with a Tukey test at 5% probability. For the quantitative factor (planting spacing), the means were submitted to polynomial regression fitting. Statistical analyses were performed using Statistical Analysis - SANEST (Zonta *et al.,* 1987).

RESULTS AND DISCUSSION

The variables chlorophyll, final height, final diameter, height of the first inflorescence, number of flowers, growth rate, and stem growth rate had differences in the treatments (Tab. 1). The characteristics were statistically significant for one or more sources of variation, with a significant interaction for the absence or presence of growth regulator and planting spacing (Tab. 1).

The chlorophyll content of the tomato plants was affected significantly by the application of the growth regulator. The plants subjected to paclobutrazol had a higher chlorophyll content than the plants that did not receive the growth regulator (Tab. 1). These results corroborate those found by Ferreira *et al.* (2017), who also found higher contents of chlorophyll in plants subjected to applications of paclobutrazol 15 d after transplanting. Paclobutrazol increases photosynthetic rates and, consequently, the chlorophyll content of leaves (Berova and Zlatev, 2000). There was no statistical difference between the different spacings, and there was also no significant interaction between the plant spacings and use of growth regulator (Tab. 1).

For final height and final diameter, there was no significant effect from the use of the growth regulator and planting spacings. The means did not differ statistically. It was observed that there was no significant interaction between the use of the growth regulator and planting spacings. The values for final height and final diameter were between 96.42 and 102.06 cm and 16.06 and 16.30 mm, respectively (Tab. 1). These results differ from those of Muller and Wamser (2009), who observed a linear decrease in plant height with increases in spacing between plants. Silva *et al.* (2008) reported that the use of paclobutrazol at a concentration of 150 ppm reduced the size of the plants when analyzing the concentrations of 0; 50; 100 and 150 ppm in a tomato crop. Ferreira *et al.* (2017) verified an increase in stem diameter using paclobutrazol at a concentration of 42.5 mg L^{-1} at 15 and 30 d after transplantation. Stems with a larger diameter favor the maintenance and sustentation of the plant and production during the crop cycle because of factors such as winds and rains.

For the analysis of the height of insertion of the first inflorescence, there was a significant effect from the use of growth regulator. The means differed statistically. The plants subjected to paclobutrazol had a higher insertion height of the first inflorescence (36.89

Table 1. Chlorophyll (CHLOR), final height (FHE), final diameter (FD), height of the first inflorescence (HFI), number of flowers (NF), plant height growth rate (PHG), and stem diameter growth rate (SDGR) of tomato plants in the presence or absence of growth regulator and different plant spacings. Ipameri-GO.

PBZ	CHLOR	FHE	FD	HFI	NF	PHG	SDGR
	(Spad)	cm				cm d^{-1}	
Presence	43.97 a	99.52 a	16.10 a	38.89 a	95.17 a	1.37 a	0.203 b
Absence	42.33 b	96.73 a	16.22 a	35.19 b	91.11 b	1.31 a	0.211 a
F value	7.06*	2.44^{NS}	0.577^{NS}	10.18*	6.08*	3.05^{NS}	9.44*
Spacings (m)							
0.15	42.75	102.06	16.30	-	-	1.40	0.210
0.20	43.61	97.56	16.07	-	-	1.32	0.208
0.25	42.33	97.22	16.06	-	-	1.31	0.208
0.30	44.72	97.36	16.12	-	-	1.33	0.205
0.35	42.35	96.42	16.25	-	-	1.33	0.205
F value	2.18^{NS}	1.25^{NS}	0.337^{NS}	15.25*	7.85*	0.92^{NS}	0.64^{NS}
Regression	-	-	-	(2)	(2)	-	-
Interaction	-	-	-	(1)	(1)	-	-
CV (%)	4.52	5.75	3.21	9.88	5.58	7.41	3.96

Means with different lowercase letters in a column for each studied factor indicate a significant statistical differences according to the Tukey test ($P \leq 0.05$). ^{NS} = not significant; * = significant at 5% probability. (1) Significant interaction for presence or absence of growth regulator and planting spacing and (2) Significant regression for effects of spacing.

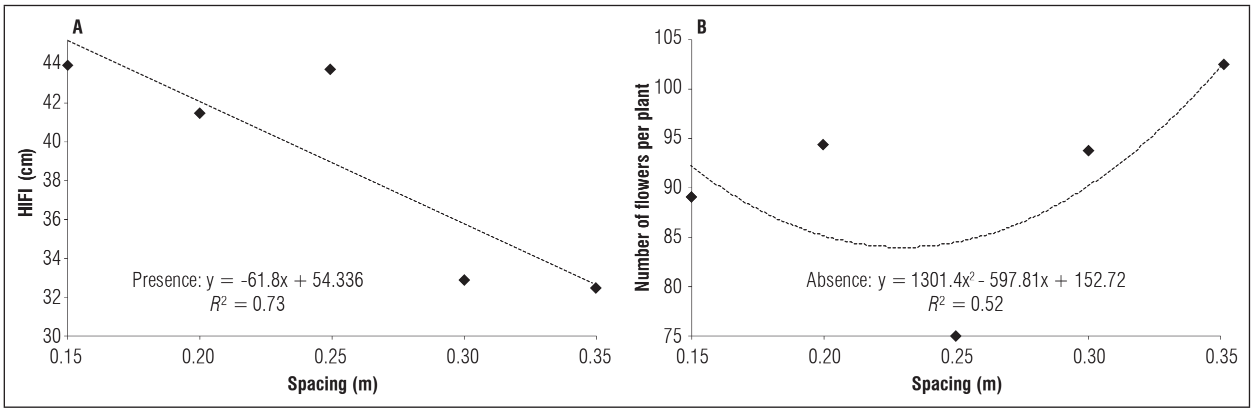

Figure 1. (A) Height of insertion of the first inflorescence (HIFI), and (B) number of flowers per plant of tomato plants in the presence or absence of growth regulator and planting spacing. Ipameri-GO.

cm) than the plants that did not receive the growth regulator (35.19 cm) (Tab. 1). Seleguini *et al.* (2016) reported that there was a decrease in the height of the first inflorescence when using paclobutrazol at 50 and 100 mg L^{-1}. When evaluating planting spacing and the absence or presence of paclobutrazol, there was a significant interaction only for the spacing in the presence of paclobutrazol. The values fitted a decreasing linear regression (Fig. 1A); as the plant spacing increased, the height of the first inflorescence decreased.

The number of flowers had statistically significant results for the use of growth regulator. The presence of paclobutrazol resulted in a greater number of flowers than the absence of paclobutrazol (Tab. 1). Thus, there was a significant interaction between planting spacing and growth regulator only in the absence of the growth regulator. The data fitted a quadratic regression with a minimum point of 0.22 m spacing (84.18 flowers) (Fig. 1B).

The plant height growth rate had no significant effect between the use of growth regulator and planting spacing. These results differ from those found by Mueller and Wamser (2009), who verified a highly significant linear fit for plant height as a function of spacing between tomato plants. For the analysis of the use of growth regulator, only the stem diameter growth rate had significant results, indicating that the presence of paclobutrazol resulted in a lower stem growth rate (0.203 cm) when compared to values found in the absence thereof (0.211 cm) (Tab. 1). Figueiredo *et al.* (2015) observed different results when studying the behavior of undetermined tomato plants in the presence of a growth regulator. The authors reported significant effects of the regulator on stem diameter.

Tab. 2 shows the production components of the tomato crop as a function of the presence or absence of paclobutrazol and planting spacing. There was a significant interaction only for maturation index, total fruit acidity, and productivity (Tab. 2).

The variables fruit length and diameter did not present a statistical difference for the presence or absence of the growth regulator. These values differ from those found by Silva and Faria Junior (2011) who observed that an increase in the concentration of a growth regulator determined a linear increase in the percentage of small fruits. Seleguini *et al.* (2016) also noted that, regardless of the method of application of paclobutrazol, an increase in concentrations increased the production of small fruits. When evaluating planting spacings, there were significantly different results only between the different spacings. There was no significant interaction between plant spacing and use of growth regulator (Tab. 2). The different planting spacings in tomato crops are of great importance since they can interfere with the plant cycle, disease control, and quality and quantity of harvested fruits (Mueller and Wamser, 2009).

The maturation index presented significant results for the use of growth regulator. In the absence of paclobutrazol, there was a higher maturation index (7.71) than in the presence of the growth regulator (7.08), with statistically different means (Tab. 2). These values differ from the results found by Seleguini *et al.* (2011). In their study, the TSS/TTA ratio was not influenced by PBZ concentrations. By evaluating planting spacing, there was a significant interaction between spacing and use of growth regulator. In the presence of paclobutrazol, the data fitted a quadratic regression with a minimum point of 0.22 m spacing

Table 2. Fruit length (FL), fruit diameter (FD) maturation index (SST/TTA), fruit texture (FT), fruit pH (pH), total soluble solids (TSS), total fruit acidity (TTA), and productivity (PROD) of tomato plants in the presence or absence of growth regulator and planting spacings. Ipameri, GO.

PBZ	FL	FD	TSS/TTA	FT	pH	TSS	TTA	PROD
	cm			lb pol^{-2}		º Brix	%	t ha^{-1}
Presence	5.54 a	4.50 a	7.08 b	10.84 a	4.50 a	4.42 b	0.64 a	92 a
Absence	5.41 a	4.39 a	7.71 a	8.59 b	4.45 b	4.60 a	0.60 b	67 b
F value	2.05^{NS}	2.33^{NS}	11.72*	18.72*	4.68*	8.69*	4.69*	84.82*
Spacings (m)								
0.15	5.57	4.47	-	9.42	4.47	4.48	-	-
0.20	5.72	4.57	-	10.27	4.40	4.63	-	-
0.25	5.10	4.22	-	9.28	4.46	4.38	-	-
0.30	5.47	4.53	-	9.77	4.57	4.56	-	-
0.35	5.51	4.43	-	9.81	4.47	4.48	-	-
F value	4.84*	2.88*	9.32*	3.62*	7.28*	1.77^{NS}	8.18*	10.17*
Regression	-	-	(2)	-	-	-	(2)	(2)
Interaction	-	-	(1)	-	-	-	(1)	(1)
CV (%)	5.43	5.11	7.92	5.41	1.46	4.39	9.41	10.69

Means with different lowercase letters in a column for each studied factor indicate a significant statistical differences according to the Tukey test ($P \leq 0.05$). ^{NS} = not significant; * = significant at 5% probability. (1) Significant interaction for presence or absence of growth regulator and planting spacing, and (2) = Significant regression for effects of spacing.

(6.46) (Fig. 2A). In the absence of paclobutrazol, the data also fitted a quadratic regression with a minimum point of 0.26 m spacing (6.51) (Fig. 2B). These values differ from the results found by Ferreira *et al.* (2017), who did not report influence from different planting spacings on the variable maturation index.

The variables fruit texture and fruit pH presented significant results for the use of the growth regulator; the best results were found with the presence of paclobutrazol (10.84 and 4.50) for fruit texture and pH (respectively) (Tab. 2). However, for planting spacing, there was no significant interaction between planting spacing and the presence or absence of PBZ in the seedlings (Tab. 2). The pH value is very important when the fruit is destined for processing, with a pH below 4.5 being desirable for preventing the proliferation of microorganisms (Monteiro *et al*. 2008); results within this standard were observed in this study (Tab. 2).

The fruit TSS had significant results only for the use of the growth regulator (Tab. 2). In the absence of paclobutrazol, the fruits had a higher TSS (4.60), differing statistically from the values found in the presence of paclobutrazol (4.42). There was a significant interaction between planting spacing and use of the growth regulator (Tab. 2).

The total fruit acidity also presented significant results for the use of the growth regulator. The total fruit acidity was higher in the presence of paclobutrazol (0.64), differing statistically from the results found in the absence of paclobutrazol (0.60) (Tab. 2). However, when evaluating the planting spacings, there was a significant interaction between the spacings and the use of the growth regulator for the total acidity (Tab. 2). The data fitted a quadratic regression for the presence and absence of paclobutrazol, with maximum points of 0.21 m spacing (0.69) and 0.27 m spacing (0.67), respectively (Fig. 2B).

For productivity, there were significant results. The averages differed statistically for the use of the growth regulator. The crop productivity was higher with the presence of paclobutrazol (92 t) and lower with the absence of paclobutrazol (67 t) (Tab. 2). There was a significant interaction between planting spacing and use of growth regulator. The values in the presence of paclobutrazol fitted a linear regression. The values were between 124 and 58 t. In the absence of paclobutrazol, the values fitted a quadratic regression, with a maximum point of 0.18 m planting spacing (76.48 t ha^{-1}) (Fig. 2C), indicating that, in the absence of the regulators, the increase in the spacing resulted in a decrease in productivity. Hachmann *et al.* (2014) obtained a higher productivity when plants were planted at a smaller spacing. After comparing the crop conduction of super-dense tomatoes with the traditional density of plants in the 2008/2009 harvest, Wamser *et al.* (2012) reported a higher productivity in the super-dense tomatoes. Benetti *et al*. (2018) observed that tomato productivity was

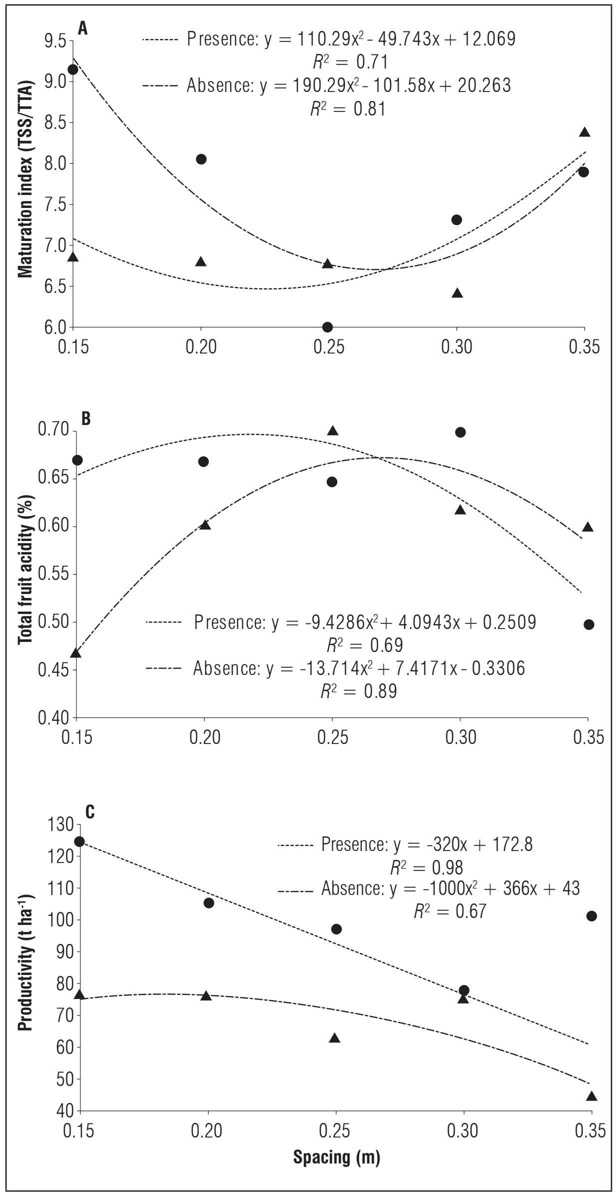

Figure 2. (A) Maturation index, (B) total titratable acidity, and (C) productivity of tomato plants as a function of the presence or absence of growth regulator and planting spacing. Ipameri, GO.

significantly modified by different spacings, with the highest productivity obtained when tomato plants were conducted at a 50 cm spacing between plants. Thus, a greater number of plants per area offsets competition between plants in dense planting.

CONCLUSIONS

It was observed that the application of paclobutrazol influenced the phylotechnical characteristics and technological quality of tomato fruits grown for industrial processing. For the planting density, the planting spacing between 0.21 and 0.27 m had better crop development and productivity.

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Salt water and silicon application on growth, chloroplastid pigments, chlorophyll fluorescence and beet production

Aplicación de agua salobre y silicio en el crecimiento, pigmentos cloroplastídicos, fluorescencia de clorofila y producción de remolacha

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Beet experiment view.

Photo: M.L.M. Véras

ABSTRACT

In recent years, the use of saline water in agriculture has become an alternative mainly because of water scarcity. However, plants do not tolerate high salt contents; so, the use of salt stress attenuators could enable saline water usage in agriculture. This study aimed to assess the effect of saline water and silicon applications on growth, chloroplastid pigments, chlorophyll fluorescence a and beet production. The experiment was conducted with complete randomized blocks in a 5 × 5 combined factorial arrangement according to the Central Composite of Box experiment matrix for the electrical conductivity in the irrigation water (ECw) and silicon doses (Si), with minimum (- α) and maximum (α) values from 0.5 to 6.0 dS m^{-1} and from 0.00 to 18.16 mL L^{-1}, totaling nine treatments, with four replicates and three plants per plot. The irrigation water ECw increase reduced growth and beet production, but the chlorophyll contents, biomass and fluorescence production were not affected by salinity. Silicon applications via the soil increased growth and chlorophyll fluorescence a but did not reduce the harmful effect of the salt stress. The irrigation water ECw above 0.50 dS m^{-1} negatively affected the beet crop. The silicon dose of 9.08 mL L^{-1} is the most recommended application.

Additional keywords: *Beta vulgaris* L.; abiotic stress; potassium silicate; photosynthesis.

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RESUMEN

En los últimos años el uso de aguas salinas en la agricultura es una alternativa, principalmente en virtud de la escasez hídrica. Sin embargo, las plantas no toleran altos niveles de sales, por lo que el uso de atenuadores de estrés salino puede ser una estrategia para posibilitar el uso de aguas salinas en la agricultura. En este sentido, este trabajo tiene como objetivo evaluar el efecto de aguas salinas y aplicación de silicio sobre el crecimiento, pigmentos cloroplatísdicos, fluorescencia de la clorofila a y producción de remolacha. El experimento fue conducido en un diseño de bloques al azar, en factorial 5 × 5, referente a cinco niveles de conductividad eléctrica del agua de riego (CEa): (0,5; 1,3; 3,25; 5,2 y 6 dS m^{-1}) y cinco dosis de silicio (0,00; 2,64; 9,08; 15,52 y 18,16 mL L^{-1}), combinadas según la matriz experimental Compuesto Central de Box, con cuatro repeticiones y tres plantas por parcela. El aumento de la conductividad eléctrica en el agua de riego reduce el crecimiento y la producción de remolacha, pero los índices de clorofila, la producción de biomasa y la fluorescencia no están influenciados por el riego con aguas salinas. La aplicación de silicio a través del suelo promueve un incremento en el crecimiento y la fluorescencia de la clorofila a, sin embargo, no reduce el efecto nocivo del estrés salino. La conductividad eléctrica en el agua de riego por encima de 0,50 dS m^{-1} es suficiente para afectar negativamente el cultivo de la remolacha y la dosis de 9,08 ml L^{-1} de silicio es la más recomendada para su aplicación.

Palabras clave adicionales: *Beta vulgaris* L.; abiotic estrés; potasio silicato; photosynthesis.

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INTRODUCTION

The agronomic performance of crops depends on edaphoclimatic factors, which are balanced under optimal conditions. Crops are subject to various types of biotic stresses (pests and diseases) and abiotic stress, isolated or combined. But in recent years, high contents of salts in water and in soil have become a significant problem in agriculture, especially in arid and semi-arid regions (Syvertsena and Garcia-Sanchez, 2014).

In arid and semi-arid regions, low rainfall and irregularity in rainfall mean that supplementary irrigation is almost mandatory, which, along with the need to expand cultivated areas, make low-quality water an alternative for irrigation (Lima *et al*., 2014).

Plants behave differently when saline water is used, and sensitivity to salinity depends on various factors, such as variety and plant exposure time (Pedrotti *et al*., 2015). When subjected to salt stress, plants experience change at the cellular level, including destruction of root plasma membrane and ionic stress on stem cells (Reis *et al*., 2016); therefore, a major symptom is stomatal closure, with a consequent lower photosynthetic activity, causing decreases in growth and productivity (Fraire-Velázquez and Balderas Hernández, 2013; Reis *et al*., 2016).

In recent years, one of the strategies to reduce the harmful effects of salt stress is using fertilization with silicon (Sahebi *et al*., 2016). Studies indicate that silicon can increase plant tolerance to salt stress since absorption of K and Ca ions contributes to the maintenance of K and Na contents in plants; however, mechanisms that contribute to increased tolerance to salt stress have not been discovered (Dias and Blanco, 2010; Shi *et al*., 2013; Castellano *et al*., 2016)

There are a few studies on some of the effects of Si in plants. These studies have shown that this element could mitigate the deleterious effects of salts in plants, but, for beets, there is little research that elucidates attenuator effect on salt stress. Thus, this study aimed to evaluate the effect of saline water and silicon applications on growth, chloroplastid pigments, fluorescence chlorophyll a and beet production.

MATERIAL AND METHODS

This experiment was conducted from January to March, 2018 in a greenhouse located in the Fruit sector belonging to the Federal University of Paraíba in Areia-PB, Brazil, located at 6º51'47" and 7º02'04"

South latitude and West longitude, 35°34’13” and 35°48’28” Greenwich meridian.

The experiment design used randomized blocks and a 5 × 5 factorial arrangement, combined according to the Central Composite of Box experiment matrix (Mateus *et al*., 2011) for the electrical conductivity of the irrigation water (ECw) and silicon doses (Si), with minimum values (-α) and maximum values (α) of 0.5 to 6.0 dS m^{-1} and 0.00 to 18.16 mL L^{-1}, totaling nine treatments, with four replicates and three plants per plot (Tab. 1).

Table 1. Combinations of treatments with the central compound matrix of box.

Levels		Doses	
Si	ECw	Si	ECw
-1	-1	2.64	1.30
-1	1	2.64	5.20
1	-1	15.52	1.30
1	1	15.52	5.20
- α	0	0.00	3.25
α	0	18.16	3.25
0	α	9.08	6.00
0	- α	9.08	0.50
0	0	9.08	3.25

Beet cv. Wonder seedlings were grown in trays and planted in pots with a 22 cm top diameter, 16 cm bottom diameter, 18 cm height, 8 dm^3 volumetric capacity, and 1 cm circular holes in the bottom to allow better root aeration and percolation of excess water.

The containers were filled with horizon A soil, collected at a depth of 0-20 cm, classified as Planossolo Háplico Eutrófico êndico/Alfisol (Embrapa 2014). The chemical and physical characteristics (Tab. 2) were analyzed according to the methodology of Embrapa (2014). The soil was air dried, homogenized, and placed in containers accommodated previously with screen (tulle fabric) and 200 g of crushed rock, while preventing the soil from coming out of the holes in the bottom.

The plants were irrigated daily, bringing the soil moisture to about 80% of field capacity (CC). The different ECw were obtained by with the salts NaCl, $CaCl_2$ $2H_2O$ and $MgCl_2$ $6H_2O$, at a ratio of 7:2:1 according to the characteristics (Tab. 3). Irrigation with water with different salinities was initiated 10 d after emergence. On the first day after emergence (DAE), the blade was calculated with the equation proposed

Table 2. Chemical and physical characteristics of the soil.

Chemicals attributes		Physical attributes	
pH	6.26	Ds	1.38
P (mg dm^{-3})	11.35	Pd	2.67
K^+ (mg dm^{-3})	40	Tp	0.48
Na^+ (cmol dm^{-3})	0.22	CC	78
$H^+ + Al^{+3}$ (cmol dm^{-3})	1.82	PMP (g g^{-1})	43
Al^{+3} (cmol dm^{-3})	0	Sand (g kg^{-1})	756.9
Ca^{+2} (cmol dm^{-3})	3	Silt (g kg^{-1})	59.1
Mg (cmol dm^{-3})	1.9	Clay (g kg^{-1})	184
BS (cmol dm^{-3})	5.22	-	-
CEC (cmol dm^{-3})	7.03	-	-
V (%)	74.34	-	-
M (%)	0	-	-
OM (g Kg^{-1})	17.53	Textural classification	Sandy franc

Base sum (BS) = ($Na^+ + K^+ + Ca^{2+} + Mg^{2+}$); CEC = cation exchange capacity; EC = BS + ($H^+ + Al^{3+}$); V = (100 x BS/CEC); OM = organic matter. Ds = density of the soil; Pd = particle density; Tp = total porosity; (1- (Ds/Dp) * 100) Ucc = volumetric humidity level of field capacity - 0.033 MPa; Upmp = humidity level of the permanent wilting point - 1.5 MPa.

Table 3. Chemical characteristics of the water.

Attributes	Electric conductivity (dS m^{-1})				
	0.50	1.30	3.25	5.20	6.00
	Values				
pH	7.00	7.50	7.40	7.30	7.40
$SO4^{-2}$	3.22	3.70	3.67	3.35	3.90
Mg^{2+}	1.33	1.78	1.93	2.03	2.98
Na^+	1.70	5.92	12.57	20.5	24.20
K^+	0.20	0.21	0.20	0.20	0.21
Ca^{2+}	0.73	1.58	1.78	1.88	2.53
CO_3^{-2}	0.00	0.00	0.00	0.00	0.00
HCO_3^-	2.75	3.50	4.00	4.25	4.25
Cl^-	3.40	10.90	30.40	48.90	58.15
SAR (mmol L^{-1}) $^{0.5}$	1.28	1.87	2.60	3.23	2.96
Classification	*C2S1*	*C3S3*	*C4S4*	C4S4	C4S4

EC= electrical conductivity at 25 °C; SAR = sodium adsorption ratio [Na^+/($Ca^{2+} + Mg^{2+}/2)^{1/2}$]; CO_3^{2-} = Absent. Water classification according to Richards (1954).

by Mantovani *et al.* (2009); the total required irrigation (TRI) was calculated with the equation by Bernardo *et al*. (2008), considering 100% of irrigation application efficiency.

The silicon was applied in the liquid potassium silicate form (K_2SiO_3) with 12% Si and 15% K_2O. The Si doses were applied via the soil. However, there was compensation of K_2O via soil, the application used in the beet aiming to provide the same amount of potassium to all plants. The application was done weekly, totaling 7 applications during the development and production of beets. The doses (Si) were diluted in 1.2 L of distilled water, and 50 mL of this solution were applied to each plant.

During the experiment, weather data (Fig. 1) were recorded daily with a digital thermo hygrometer, HT-600 Instruthermr®, installed in the experimental area, at the height of the plants. The average air temperature was near the ideal range (25°C) during the crop cycle, according to Filgueiras (2008).

Figure 1. Graphical representation of relative humidity and temperature in the experiment. Mean Air temperature (Tmed) and mean relative humidity (URmed).

The fertilizer at sowing and covering was done with 40, 180 and 90 kg ha^{-1} of NPK, with urea, superphosphate and potassium chloride, according to the chemical analysis of the soil and fertilizer recommendations for State of Pernambuco, Brazil (IPA, 2008). During the conduction of the experiment phytosanitary controls of pests and weeds were made.

The growth of the beet plants was evaluated by measuring plant height (measured with a ruler graduated in cm), leaf area ((by measuring the length (C) and width (L), and by applying in the AF formula = C * L * f, with AF = leaf area in m^2; C = length of the leaf in m; L = leaf width in m; and f = correction factor for beets (0.692), according to Simões *et al*. (2016)), number of leaves, and leaf length and width (using digital caliper) at 60 d after emergence.

At the end of the experiment, measurements were taken: longitudinal and transversal diameter of the bulb (using digital caliper); bulb fresh mass, leaf fresh mass, leaf dry mass, root dry mass, fresh mass bulb (through weighing on an analytical balance), leaf chlorophyll indices a, b, total and a/b ratio with a Clorofilog® chlorophyll meter (Falker). The readings were taken with intermediate leaves of the four central plants in the experiment area, performing four readings per plant.

The initial fluorescence (F_0), maximal fluorescence (F_m), variable fluorescence (F_v) and quantum efficiency of photosystem II (F_v/F_m) were also evaluated using a portable fluorometer (PEA – Plant Efficiency Analyzer, Hansatech).

The data were subjected to analysis of variance, and, when significant, the data were subjected to polynomial regression analysis ($P \leq 0.05$). For the data that were not significant, standard deviations of the mean were carried out. The SAS University (Cody, 2015) software was used for these analyses.

RESULTS AND DISCUSSION

Plant height was adjusted to a quadratic model as a function of salinity in the irrigation water. There was a reduction in plant height with the increasing salt concentrations, reaching the point of maximum efficiency when the plants were irrigated with 0.40 dS m^{-1}, corresponding to a height of 27.50 cm; the plant height was reduced when irrigation was done with high salinity water (Fig. 2A).

A reduction in plant height was also observed by Silva *et al*. (2015), who found that the greatest height of beet cv. Itaapuã plants was 43.6 cm with 6 dS m^{-1} irrigation water, with a reduction with increasing salinity. Santos *et al*. (2016) observed that the maximum height was obtained with 3.11 dS m^{-1}, 12.8 cm, confirming the results obtained in this study, i.e. the increased salinity in the irrigation water significantly reduced the height of the plants.

The same behavior was observed for the leaf area, which decreased as salinity increased. The largest leaf area was 3720.75 cm^2, in the plants irrigated with 0.5 dS m^{-1} water (Fig. 2B). This reduction may have been caused by morphological and physiological changes, one of the first symptoms being a reduction in leaf area, which is a way to increase tolerance to salinity.

Santos *et al*. (2016) observed that the maximum leaf area was obtained with a salinity of 2.85 dS m^{-1}, 21 4 cm^2. This result is lower than that obtained in the present study. In studies on radish crops, Oliveira *et al*. (2012) found that the greatest leaf area value was observed in plants irrigated with lower salinity water (2 dS m^{-1}), yielding 497.20 cm^2, while the highest ECw (10 dS m^{-1}) resulted in a smaller leaf area, 220 cm^2, a reduction of 55.75%, corroborating the data obtained in this study.

The bulb diameter and the bulb longitudinal diameter decreased when the beet plants were irrigated with saline water, decreases with increasing salt. Larger diameters were obtained with plants irrigated with water with a lower salinity, yielding 31.26 mm with 0.10 dS m^{-1} irrigation water (Fig. 2C) and 32.56 mm with 0.15 dS m^{-1} water (Fig. 2D).

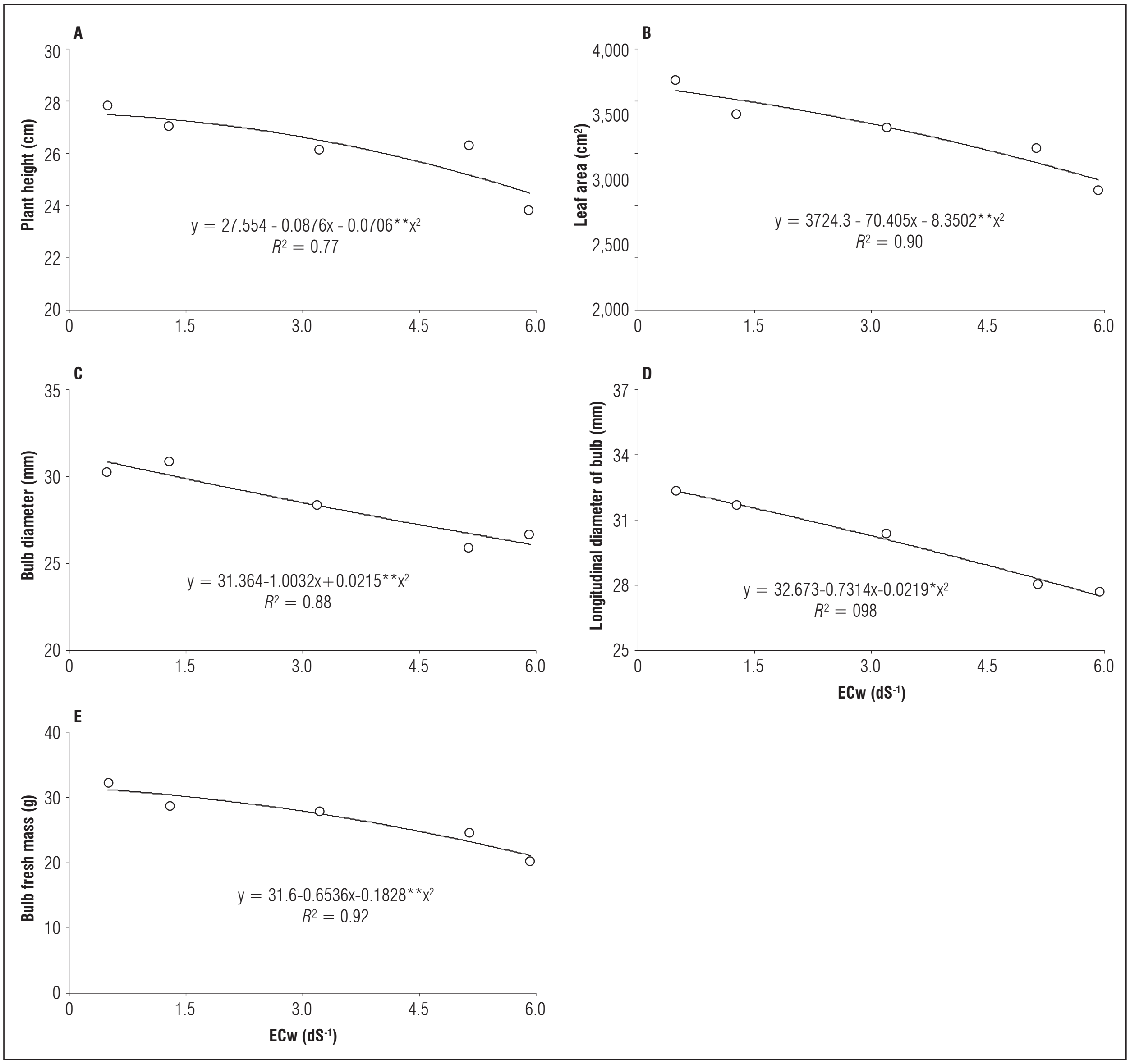

Figure 2. Plant height (A), leaf area (B), bulb diameter (C), bulb longitudinal diameter (D) and fresh bulb mass (E) of beet plants irrigated with water with different electrical conductivities.

The bulb fresh mass also decreased with the increased salts in the irrigation water; the largest bulb fresh mass was 31.49 g when the beet plants were irrigated with 0.14 dS m^{-1} (Fig. 2E).

The results obtained in this present study agree with those of Silva *et al*. (2015) who studied the management of fertigation and soil salinity in terms of growth in a beet culture, observing that increasing the salinity of the water reduced the beet plant bulb diameter.

The salinity in the irrigation water did not significantly affect the analyzed variables (Tab. 4). However, the highest values were observed in the beet plants irrigated with low salinity water (0.50 dS m^{-1}), and, although no significant effect was found, the lowest values were observed with higher salinity irrigation water (6.00 dS m^{-1}), proving that the salt stress dramatically reduced the growth and development of the plants, as was observed by Silva *et al*. (2015) and Santos *et al*. (2016) in beets, and by Shi *et al*. (2013), Lima *et al*. (2014) and Reis *et al*. (2016) in rice, roses and castor, respectively.

By irrigating the beet plants with 6.00 dS m^{-1} water, smaller values were obtained for leaf fresh mass, leaf dry mass and root dry mass, which may have been caused by a decrease in osmotic potential, the soil solution, and the excessive accumulation of salts, inhibiting the absorption of water from the beet plants and, as a result, requiring greater energy for the absorption of water and nutrients and reducing growth and biomass accumulation (Sá *et al*., 2015; Bertazzini *et al*., 2018).

Table 4. Mean ± standard deviation of the variables leaf width (Wl), leaf length (Ll), leaf area (La), number of leaves (Nl), chlorophyll content A (Cl *a*), chlorophyll B (Cl *b*), total chlorophyll (Total Cl), chlorophyll *a/b ratio* (Cl *a/b*), leaf fresh mass (Lmf), leaf dry mass (Ldm), root dry mass (Rdm), cross bulb diameter (Cbd), initial fluorescence (F_0), maximal fluorescence (F_m), variable fluorescence (F_v) and quantum efficiency of photosystem II (F_v/F_m) of beet plants as a function of irrigation with saline water (ECw).

ECw (dS m^{-1})	Variables			
	Wl	Ll	La	Nl
0.50	62.71±9.51	123.06±15.48	5,398.06±15.02	9.91±1.67
1.30	60.52±15.22	105.72±22.01	4,762.31±10.02	10.20±1.92
3.25	56.09±16.23	95.14±21.99	3,821.68±18.23	10.11±1.08
5.20	55.88±13.56	103.82±18.85	3,822.55±17.15	9.95±1.57
6.00	51.65±17.51	83.99±26.40	3,284.99±9.16	9.91±1.67
	Cl *a*	Cl *b*	Total Cl	Cl *a/b*
0.50	38.00±4.84	17.67±4.53	53.67±9.10	2.15±0.51
1.30	36.62±2.62	18.40±4.08	55.03±6.11	2.08±0.32
3.25	35.36±4.31	17.70±3.92	53.06±8.08	2.13±0.49
5.20	35.23±2.79	17.16±4.50	52.40±6.95	2.20±0.33
6.00	35.42±3.88	13.06±7.35	51.49±10.81	1.76±0.36
	Lmf	Ldm	Rdm	Cbd
0.50	32.80±10.92	11.72±6.35	0.12±0.09	55.32±9.65
1.30	29.50±13.76	9.40±6.35	0.17±0.17	54.19±7.07
3.25	27.93±9.17	9.20±4.43	0.13±0.08	53.85±8.97
5.20	28.44±15.67	11.29±7.92	0.17±0.08	54.52±11.37
6.00	21.37±7.89	8.74±5.82	0.11±0.11	46.35±5.26
	F_o	Fm	Fv	Fv/Fm
0.50	78.19±12.8	332.38±10.52	294.19±11.17	0.95±0.07
1.30	62.88±10.51	318.97±11.17	256.09±12.10	0.79±0.09
3.25	65.75±11.87	317.77±11.53	252.02±9.14	0.80±0.06
5.20	74.13±15.71	313.66±12.19	239.53±13.51	0.77±0.08
6.00	56.94±9.17	318.44±14.28	2,371.50±10.19	0.73±0.02

The smallest values for the chlorophyll contents a, b and total chlorophyll a and fluorescence photochemical efficiency of photosystem II when the plants were irrigated with water with higher salinity (6.00 dS m^{-1}) were due to the reduction in the activity of photosynthetic enzymes, limiting the electron transport in the chloroplasts. The accumulation of Na^+ and Cl^{-1} in chloroplasts changes the photosynthetic activity of plants, which reduces the photochemical efficiency of photosystem II (Huang *et al*., 2012).

The same behavior was observed by Silva *et al*. (2013), who found a decrease in the growth and physiological parameters of beet cv. Early Wonder with increased salinity. Silva *et al*. (2015) also found that the salinity in irrigation water reduced the growth of beet plants.

It was observed that the leaf area decreased with the increase of the silicon doses, up to 9.08 mL L^{-1}, with a subsequent increase up to 18.16 mL L^{-1}, reaching a maximum leaf area of 3591.15 cm^2 with 0.26 mL L^{-1} (Fig. 3A). The longitudinal diameter of the bulbs presented the same behavior, with the greatest longitudinal diameter, 32.45 mm, observed in the plants treated with 0.24 mL L^{-1} of silicon (Fig. 3B).

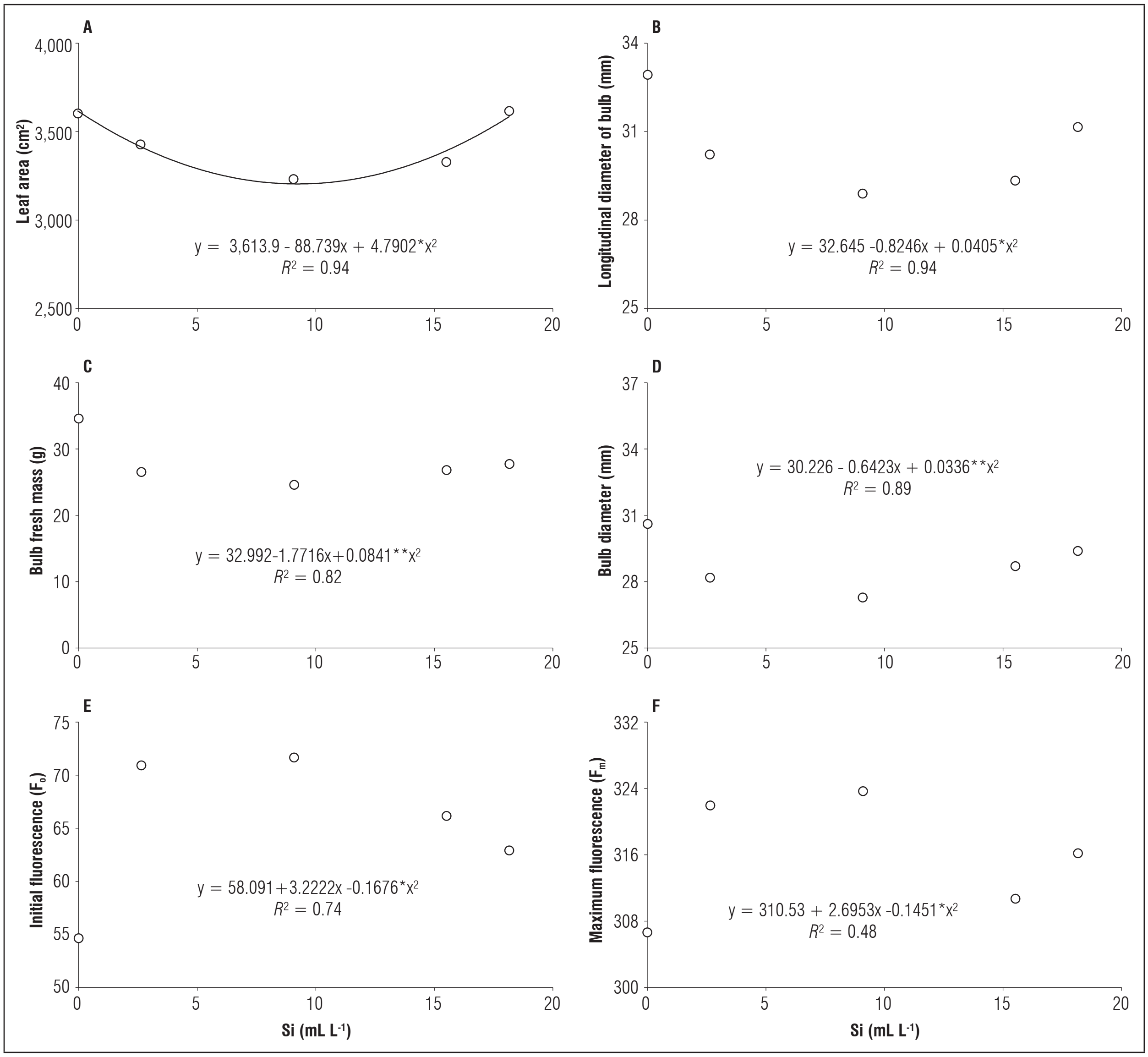

Figure 3. Longitudinal diameter of the bulbs (B), bulb fresh mass (C), bulb diameter (D), initial fluorescence (E) and maximum fluorescence (F) in beet plants for the different doses of leaf silicon.

The fresh mass of the bulb and the bulb diameter decreased with increased doses of silicon, up to 9.08 mL L^{-1}; however, higher doses of Si increased these variables. The highest values for bulb fresh mass (Fig. 3C) and bulb diameter (Fig. 3D) were obtained when the plants received 0.23 mL L^{-1} and 0.26 ml L^{-1}, yielding 32.60 g and 30.06 mm.

The initial fluorescence and maximum fluorescence increased with doses up to 9.08 mL L^{-1}, with reductions over this dose. The highest values were observed when applying 9.08 mL L^{-1} with values of 71.63 (Fig. 3E) and 323.75 (Fig. 3F), respectively.

The silicon doses did not influence the analyzed variables (Tab. 5). However, the dose of 9.08 mL L^{-1} promoted higher contents of chlorophyll a, b, and total, and a higher dose (18.16 mL L^{-1}) provided higher chlorophyll fluorescence (F_v) and quantum efficiency of photosystem II (F_v/F_m), indicating that Si may mitigate the effect of salt stress because of the increase of photosynthetic pigments (Rezende *et al.*, 2018).

Although there was not a significant effect on the analyzed variables, greater values of chlorophyll a, b and total, fluorescence (F_v) and quantum efficiency of photosystem II (F_v/F_m) confirmed that Si has a significant influence on the photosynthesis and biochemistry of plants. Several studies have demonstrated the positive effect of Si, as reported by Bae *et al*. (2012), Tahir *et al*. (2012) and Yin *et al*. (2013), who found that an increased availability of Si increases growth, photosynthetic and biochemical aspects of plants.

As noted in this study, several studies have shown that Si promotes growth and photosynthetic activity (Bae *et al*., 2012; Tahir *et al*., 2012; Yin *et al*., 2013); leaves become more erect, allowing a greater absorption of CO_2 and higher photosynthetic efficiency and chlorophyll contents. Si accumulation on the leaf surface may have promoted a physical barrier in the leaves of the beet plants, playing an important role in osmotic adjustment (Heckman, 2013; Cantuário *et al*., 2014).

Table 5. Mean ± standard deviation of the variables plant height (Ph), leaf width (Wl), leaf length (Ll), number of leaves (Nl), chlorophyll content *a* (Cl *a*), chlorophyll b (Cl *b*), total chlorophyll (Total Cl), chlorophyll *a/b* ratio (Cl *a/b*), fluorescence (F_v), quantum efficiency photosystem (F_v/F_m), leaf fresh mass (Lfm), leaf dry mass (Ldm), root dry mass (Rdm) and transverse bulb diameter (Tdm) of beet plants under silicon applications via the soil.

Si (mL L^{-1})	Variables				
	Ph	Wl	Ll	Nl	Cl *a*
0.00	26.05±2.93	59.55±15.07	98.90±24.44	10.25±1.22	35.05±3.00
2.64	27.06±3.86	58.90±23.41	101.10±27.04	10.08±1.56	36.02±4.54
9.08	25.82±3.50	54.88±16.45	90.83±26.46	9.94±1.45	36.80±4.17
15.52	26.25±3.95	57.50±18.41	128.43±28.10	10.08±1.61	35.84±3.78
18.16	26.43±3.63	58.42±16.09	101.08±26.15	10.08±1.73	35.05±6.53
	Cl *b*	Total Cl	Cl *a/b*	F_v	F_v/F_m
0.00	18.87±6.54	53.93±9.15	2.01±0.52	222.25±12.1	0.80±0.51
2.64	18.18±5.34	54.21±9.54	2.12±0.52	233.63±10.5	0.79±0.43
9.08	19.31±5.84	56.11±9.50	2.02±0.43	235.75±12.14	0.77±0.86
15.52	17.38±5.04	53.22±8.36	2.16±0.39	198.50±12.75	0.69±0.74
18.16	17.03±6.10	52.09±12.34	2.25±0.61	238.00±12.53	0.81±0.17
	Lfm	Ldm	Rdm	Tdm	
0.00	31.77±11.79	10.59±5.55	0.17±0.11	57.39±7.66	
2.64	30.22±15.83	10.66±6.88	0.16±0.15	55.40±11.58	
9.08	26.57±11.17	9.89±5.75	0.13±0.09	51.03±7.98	
15.52	27.72±13.39	10.03±6.68	0.18±0.13	53.31±9.47	
18.16	26.48±11.17	7.79±4.10	0.11±0.05	50.74±9.31	

CONCLUSION

The increase of electrical conductivity in the irrigation water reduced the growth and production of the beets, but the chlorophyll contents, biomass production and fluorescence were not influenced by irrigation with saline water.

The application of silicon in the soil promoted increases in growth and chlorophyll fluorescence a; however, it did not reduce the harmful effect of salt stress.

Electrical conductivity in the irrigation water above 0.50 dS m^{-1} adversely affected the beet cultivation, and the silicon dose of 9.08 mL L^{-1} is recommended for applications.

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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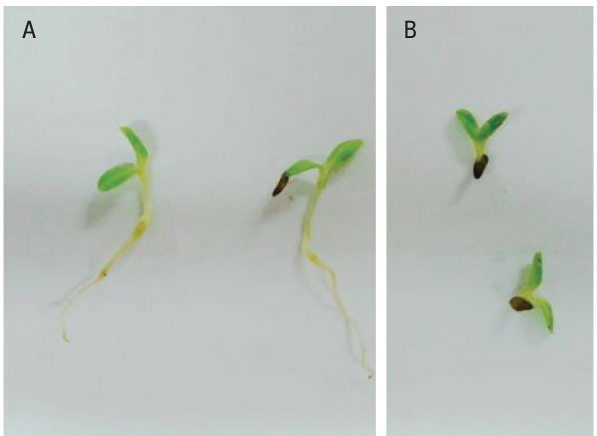
Essential oil of *Lippia alba* (Mill.) N.E.Br. influences the germination, vigor and emergence of lettuce seeds

El aceite esencial de *Lippia alba* (Mill.) N.E.Br. influye en la germinación, vigor y emergencia de semillas de lechuga



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Normal (A) and abnormal (B) lettuce seedlings.

Photo: L.A. Thiesen

ABSTRACT

Secondary metabolites are produced by certain plant species and may influence the development of some species through the action of allelopathic effects. Thus, the objective of this study was to evaluate the allelopathic effect of *Lippia alba* essential oil on the germination, emergence and vigor of seedlings of different batches of lettuce seeds. This experiment was conducted in a completely randomized design with a 3×5 factorial scheme consisting of three batches of seeds of the cultivar Grand Rapids® and five doses of *L. alba* essential oil (0, 0.25, 0.50, 0.75 and 1.00%) diluted in distilled water and homogenized in Tween® 80. Seed germination and vigor, in the laboratory (experiment I), and emergence, in a protected environment (experiment II), were evaluated. The results demonstrated that there was an inhibitory effect with a significant reduction of germination, vigor and seed emergence when *L. alba* essential oil was added starting at concentrations of 0.25%. The essential oil showed allelopathic potential over different lettuce seed lots, affecting germination, vigor and seed emergence.

Additional keywords: *Lactuca sativa* L.; false lemon balm; allelopathy; secondary metabolites.

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RESUMEN

Los metabolitos secundarios son producidos por ciertas especies de plantas, y pueden influir en el desarrollo de algunas especies, debido a la acción de los efectos alelopáticos. Por lo tanto, el objetivo del trabajo fue evaluar el efecto alelopático del aceite esencial de *Lippia alba* en la germinación, emergencia y vigor de las plántulas de diferentes lotes de semillas de lechuga. El experimento se realizó en un diseño completamente al azar, en un esquema factorial 3×5, que consta de tres lotes de semillas del cultivar Grand Rapids® y cinco dosis de aceite esencial de *L. alba* (0; 0,25; 0,50; 0,75 y 1,00%) diluido en agua destilada y homogeneizado en Tween® 80. Se evaluó la germinación y el vigor de la semilla en el laboratorio (experimento I) y la emergencia en un ambiente protegido (experimento II). Los resultados obtenidos mostraron que hubo un efecto inhibitorio con una reducción significativa de la germinación, el vigor y la emergencia de la semilla cuando se añadió aceite esencial de *L. alba* a partir de concentraciones de 0.25%. El aceite esencial mostró potencial alelopático sobre diferentes lotes de semillas de lechuga, afectando la germinación, el vigor y la emergencia de las semillas.

Palabras clave adicionales: *Lactuca sativa* L.; falso bálsamo de limón; alelopatía; metabolitos secundarios.

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INTRODUCTION

Allelopathy is a chemical phenomenon in plants that produces certain secondary metabolites that, when released into the environment, positively or negatively influence the germination and development of other species that inhabit the same environment, which has contributed significantly to the adaptive evolution of vegetables (Ferreira *et al*., 2007; Gliessman, 2009; Taiz *et al.*, 2017). This allelopathic effect occurs as a result of the presence of these secondary metabolites in different plant structures, with different active ingredient composition for each species (Mirmostafaee *et al.*, 2020).

Usually, secondary metabolites are produced as a defense mechanism against biotic or abiotic stress conditions or are synthesized to provide plant-plant, plant-microorganism and plant- animal interactions (Arceo-Medina *et al.*, 2016). Because of these interactions, the volatile compounds of secondary metabolites can diffuse in the air and affect neighboring organisms (Mirmostafaee *et al.*, 2020). Also, they can attract insects, promoting pollination and seed dispersion. Among these secondary metabolites, essential oil stands out, which can perform different biological functions depending on the different constituents produced. For these reasons, the effect of essential oils has been studied in the search for new alternatives that are more sustainable and less toxic to the environment and humans, geared towards management and diversification of agricultural crops (Li *et al.*, 2011; Gusman *et al.*, 2012).

Among the species that produce essential oils, mainly aromatic plants, *Lippia alba* (Verbenaceae) is notable, popularly known as false lemon balm, lemon balm, melissa false, lemon tree, Brazilian lemongrass, lemongrass, board tea or wild rosemary (Oliveira *et al.*, 2019; Santos *et al*., 2019). It is a plant rich in essential oil with notable chemotype limonene, citral, cavone and linalool levels, with bactericidal, fungicidal, nematicidal, mild sedative and anticancer action (Tavares *et al.*, 2005; Fabri *et al.*, 2011; Oliveira *et al.*, 2018) and analgesic, anti-inflammatory, sedative and antispasmodic activities (Tavares *et al.*, 2011; Mamun-Or-Rashid *et al.*, 2013). *L. alba* essential oil can also cause phytotoxicity, as seen in watermelon seedlings (*Citrullus lanatus* Thunb.) and rice (*Oryza sativa* L.) with concentrations of 4% (Brum *et al.,* 2014).

The resistance or tolerance to certain secondary metabolism compounds is characteristic of each plant species. Among the species most sensitive to the effects of secondary metabolites, cucumber, tomato and lettuce stand out (Garbim *et al.*, 2015). Lettuce is considered a model plant for assessing allelopathic potential because of its fast and uniform germination and high sensitivity to allelopathic substances (Mirmostafaee *et al.*, 2020). Therefore, the objective of the study was to evaluate the influence of *L. alba* essential oil on the germination, vigor and emergence of lettuce seeds from different commercial lots.

MATERIALS AND METHODS

This study was conducted at the Federal University of Santa Maria, Frederico Westphalen *Campus*, geographically located at 27º 23'S, 53º 25'W and 493 m altitude. The climate of the region is of the Cfa type - humid subtropical, rainy, temperate, and average annual precipitation of 1,800 mm that is well distributed throughout the year according to the Köppen classification (Alvares *et al.*, 2013).

For the allelopathy test of the essential oil of *Lippia alba,* lettuce cultivar Grand Rapids® was used. The seeds came from three commercial lots, with lot 1 showing germination of 92 and 100% purity and lots 2 and 3 showing germination and purity of 97 and 99.9%, respectively.

The essential oil used in the tests was extracted from fresh L. *alba* leaves taken from adult plants without flowering collected from the medicinal garden located at the Federal University of Santa Maria, Frederico Westphalen C*ampus*. The extraction of the essential oil was carried out with the hydrodistillation process, with a modified Clevenger apparatus, for a period of 2 h. For this, the plant material was chopped and added in a volumetric flask together with distilled water. The balloon was placed in thermal blankets for heating. After detecting the beginning of the boil, 2 h were timed, corresponding to the period of hydrodistillation. After this period, the oil was removed, added to amber glass bottles and stored in the dark in a refrigerator at 5ºC.

Experiment I: Allelopathy of the essential oil of *L. alba* in the germination and vigor lettuce seeds

Experiment I was carried out from January 28 to February 11, 2016 at the Seed Laboratory with a completely randomized experiment design in a 3×5 factorial arrangement with three commercial seeds lots and five doses of *L. alba* essential oil [0%; 0.25%; 0.50%; 0.75% and 1%, (v/v)], totaling 15 treatments, with four repetitions per treatment. The experiment unit was composed of 100 seeds per repetition.

The solutions in the different concentrations of *L. alba* essential oil were prepared by diluting the essential oil in distilled water, homogenized in Tween® 80, at a concentration of 1% (v/v). Sowing of the lettuce was carried out in a transparent acrylic gerbox box, 11×11×4 cm, with a substrate of two sheets of germitest paper. The paper was moistened with a solution of different concentrations of essential oil in an amount of 2.5 times the mass of the dry germitest paper. In each box, 33 to 34 seeds were added with homogeneous spacing, totaling 100 seeds per repetition and 400 seeds per treatment. The gerboxes remained in a B.O.D. with a temperature of 25ºC and photoperiod of 12 h.

After 7 d of sowing, the following variables were evaluated: percentage of germinated seeds, first count of normal seedlings, percentage of normal seedlings, percentage of abnormal seedlings, radicle length, shoot length and dry mass of seedlings. In addition, the number of seeds germinated daily was counted to determine the germination speed index (GSI). After obtaining the daily data, the GSI was calculated using the formula established by Maguire (1962), which is based on the sum of emerged seedlings divided by the days after sowing. For the other evaluated variables, those seeds that showed radicle protrusion were considered germinated, expressed as a percentage. The evaluation of abnormal, normal seedlings and germinated seeds was carried out according to the criteria established by MAPA (2009), expressed as a percentage.

For evaluations of radicle length, shoot length and dry mass of seedlings, 20 random seedlings (considered normal) per repetition were selected. To be considered normal seedlings, these must have a root system in perfect condition, normal seedlings germinated on the fourth day after sowing were quantified, expressed as a straight hypocotyl, two green cotyledons and a well-developed coleoptile. Therefore, seedlings that showed different characteristics were considered abnormal following the recommendations of MAPA (2009).

Experiment II: Influence of seed treatment with essential oil of *L. alba* during emergence and growth of lettuce in a protected environment

Experiment II was conducted from September 18, 2015 to October 9, 2015 in a protected environment, built with galvanized steel and a pampean arch, 20 m long, 10 m wide and 3.5 m high.

The experiment was conducted in a completely randomized design in a 3×5 factorial arrangement with three commercial seed lots and five doses of the essential oil of *L. alba* (Mill.) [0%; 0.25%, 0.50%, 0.75% and 1%, (v/v)], totaling 15 treatments, with four repetitions per treatment. The experiment unit consisted of 50 seeds per repetition.

The sowing was carried out in expanded polystyrene trays with 200 cells, filled with commercial substrate and with 60% field capacity. Each cell received a seed, allocated at a depth of three times its size. To evaluate the effect of *L. alba* essential oil, the seeds were kept submerged in the different concentrations of essential oil solution for one minute, then they were dried on paper towels to remove the excess essential oil and sown (methodology planned by the group). Irrigation was carried out manually with the aid of a watering can, observing the water need of the crop.

After 21 d of sowing, the following variables were evaluated: percentage of seedling emergence, Emergence Speed Index (ESI), root length, shoot length and dry mass of seedling. For evaluation of emergence, the emerged seedlings were counted, transformed into a percentage. The ESI was determined by counting seedlings that emerged daily, and evaluations were carried out at the same time. After obtaining the daily data, the variable ESI was calculated using the formula established by Maguire (1962), based on the sum of emerged seedlings divided by the days after sowing. The root length, shoot length and dry mass of seedling were determined with a random selection of 20 seedlings per treatment, with subsequent washing of the root system.

The results of both experiments were subjected to analysis of variance to evaluate the effect of the different commercial seed lots and the concentrations of the L. *alba* essential oil. When significant, the averages of the qualitative data of the variables were compared with the Tukey test at 5% probability, and, for the quantitative data, a regression analysis was performed, with the significance of the regression and coefficients being verified with the F test ($P \leq 0.05$) and t test ($P \leq 0.05$), respectively. The regression adjustment was performed using the adjusted determination coefficient (R^2). The data of the variables were analyzed using the statistical program SISVAR (Ferreira, 2011).

RESULTS AND DISCUSSION

Experiment I: Allelopathy of the *L. alba* essential oil in the germination and vigor of lettuce seeds

In the analysis of variance for the variables percentage of normal seedlings, percentage of abnormal seedlings, GSI and first count of normal seedlings, there was a significant interaction between the factors according to the F test; that is, between the doses of essential oil of *L. alba* and seed lots. For the variables percentage of germinated seeds and radicle length, there was a difference only for the individual factors for the doses of essential oil of *L. alba* and seed lots ($P \leq 0.05$). For the variables shoot length and dry mass of seedlings, there was a significant difference only for the dose of essential oil.

The variable percentage of germinated seeds had a cubic tendency, with a high percentage of germinated seeds in the environment without the presence of essential oil (90%). When L. *alba* essential oil was added, there was a drastic reduction in the percentage of germinated seeds (Fig. 1). Thus, 0.25% of the essential oil had an inhibitory effect on the germination of the lettuce seeds.

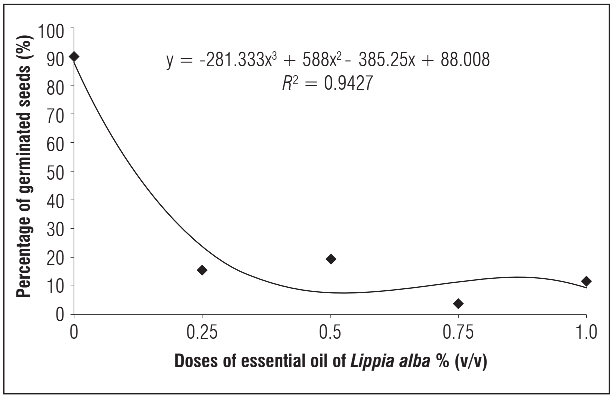

Figure 1. Percentage of germinated lettuce seeds at different doses of *L. alba* essential oil.

The essential oil produced by aromatic plants can perform several functions related to plant interactions and are also important sources of allelochemicals (Saharkhix *et al*., 2010). These allelochemicals are produced through the secondary metabolism of plants, forming complex compounds (Ma *et al*., 2012) that can affect the germination of some species.

Therefore, the essential oil of *L. alba* tends to have allelochemicals with the ability to reduce seed germination, as was observed in the lettuce seeds in the present study. Research results with lemongrass (*Cymbopogon citratus*), alfavaca (*Ocimum gratissimum*) and basil (*Ocimum basilicum*) have shown that, with an increase in essential oil doses, the values related to the percentage of seeds germinated in the first count increased (Miranda *et al.*, 2015b). Likewise, Silva *et al.* (2009) found that, with the increase in the concentration of the essential oil of acariçoba (*Hydrocotyle bonarensis*), as a result of the volatile effect, there was a restriction on germination, root length and shoot length in lettuce. The essential oil of thyme (*Thymus vulgaris*) also caused a negative allelopathic effect on the germination and vigor of lettuce seeds (Miranda *et al.*, 2015a).

Results diverging from that observed in this study were found by Marco *et al.* (2015), in which there was no significant difference in germination and in the emergence speed index of lettuce and cabbage seeds submitted to germination in a substrate moistened with essential oil from candela (*Vanillosmopsis arborea*) at doses of 0.25, 0.5 and 1 ml L^{-1}. However, germination and Emergence Speed Index were higher for lettuce than for cabbage. These results demonstrate that there is variability in the essential oil components of plants, some of which may have an allelopathic effect on other crops, while other plants do not have or require doses high enough to cause significant effects.

For the variable percentage of normal seedlings, it was observed that all the lots, despite presenting small differences, also adjusted to the cubic trend curve, proving that when L. *alba* essential oil was added, there was a decrease in this variable (Fig. 2A). For the values of percentage of abnormal seedlings, lots 2 and 3 followed the same response; however, with a quadratic trend line, a similar performance was observed, reducing the percentage of abnormal seedlings with increasing doses of *L. alba* essential oil. For lot 1, there was a decrease in the percentage of abnormal seedlings at a dose of 0.5% (Fig. 2B). This decrease in the percentage of abnormal seedlings is directly related to the reduction in the percentage of germinated seeds as a result of the increase in essential oil doses.

For the variables first count of normal seedlings (Fig. 3A) and GSI (Fig. 3B), the tendency of the equations was similar, in which it was possible to adjust the cubic response. For both variables, there was a drastic reduction in the average already at the dose of 0.25%. The application of *L. alba* essential oil significantly reduced the vigor of the seed lots, demonstrating that the lettuce is affected by application of this essential oil.

In a study evaluating the allelopathic effect of 112 essential oils, there was a significant reduction in the percentage of germination and root length of lettuce for most treatments with essential oil (Mirmostafaee *et al.*, 2020). These results agree with those observed in the present study, in which the dose of 0.25% already caused inhibition of the germination of the lettuce seeds and, consequently, decreased both the root length and the shoot length. This can be explained by the possibility of the essential oil having major

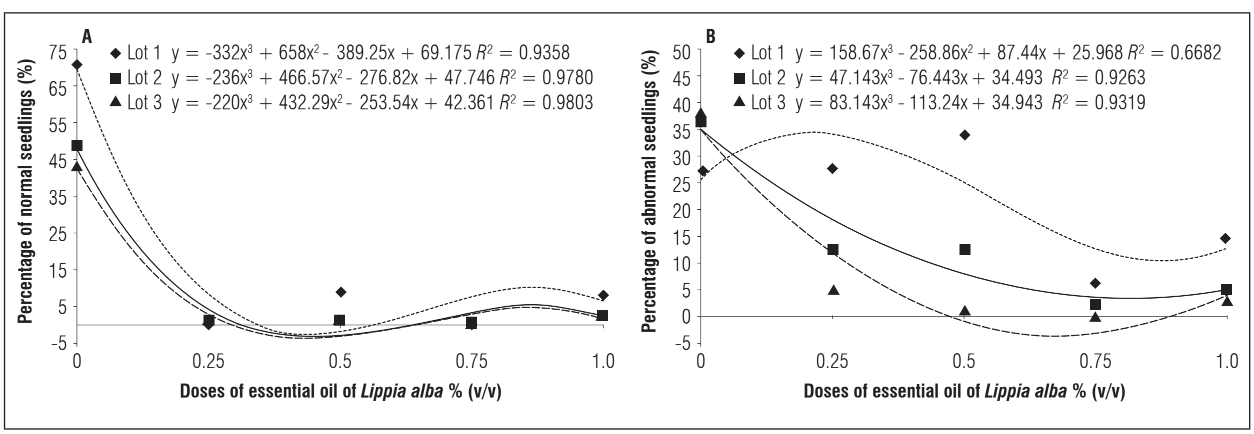

Figure 2. Percentage of normal (A) and abnormal seedlings (B) of commercial lettuce seed lots submitted to different doses of *L. alba* essential oil.

allochemical components with a greater inhibiting action, thus providing results even with the addition of small concentrations of the oil. The increase in the concentrations of essential oil tends to provide a reduction in germination and GSI precisely because of the increase in the concentration of the components responsible for causing an allelopathic effect. Rosado *et al*. (2009) observed phytotoxicity from basil essential oil (*Ocimum basilicum*) in the solution, noting that, with the increase in essential oil concentrations, there was a reduction in the GSI and the percentage of germination lettuce seeds.

For radicle length (Fig. 4A) and shoot length (Fig. 4B), it was observed that the lettuce seeds submitted to treatments with essential oil showed lower values. Similar results were found by Rosado *et al.* (2009), who found a shorter length of lettuce roots with an increase in the doses of basil essential oil (*Ocimum basilicum*), obtaining null values for a concentration of 1%. Root deformity is a good parameter for recording seedling abnormality, as this organ is more sensitive to allelopathic action than the shoot (Pires and Oliveira, 2001), in addition to being the organ that remains in direct contact with the essential oil or allelochemical compound.

It was found that the dry mass of the seedling (Fig. 4C) followed the same pattern of reduction as the vigor variables mentioned above; that is, when the essential oil of *L. alba* was added, there was a reduction in the germinative potential of the seeds and the percentage of normal seedlings. Similar results were obtained by Miranda *et al.* (2015b), who observed that the root and shoot length and the dry mass of the

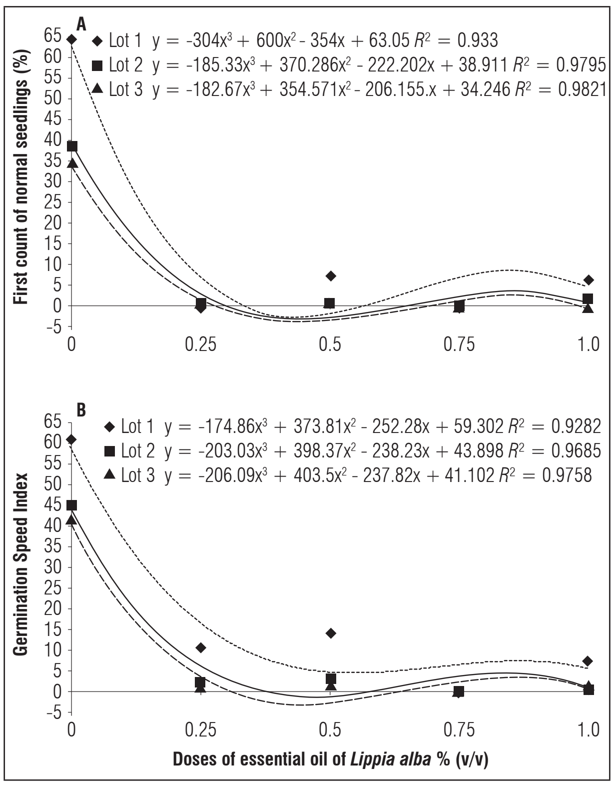

Figure 3. First count of normal seedlings (A) and germination speed index (B) of commercial lettuce seed lots submitted to different doses of *L. alba* essential oil.

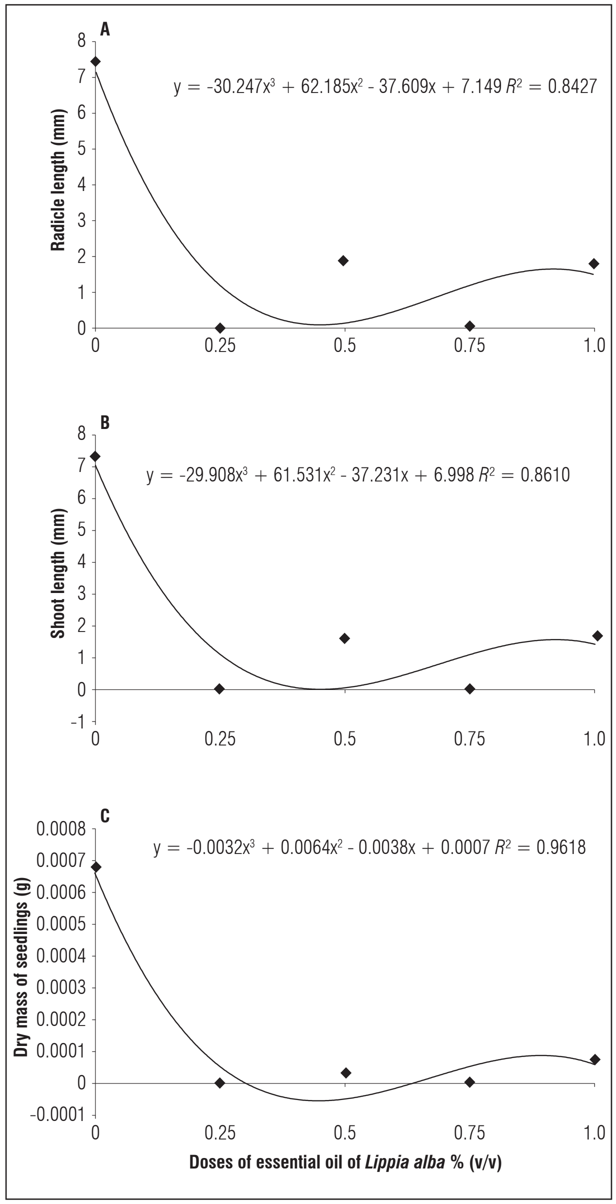

Figure 4. Radicle length (A), shoot length (B) and dry mass of lettuce seedlings (C) in different doses of *L. alba* essential oil.

seedlings were reduced by an increase in the concentrations of locust essential oil (*Ocimum gratissimum*). In the present study, the use of essential oil also significantly reduced the root length and the shoot length, with negative consequences in the increase of dry mass of seedlings since it negatively influenced their growth, providing inferior biomass results. That is, it was observed that the *L. alba* essential oil negatively influenced the variables of first count of normal seedlings, percentage of normal and abnormal seedlings, GSI, radicle length, shoot length and dry mass of seedling, variables that demonstrate the vigor of lettuce seeds.

The averages of the percentage of germinated seeds (Fig. 5A) and the radicle length (Fig. 5B) were significantly higher for lot 1 than the other lots. The reverse was observed for the variable percentage of non-germinated seeds, in which lot 1 had lower values than lots 2 and 3 (Fig. 5A). For the radicle length variable, lot 1 was significantly higher than the other lots (Fig. 5B). This shows that there is variation in commercial seed lots, and the potential for germination and seed vigor of the same species may vary depending on the different commercial lots (Souza Grzyboyski *et al.*, 2015).

Experiment II: Influence of seed treatment with *L. alba* essential oil during emergence and growth of lettuce in a protected environment.

The analysis of variance showed a significant interaction between the lettuce lots and the doses of *L. alba*

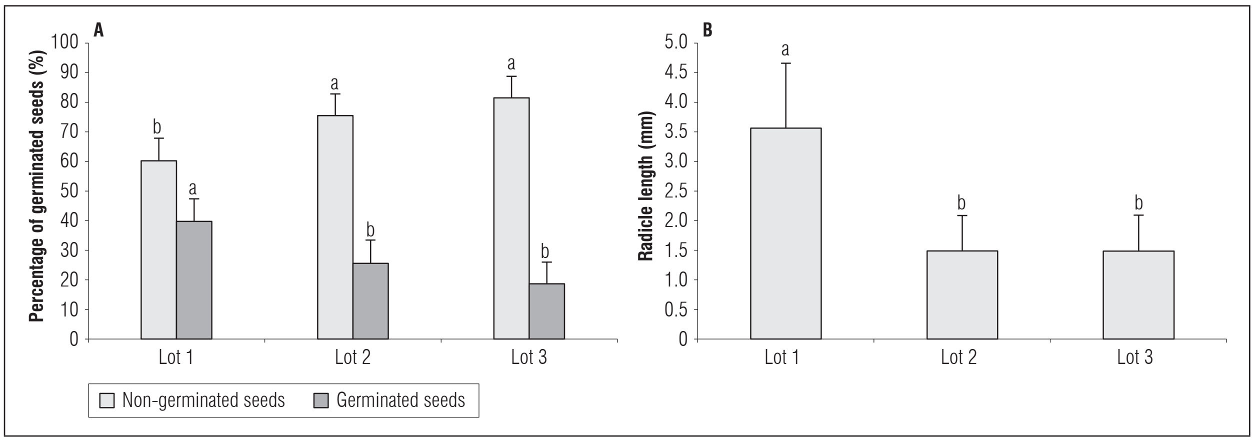

Figure 5. Percentage of germinated and non-germinated seeds(A) and radicle length (B) in the different batches of lettuce seeds. Means with different letters indicate significant statistical difference according to the Tukey test ($P\leq0.05$; $n=4$). The vertical bars indicate ± standard error.

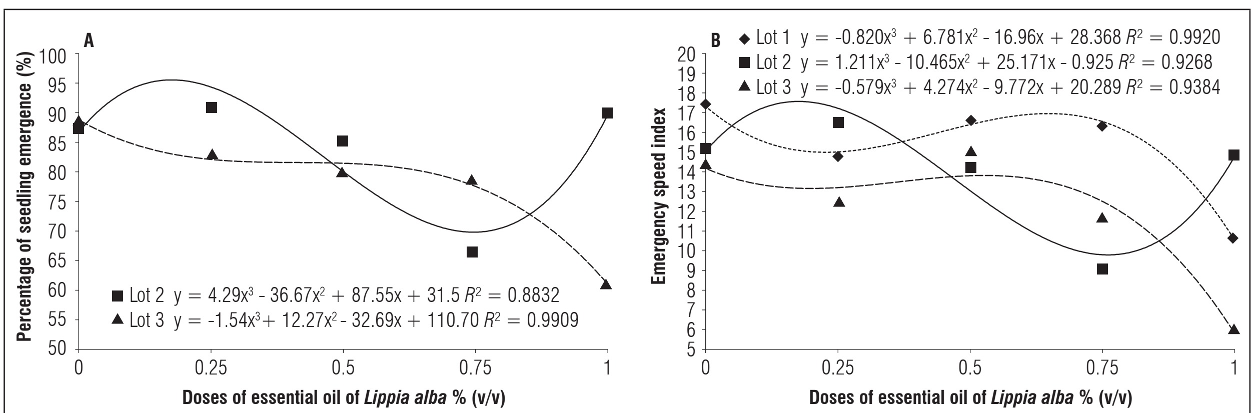

Figure 6. Percentage of seedling emergence (A) and emergence speed index (B) of commercial lettuce seed lots submitted to different doses of *L. alba* essential oil.

essential oil ($P \leq 0.05$) for all analyzed variables. For seedling emergence, lots 2 and 3 showed a trend line with cubic performance, with a reduction in emergence with increasing doses of *L. alba* essential oil (Fig. 6A). For variable ESI, lots 1 and 3 showed the same cubic performance, and there was a reduction in this variable when treatments with essential oil were added. Lot 2 had a sharp drop in ESI up to the dose of 0.75%, and, for the dose of 1%, there was an increase, reaching values close to the treatment without essential oil (Fig. 6B).

Maia *et al.* (2011) observed an allelopathic effect from mint (*Mentha x vilosa*) on the emergence of lettuce seedlings in two types of soil, without and with previous mint cultivation, in which there was a difference for seedling emergence. In the present study, the results indicated the allelopathic potential of *L. alba* in seedling emergence and in lettuce seed ESI.

For the root length variable (Fig. 7A), a quadratic trend was observed for lot 2, with a small increase in root length for doses 0.25 and 0.5%, and, after these doses, it was possible to observe a root length reduction. For lot 3, there was a cubic trend line (Fig. 7A), with a reduction in the root length for all tested essential oil doses, and, after the 0.5% dose, some stabilization was observed.

For shoot length, it was possible to observe a quadratic response for lots 2 and 3 (Fig. 7B), in which there was a reduction in this variable starting with the 0.25% dose. For lot 1, a cubic trend was observed, in which, starting with the 0.25% dose, there was a reduction in this variable (Fig. 7B). For the variable dry mass of seedling (Fig. 7C), lot 2 showed linear performance, with a positive response for this variable. For lots 1 and 3, a cubic trend was observed, reducing the dry mass of seedlings at a dose of 1%. The fact that no direct differences were observed between the variables root length, shoot length and dry mass of seedlings can be explained by the fact that plants normally intensify enzymatic processes and can resist the effect of inhibitory substances by degrading them through of enzyme activation (Almeida *et al*., 2011). In addition, the effect of essential oil allelochemicals may present divergent responses for the concentrations used, where low concentrations can stimulate physiological processes and high concentrations tend to inhibit them (Tigre *et al.*, 2012; Saharkhiz *et al.*, 2010), changing the responses of vegetables to different concentrations and composition of essential oils.

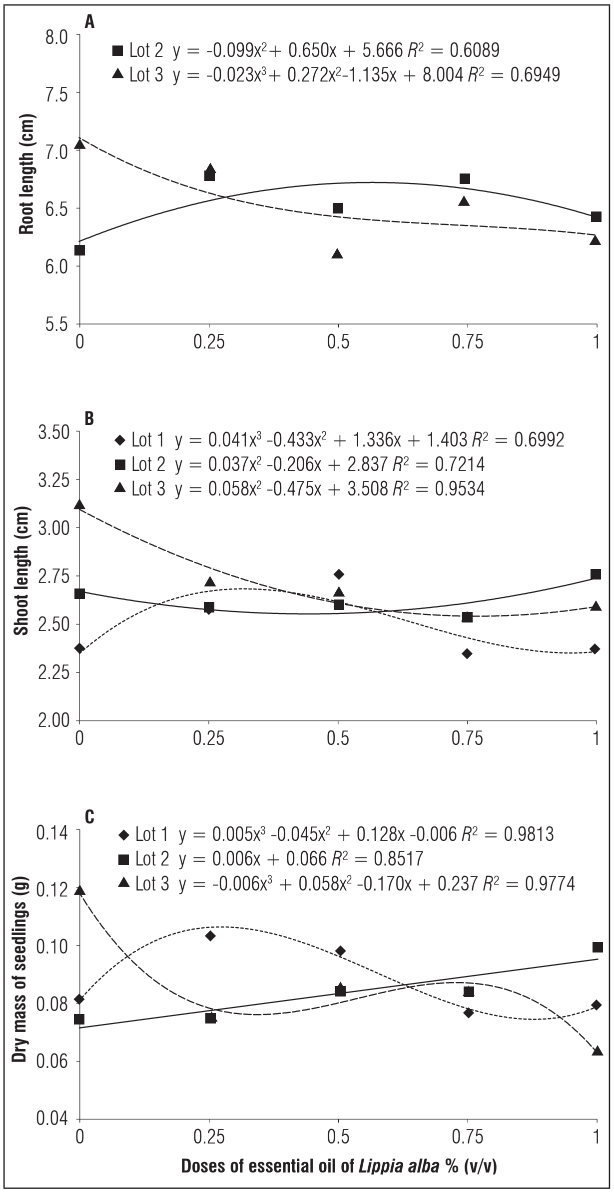

Figure 7. Root length (A), shoot length (B) and dry mass of seedlings (C) of commercial lettuce seed lots submitted to different doses of *L. alba* essential oil.

Therefore, *L. alba* essential oil has characteristics that inhibit germination and seedling growth, mainly in sensitive species such as lettuce (*Lactuca sativa*). Because of the sensitivity to the effect of different metabolites, lettuce is commonly used since it is a model species for evaluating the effect of certain allelopathic substances. The responses observed in this study are very promising; however, it is difficult to recommend an ideal concentration or dose that causes greater allelopathic effect since the highest doses were more favorable for reducing germination, emergence and seedling growth. Therefore, the allelopathic effect of *L. alba* can be evaluated as a potential herbicide, using it in the control of weed growth in order to avoid unwanted competition with crops of interest. In addition, the composition of the essential oil may vary depending on phytotechnical, physiological and environmental factors, and responses may vary by crop. For this, further research is needed to prove which chemical components present in essential oils have this allelopathic effect, in addition to assessing the potential effect on other species of economic and social importance.

CONCLUSIONS

Lippia alba essential oil has an allelopathic potential in lettuce culture, negatively interfering with germination and vigor under laboratory conditions and with emergence under protected environment conditions.

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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Characterization of three *Arracacia xanthorrhiza* Bancroft genotypes using morphological and color parameters

Caracterización de tres genotipos de *Arracacia xanthorrhiza* Bancroft mediante parámetros morfológicos y de color

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Arracacha genotypes evaluated in the study.

Photo: Y.L. Pinto-Acero

ABSTRACT

In Colombia, the arrachacha is a crop with high economic and nutritional value. Despite its agricultural importance, few studies have focused on characterizing morphological parameters between genotypes. Some arracacha genotypes have been classified based on their qualitative traits such as coloration of the root, stem and leaf. Because of the variability of this characteristic, it is necessary to use the colorimetric system for greater precision. Color characterization was performed using the CIELab colorimetric system and morphological parameters (plant height, root diameter and number of fleshy roots) on three different genotypes of *A. xanthorrhiza* Bancroft (Yema de huevo, Paliverde and Yucatana) in the municipalities of Boyaca and Somondoco in the Department of Boyaca, Colombia. The results showed differences in the morphological characteristics plant height, root diameter and number of fleshy roots. The Yema de huevo genotype had a greater root number and diameter and a higher height, closely followed by the Paliverde genotype. This response depended on the environmental conditions of each municipality. The root and stem coloration had differences between the three genotypes, meaning these morphological characteristics can differentiate these genotypes. The Yema de huevo and Paliverde genotypes had the highest color index, a response that depended on the characteristics of each municipality. This research provides information on the physical characteristics of each genotype, which allows for easier visual identification.

Additional key words: arracacha; color index; leaf color; root color; stem color; Andean root.

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RESUMEN

En Colombia, la arrachacha es un cultivo con un alto valor económico y nutricional. A pesar de su importancia agrícola, pocos estudios se han enfocado en la caracterización de parámetros morfológicos entre genotipos. Algunos genotipos de la arracacha han sido clasificados con base en sus características cualitativas como color de la raíz, tallo y hojas. Debido a la variabilidad de esta característica se considera necesario el uso del sistema colorimétrico para mayor precisión. Se realizó la caracterización del color mediante el sistema colorimétrico CIELab y de algunos parámetros morfológicos en tres diferentes genotipos de *A. xanthorrhiza* Bancroft (Yema de Huevo, Paliverde y Yucatana), en los municipios de Boyaca y Somondoco del departamento de Boyacá, Colombia. Los resultados mostraron diferencias en características morfológicas tales como altura de la planta, diámetro de la raíz y número de raíces carnosas. El genotipo Yema de huevo presentó mayor número y diámetro de raíces, y mayor altura, seguido muy de cerca del genotipo Paliverde, esta respuesta dependió de las condiciones ambientales de cada municipio. La coloración de raíz y tallo registró diferencias de color entre los tres genotipos, siendo estas características morfológicas las que pueden servir para diferenciarlos. Los genotipos yema de huevo y paliverde fueron los que presentaron un mayor índice de color, respuesta que depende de las características de cada municipio. Esta investigación proporciona información relacionada con las características físicas propias de cada genotipo que puede permitir más fácilmente su identificación visual.

Palabras clave adicionales: arracacha; índice de color; color de la hoja; color del tallo; color de la raíz; raíces Andinas.

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INTRODUCTION

Roots are one of the more important food products, after cereals in tropical regions (Chandrasekara and Kumar, 2016). In Colombia, arracacha (*Arracacia xanthorrhiza* Bancroft) is a product with high socio-economic importance (Muñoz *et al*., 2015), like other crops in the zone (Gallo *et al*., 2018). In recent years, this crop has increased in the Boyaca Department, mainly in zones at altitudes between 2200 and 2800 m a.s.l. (Alvarado *et al*., 2016). In 2016, more than 6,500 ha of arracacha were planted in Colombia, with a total production of 70,000 t, an average yield of 9.3 t ha^{-1}. In the case of Boyaca in 2016, production rates for arracacha, which represents a significant percentage of the cultivated land area (12.4%), reached 13.47 t ha^{-1} (Agronet, 2018). This root has a high nutritional value because of its high content of protein and macronutrients, such as calcium and phosphorous. Leidi *et al*. (2018) indicated that it has potential as functional foods. Moreover, this root is used in the pharmaceutical and cosmetic industries (Gutiérrez, 2011), and it can be used as an alternative source for starch extraction because of its attractive properties (Castanha *et al*., 2018; Londoño-Restrepo *et al*., 2018; Cruz-Tirado *et al*., 2019).

The arracacha is a minor ecosystem (Cleves-Leguízamo *et al*., 2017), one of the more ancient domesticated plants in the Americas; different types or varieties of arracacha genotypes have been characterized according to the descriptors developed by International Potato Center (CIP, Peru), with partial modifications done by CORPOICA's Plant Genetic Resources and Biotechnology program; these descriptors have exhibited a discriminatory power and have been easy to record at field and laboratory levels in Colombia (Lobo *et al*., 2002). Ignacio *et al*. (2017) found three different morphotypes of grown arracacha with 21 descriptors. Alvarado and Ochoa (2010) characterized some arracacha genotypes and found that one variety was white like radishes and turnips, another was yellow, and the last one was white with a violet ring around the insertion of the crown. This authors also reported that the root characterization was based mainly on the physical appearance, but there was not a morphological and genetic classification (Alvarado and Ochoa, 2010). Likewise, Muñoz *et al*. (2015) reported the preliminary characterization of six genetic materials in the Boyaca Department, which were named Huevo, Paliverde, Palirrusia, Palinegra, Sata, and Satamorada. This study was based on basic traits such as coloration of leaves, stem and roots, but the color was an estimate of the visual and qualitative form. This characterization leads to confusion among farmers when choosing a genotype. Because of the variability of this characteristic, it is necessary to use the colorimetric system for greater precision in physical characterizations.

On the other hand, environmental variations influence root quality, and differences between genotypes are not considered when these varieties are cultivated in different regions (Oliveira *et al*., 2005). Indeed, farmers tend to cultivate only two genotypes, not considering whether the light, soil and temperature are suitable for arracacha crops, which leads to a reduction in the number of genotypes and, in turn, an agro-biodiversity loss (Alvarado and Ochoa, 2010). In the last decade, this crop has seen growing demand, being cultivated in countries such as Australia, the United States and different Central American countries. Despite being long considered a crop with potential economic value, the plant is still almost exclusively produced by smallholder farmers, where 80% of farmers have farms under 3 ha (Alvarado *et al*., 2016). For these reasons, knowledge on arracacha biology, conservation, and the implementation of tools that allow scientists and farmers to recognize existing genetic materials or genotypes and their yield in different climate conditions is essential. This knowledge will allow farmers to reduce the loss of agrobriodiversity and increase arracacha crop production (Alvarado and Ochoa, 2010). Moreover, knowing the effects that different growing environments have on crops may provide information about changes related to pigments, nutrients, and others important aspects (Haynes *et al*., 2010).

The objective of this study was to carry out a characterization of color and morphologic parameters of three genotypes of *Arracacia xanthorrhiza* Bancroft (Yema de huevo, Paliverde and Yucatana) in Somondoco and Boyaca, Boyaca Department, Colombia.

MATERIALS AND METHODS

Location

The study was carried out in Somondoco and Boyaca, Boyaca Department (Colombia). Somondoco is localized to latitude 4º58'59'' N, longitude 73º25'49''W, altitude: 1,670 m a.s.l., with solar radiation of 3.5-4 kWh m^{-2}, mean temperature of 19.9°C and precipitation of 1,340 mm y^{-1}. Boyaca (latitude 5°27'24'' N, longitude 73°21'32'' W, 2,420 m a.s.l.) has a solar radiation of 4.5-5 kWh m^{-2}, mean temperature of 15.2°C and precipitation of 992 mm y^{-1}. The analyses were done in the Laboratory of Plant Physiology in the Faculty of Agricultural Sciences at the Universidad Pedagógica y Tecnológica de Colombia, Tunja.

Morphological characterization

Plant samples in the vegetative stage (which corresponds to 10 months after sowing) were collected. Five samples of plant material, from randomly selected batches (n=30), were collected. Three genotypes: Yema de huevo, Paliverde and Yucatana of *A. xanthorrhiza* were characterized using morphology parameters. Yema de huevo and Paliverde are the most commercial genotypes, while Yucatana is a less commercial genotype and is mainly cultivated for self-consumption (Alvarado and Ochoa, 2010; Alvarado *et al*., 2016).

Arracacha samples were washed in the laboratory and air dried before taking the measurements. The color was determined on the surface of the plant with the CIELab coordinates system L*, a* and b* (Mendoza *et al*., 2006; Balaguera-López *et al*. (2015) using a digital colorimeter, Minolta CR-300 (Minolta, Osaka, Japan). Each value was the average of three readings. L* indicates luminosity and, a* and b* chrome. Negative a* values mean a green color, and positive values indicate a red color. For b*, negative values mean a blue color, and positive values mean a yellow color. With the data, the color index was calculated using the formula $CI= (1000 \times a^*) / (L^* \times b^*)$. The secondary color index in the root (SCIR), primary color index in the root (PCIR), root pulp color index (RpCI), color index of upper side of leaf (CIUL), color index of back side of the leaf (CIBL), primary color index of the stem (PCIS) and secondary color index of the stem (SCIS) were measured. The predominant color in tissue was considered primary. All measurements were taken using the same parameter to measure surface color. The measurements were taken on fully expanded leaves, in the middle part of the stem and the roots.

The plant length was determined by measuring from ground level to the apex using a flexometer. The diameter was measured at the basal part of the fleshy root with a vernier caliper, and the number of fleshy roots was determined with direct counting.

Statistical analysis

The assumptions of the model were visually inspected, and the correct error distribution was chosen. A two-way ANOVA for medians in a completely random design using the WRS2 package (Mair and Wilcox, 2019) was performed in order to compare the effect of genotype, site, and their interaction on the different morphological variables (color index of the root, leaves and stem; length and number of roots and root diameter). The post hoc tests used to compare groups were percentile bootstrap (function mcp2a) and Tukey. The summary statistics were presented as median ± IQR (interquartile range). Generalized linear models were used for analyzing the number of roots (Poisson); the mean ± SD (standard deviation) were shown for this test. The software R, version 3.5.1 (R Development Core Team 2018), was used for all Statistical analysis.

RESULTS

Significant differences in the secondary color index of the root between the genotypes and site were observed (Tab. 1). The Paliverde genotype had a higher index value (4.984) than 'Yema de huevo' and 'Yucatana'. Moreover, in Boyaca, higher coloration values (5.237) were observed than in Somondoco. An effect from the interaction on the secondary index color was not observed (Tab. 1).

There were differences between genotypes for the primary color index of the root, where 'Yema de huevo' (0.782) had higher values than 'Yucatana' (-0.204). The site did not have any effect on the color, but the interaction was statistically significant. This means that the genotype differed in respect to each province. In Boyaca, 'Yema de huevo' had higher values (2.085; Tab. 1).

Statistical differences were found in the root pulp color index between the genotypes, where 'Paliverde' had higher color values (1.02) than 'Yema de huevo' (-0.3975), but not Yucatana. The site and the interaction did not have any effect on the root pulp color index (Tab. 1).

In the color index of upper side of the leaf, differences between genotypes were not observed, but, between sites, there were differences (Tab. 1). Higher color index values were observed in Somondoco (-9.804) than in Boyaca (-11.515). Likewise, the interaction had an effect on the upper side of the leaves, with variation between the genotypes and the sites. In the Paliverde genotype in Boyaca, lower color values (-14.3) were observed in 'Yema de huevo' and 'Paliverde' than in Somondoco. The Yucatana genotype had lower values than 'Yema de huevo' and 'Paliverde' in Somondoco (Tab. 1).

Table 1. Means and P values of the secondary color index of the root (SCIR), primary color index of the root (PCIR), root pulp color index (RpCI), color index of upper side of leaf (CIUL), color index of back side of the leaf (CIBL), primary color index of the stem (PCIS) and secondary color index of the stem (SCIS), traits evaluated in the Yema de Huevo (YH), Paliverde (PV) and Yucatana (Y) genotypes in the municipalities of Boyaca (B) and Somondoco (S).

Traits	SCIR	PCIR	RpCI	CIUL	CIBL	PCIS	SCIS
Genotype	<0.001	<0.001	<0.001	NS	NS	<0.001	<0.001
YH	3.901 b	1.685 a	-0.3975 b	-9.937	-8.851	-6.018 a	41.741 a
PV	4.984 a	0.782 a	1.02 a	-9.6695	-8.1405	-5.2015 a	29.478 b
Y	3.247 b	-0.204 b	0.686 ab	-11.4405	-8.337	-13.8585 b	19.112 b
Site	<0.05	NS	NS	<0.001	<0.001	<0.001	<0.001
B	5.237 a	-0.037	0.626	-11.515b	-8.612 b	-5.712 a	38.897 a
S	3.912 b	1.573	0.587	-9.804a	-7.86 a	-6.324 b	19.112 b
Interaction	NS	<0.001	NS	<0.001	<0.001	<0.001	NS
YH×B	4.23	2.085 a	-0.589	-11.501 bc	-9.535 b	-5.712 ab	50.081
PV×B	5.371	0.113 cd	1.158	-14.3 c	-8.324 ab	-5.065 a	32.337
Y×B	3.509	-0.312 c	0.648	-11.048 bc	-8.228 ab	-8.542 bc	23.856
YH×S	3.89	1.285 ab	-0.252	-7.162 a	-7.831 a	-6.324 ab	39.386
PV×S	4.416	1.832 ab	0.869	-7.856 a	-7.454 a	-5.531 ab	19.112
Y×S	2.985	0.11 abc	0.724	-12.377 bc	-8.446 ab	-20.062 c	12.692

*Means with different letters indicate significant statistical differences for each factor and interaction, according to Tukey's test ($P<0.05$) ($n=5$); NS = not significant.

The genotype did not have an effect on the color index of the back side of the leaf. However, a significant difference between sites was observed, where the most negative values (-8.612) were observed in Boyaca (Tab. 1). Moreover, the interaction had a significant effect on the color index of the back side of the leaf. 'Yema de huevo' showed negative values (-9.54) in Boyaca, as compared to Somondoco and the Paliverde genotype at this site.

The genotype, site and the interaction had a significant effect on the primary color index in the stem. The Yucatana genotype had low values (-13.86). Furthermore, Boyaca had less negative color values (-5.712) than Somondoco. For the interaction, 'Yema de huevo' and 'Paliverde' in Boyaca and Somondoco had higher color values than 'Yema de huevo' in Somondoco (Tab. 1).

In the secondary color index of the stem, the genotype and site had an effect on the color variation, but the interaction did not. When the genotypes were compared, 'Yema de huevo' had higher color values (41.741). Between sites, the color index in Boyaca was higher with a value of 38.897 (Tab. 1).

The plant length of the genotypes showed differences between the Paliverde and Yucatana genotypes (P=0.04), but not between the sites. Similarly, it was observed that the interaction between genotype and province for plant length was significant (P=0.001). The genotype 'Yema de huevo' had a higher plant length in Boyaca than in Somondoco and 'Yucatana' in Boyaca. In addition, the Paliverde genotype had a longer plant length than 'Yema de huevo' in Somondoco and 'Yucatana' in Boyaca.

For the number of fleshy roots, there were significant differences between all genotypes (P=0.001), where Yema de huevo had a higher number of roots than 'Paliverde' and 'Yucatana', and, in turn, 'Paliverde' had

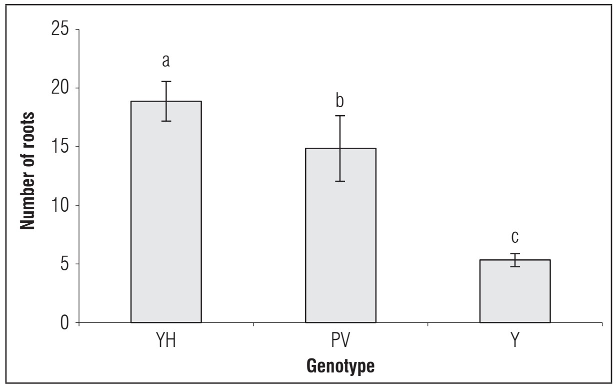

Figure 2. Differences in the number of roots between three arracacha genotypes Yema de huevo (YH), Paliverde (PV), and Yucatana (Y). Means with different letters indicate statistical differences according Tukey's test (P<0.05) (n=5). The vertical bars indicate ± standard error.

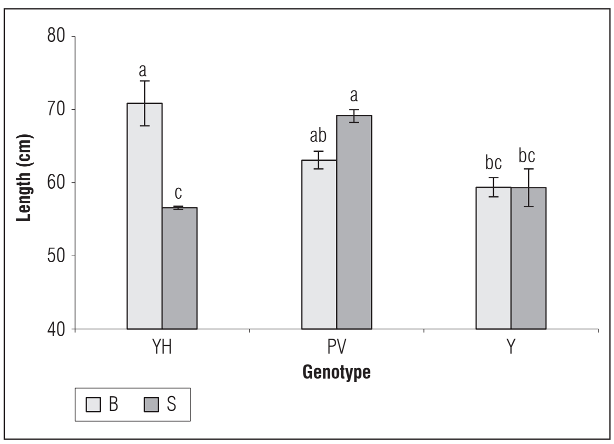

Figure 1. Effect of sites, Boyaca (B) and Somondoco (S), on plant length of three arracacha genotypes Yema de huevo (YH), Paliverde (PV), and Yucatana (Y). Medians with different letters indicate statistical differences, according to percentile bootstrap (P<0.025) (n=5). The vertical bars indicate the interquartile range.

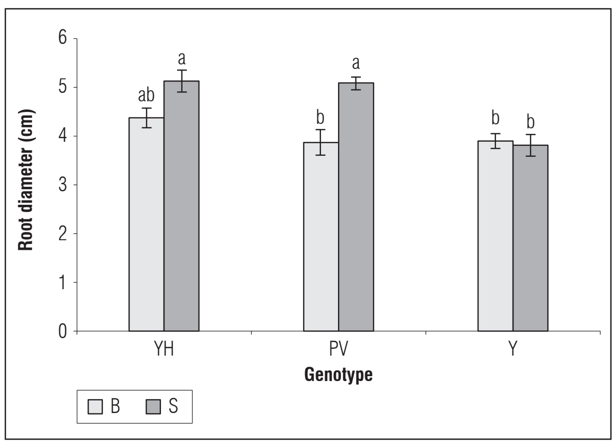

Figure 3. Effect of site, Boyaca (B) and Somondoco (S) on root diameter of three arracacha genotypes, Yema de huevo (YH), Paliverde (PV), and Yucatana (Y). Medians with different letters indicate statistical differences, according to percentile bootstrap (P<0.025) (n=5). The vertical bars indicate the interquartile range.

a higher number than 'Yucatana'. The site (P=0.169) and the interaction between genotype and site did not have an effect on the root number (Fig. 2).

When the diameter of the root was compared, it was observed that the genotype (P=0.001), site (P=0.001) and interaction (P=0.001) had a significant effect on the diameter. In Somondoco, the Yema de huevo and paliverde genotypes had a higher diameter (Fig. 3).

DISCUSSION

The change of coloration in the roots, leaves and stems was represented by the index color (Tab. 1). The CI may be correlated to pigment composition in vegetables tissues. Negative values represent green colors, while values higher than zero are related to yellow or red coloration (Mendoza *et al*., 2006; Balaguera-Lopez *et al.,* 2017). In our case, it was observed that the secondary CI of the roots had positive values and that 'Paliverde' (dark brown) had a higher value than 'Yema de huevo' and 'Yucatana' (pale brownish), possibly because of anthocyanins present in the tissues (Azcon-Bieto and Talon, 2013).

Similarly, in Boyaca, the CI in all genotypes had higher values than in Somondoco, which indicates that location had an effect on this index, but that the interaction between them did not. Boyaca is located at a high altitude and presents major radiation, which stimulate the biosynthesis of anthocyanins as a protection mechanism against damage from excess radiation, which can affect the photosynthetic apparatus (Jimenez-Suancha *et al*., 2015). Similar differences have been observed in carrots, where changes in pigments are associated with temperature, light conditions, and humidity (García de Souza *et al*., 2012). In the primary CI of the root, it was observed that the interaction affected pigmentation in 'Paliverde' and 'Yema de huevo' (pale brown), as compared to 'Yucatana' (wheat color). For this parameter, differences were observed between the genotypes. In this case, 'Yema de huevo' (dark golden rod) and 'Paliverde' (pale brownish) showed positive values, and 'Yucatana' (olive) had negative values, which means that the root pigment content was affected by the genotype (Schmidt *et al*., 2018). These results agree with Muñoz *et al*. (2015), who reported that 'Yema de huevo' and 'Paliverde' genotypes had a yellow color in the root; this measurement was taken with the qualitative method. In addition, the differences between the genotypes was also due to the fact that some have a ring at the root, as seen in 'Paliverde'.

In the leaf color index (CIUL, CIBL, Tab. 1), it was observed that the interaction between genotype and site had significant effects, where fewer negative values were observed in Somondoco than in Boyaca. Moreover, the genotypes 'Yema de huevo' and 'Paliverde' had lower values in Boyaca (dark olive green) than in Somondoco (grey green), but the location did not affect the 'Yucatana' genotype. The pigment content in leaves and its accumulation depend on the amount that is produced, but the destruction of pigments is caused by factors such as wave length of radiation, temperature, and light intensity (Taiz and Zeiger, 2010; Macedo, 2012). For instance, light affects chlorophyll production in different types of plants and also in older and younger leaves (Manrique, 2003; Li *et al*., 2018). It is possible this could explain the variation in the coloration index between the genotypes in different regions, such as Boyaca, which has higher solar radiation, which reduces chlorophyll production, as compared to Somondoco. In the Paliverde and Yema de huevo genotypes, the visual color of the leaves was green (Alvarado and Ochoa, 2010; Muñoz *et al*., 2015).

Finally, it was observed that the interaction had an effect on the stem color index (Tab. 1). For the primary color index of the stem, the genotypes in Boyaca had higher color values than in Somondoco, 'Yema de huevo' and 'Paliverde' had olive colors, as compared to 'Yucatana', which showed a pale olive color. In the secondary color index of the stem, the genotypes and location only differed with respect to 'Yucatana', where 'Yema de huevo' had lower values than all genotypes and locations. As with the root and leaf CI, the stem CI showed variation probably because of environmental variables such as temperature and solar variation. For instance, in Boyaca, an average temperature of 15.2 °C and solar radiation of 4.5 kW m^{-2} were observed, as compared to Somondoco where the temperature is higher (20°C) with a lower level of solar radiation (3.5 kW m^{-2}) (Climate-Data, 2018).

It is important to highlight that a variety of environmental factors affect coloration and pigments in crops (Balaguera-López *et al*., 2017). Coloration changes in vegetables and fruits are correlated with pigment changes such as chlorophyll degradation (Quesada and Valpuesta, 2013; Paliyath *et al*., 2008). Knowledge on how growing environments affect individuals and coloration might provide evidence on pigment content, such as carotenoids in potatoes (Haynes *et al*., 2010). Similarly, different crop plant structures show diverse physiological, physical, chemical reactions, which in turn are affected by environmental conditions (Garcia de Souza *et al*., 2012). In the case of the arracacha, determining the optimal conditions for each genotype might increase yield, production and quality of crops.

According to the morphological variables analyzed in the genotypes Yema de huevo, Paliverde and Yucatan in Boyaca and Somondoco, variation in plant length was observed between the Paliverde genotypes with respect to 'Yema de huevo' and 'Yucatan'. The observed difference was most likely because 'Paliverde' grows better under the environmental conditions of Somondoco than the other genotypes, possibly because this municipality has a higher mean environmental temperature (19.9°C), a situation that apparently favors cell division and elongation processes that generate a higher plant height. For 'Yema de huevo', the results show that this genotype grew better in Boyaca than in Somondoco (Fig. 1) because, in Boyaca, it might capture a higher proportion of light, which influences growth. Similar results have been observed in carrot crops where climate variations in different regions can affect adaptability, stability, production and yield of genotypes (Oliveira *et al*., 2005; García de Souza *et al*., 2012). The values obtained were higher than those reported by Lobo *et al*. (2002) in the southern Nariño arracacha collection, whose average plant height at harvest was 37.57 cm.

For the number of roots, differences between genotypes were observed (Fig. 2), where 'Yema de huevo' had a higher number of roots. In this case, the location did not have an effect, meaning that the number of roots depended on the genotype, as reported by Oliveira *et al.* (2005). This parameter is important because it is a yield component, so, 'Yema de huevo' may be a more productive genotype.

On the other hand, Lobo *et al*. (2002) reported similar values for root width (root diameter) to those found in this study. It was observed that the diameter of the roots was affected by genotype, location, and the interaction between these variables. The Yema de huevo and Paliverde genotypes in Somondoco had a higher diameter than in Boyaca, The Somondoco site has a higher temperature and precipitation average than Boyaca (Climate-Data, 2018). Therefore, it is likely that the environmental conditions in Somondoco, such as temperature and humidity, can generate more root growth, mainly as a result of increased metabolic activity, according to Azcón-Bieto and Talón (2013). It has been shown that higher temperatures increase photosynthesis and the transport rate of photo assimilates from leaves to sink organs (Taiz and Zeiger, 2010). It is important to note that 'Yema de huevo' is a well-adapted genotype, with better growth at both sites (Figs. 1, 2, 3) because this genotype appears to have high rates of photosynthesis and a high percentage of photassimilates stored in sink organ, as Vega *et al.* (2012) observed in carrots.

The highest values of root diameter and number of roots have been reported for cassava at lower altitudes (Noerwijati and Budiono, 2015), probably caused by the higher temperature at lower altitudes. Similarly, it is probable that a higher production and number of roots are related to a higher photosynthetic rate, as found by Jaimez *et al.* (2008) in arracacha crops, where plants cultivated at a lower altitude (1,580 m) had a higher photosynthetic rate and yield than plants cultivated at higher altitudes (1,930). Effect of the zone was also found by Vergel *et al.* (2016).

CONCLUSION

The results show variation in all morphological traits of the genotypes Yema de huevo, Paliverde and Yucatana with respect to location. Color differences from the CIElab coordinate system were found between the genotypes, except in the leaves, indicating that the root and stem coloration can differentiate the genotypes. However, this response depended on the characteristics of each municipality, where 'Yema de huevo' and 'Paliverde' presented a higher color index than the Yucatan genotype. Also, the genotypes Yema de huevo and Paliverde had better performance in both sites (Boyaca and Somondoco), with a longer plant length, higher number of roots, and larger diameter. This information may allow for a more correct visual identification of each arracacha genotype

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Agroclimatic zoning: a planning strategy for agricultural and livestock systems in Alta Guajira, Colombia

Zonificación agroclimática: una estrategia de planificación para sistemas agrícolas y pecuarios en la Alta Guajira, Colombia

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Typical landscape in Alta Guajira.

Photo: D.A. Gómez-Latorre

ABSTRACT

One of the most important dry agroecosystems in Colombia is found in the northern Guajira region, which has native inhabitants (sociocultural aspect) and semiarid zones (ecological aspect). This condition has resulted in great vulnerability in agricultural production systems to adverse climatic events, which require large scale action. For example, the establishment of agroclimatic suitability zones are needed to access information, for decision-making. The aim of this study was to carry out agroclimatic zoning in the municipality of Uribia (La Guajira) for agricultural production systems and animal feed species. The criteria used to identify the agroclimatic suitability zones included: plant coverage present in the municipality, soil suitability, water storage under water stress, regular conditions found in the municipality, and an extreme water deficit event. The evaluated conditions showed variations in agroclimatic suitability during the periods January to April and August to November. During an extreme water-deficit event between August and November, the suitable area for the establishment of production systems with plant species (type C3 and C4) was smaller (77,000 ha) than in the period January to April (130,000 ha). The agroclimatic suitability categories in Uribia did not exhibit differences between the evaluated periods under average water-deficit conditions.

Additional key words: land cover; dry zones; water deficit; production systems.

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RESUMEN

En el norte de la Guajira encontramos uno de los ecosistemas secos más importantes de Colombia desde el punto de vista sociocultural y ecológico. Esta condición genera una gran vulnerabilidad de sus sistemas agropecuarios a eventos climáticos extremos, razón por la cual varias acciones son requeridas para reducirla. Dentro de las acciones se encuentra el establecimiento de zonas de aptitud agroclimática, una fuente de acceso a información que permite tomar decisiones. El objetivo de este estudio fue realizar una zonificación agroclimática del municipio de Uribia (La Guajira) para sistemas de producción agrícola y especies de alimentación animal. Los criterios utilizados para realizar la identificación de las zonas de aptitud incluyeron: coberturas vegetales presentes, aptitud de suelos, almacenamiento de agua bajo estrés hídrico, condiciones regulares climáticas y un evento de déficit hídrico extremo. Las condiciones evaluadas registraron variaciones en la aptitud agroclimática desde enero a abril y desde agosto a noviembre. Bajo la condición de déficit hídrico extremo entre agosto y noviembre, el área apta para el establecimiento de sistemas de producción con especies agrícolas (tipo C3 y C4) fue menor (77.000 ha) comparado con el periodo de enero a abril (130.000 ha). Las categorías de aptitud agroclimática en Uribia no mostraron diferencias entre los períodos evaluados bajo condiciones de déficit hídrico promedio.

Palabras clave adicionales: cobertura de la tierra; zonas secas; déficit hídrico; sistemas productivos.

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INTRODUCTION

There are several incompatibilities between environmental offerings and areas used for agricultural activities since, in many cases, these activities are carried out in areas that experience major climatic threats, such as water deficiencies or excess water in the soil, frosts, and floods, among others (Brown and Raymond, 2014; Kim and Arnbold, 2018). Likewise, the socioeconomic development of communities or producers located in threatened areas suffers high vulnerability, generating inequity in the distribution of resources (Magrin, 2015; Cleves-Leguízamo *et al*., 2017).

The municipality of Uribia in the Department of La Guajira has a desert climate, with annual precipitation averages between 300 and 600 mm and an evapotranspiration of 2,000 mm, which constantly generates water deficit conditions throughout the region (Corpoica, 2016). This situation puts at least 105,000 people of the indigenous Wayúu population who depend mainly on goat production systems at risk.

Rudimentary agricultural practices result in high vulnerability to seasonal rainfall variability and extreme weather events. The third national agricultural census (CNA) for year 2013 recorded 173,134 sheep (mostly *Ovies aries* "camuro" breed) and 243,351 goats (mostly *Capra aegagrus hircus* "guajira" or "criolla" breed) for this municipality, a ratio of 1:1.4 (DANE, 2014), associated with regular land use patterns such as transhumance. The main sources of animal feed are pastures and shrubs, so the mobility and diet of the animals depend on the scarce distribution and variability of plant species (Steinfeld *et al*., 1996; Shinde *et al.*, 1998; Oba *et al.*, 2000).

Agroclimatic-zoning studies contribute to agricultural system adaptation since they provide information for the planning of agricultural activities and establish incompatible uses that negatively affect sustainability and competitiveness (Gelcer *et al*., 2018). Furthermore, they incorporate climatic and agroclimatic indicators that are useful for identifying potential areas in the establishment of production systems and contribute to the generation of strategies for climate change and variability prevention and adaptation (Basualdo *et al.*, 2015).

Zoning based on the climatic variables of solar radiation, temperature, and precipitation (Arce and Uribe, 2015), as well as the evapotranspiration of the reference crop (ET_0) (Allen *et al.*, 1998), are determining factors in the behavior analysis of plants or agricultural crops of interest with climatic variability events and in the study of adaptation mechanisms for food production. Average values of these elements have been used in countless zoning studies for multiple crops, usually based on methodologies such as agro-ecological zoning or AEZ (FAO, 1996; Fischer and Velthuizen, 2002).

Some agroclimatic zoning analyses include factors such as thermal supply and water availability on daily or decadal scales and specific aspects of crop physiology and edaphoclimatic requirements (Brunini *et al.*, 2001). Others incorporate thematic layers that characterize the territory, such as land cover, obtained primarily from the performance of land resource inventories (FAO, 1996). Moreover, many studies have adopted methods for analyzing suitability through geographic information system (GIS) methodologies, following multicriteria decision-making approaches in different situations (Amiri *et al.*, 2012; Kioko *et al.*, 2012; Zhang *et al.*, 2014; Keno and Suryabhagavan, 2015).

Martínez *et al.* (2016) conducted territorial studies of water excess and deficiency susceptibility in soil in order to construct an agroclimatic zoning methodology. They performed their analysis based on the conceptualization of agroclimatic risks by Field *et al.* (2012), integrating the soil requirements of a tomato crop and the monthly probability of the occurrence of moisture conditions in a soil with a water deficit. The water deficit analysis was done with a data series from the period between 1980 and 2011 using the Palmer Drought Severity Index (PDSI) during each of the crop development-cycle months. This methodology identified "productive niches" or potential areas for tomato production, i.e. areas that exhibited low soil limitations and low water deficit probability in the soil.

Given the importance of this region in Colombia given the dry agroecosystem characteristics, the type of natural cover, and the high degree of vulnerability, the aim of this study was to carry out agroclimatic zoning of the municipality of Uribia in order to define suitable agricultural development areas under conditions of a water deficit in the soil.

MATERIALS AND METHODS

Study area

Uribia is in the northernmost region of the Department of La Guajira, Colombia, between longitudes 71°05' and 72°23' W and latitudes 11°31' and 12°28' N, with an area of 787,202 ha (Fig. 1). Strong trade winds from the northeast, sand displacement, and low total annual precipitation volumes (<500 mm) result in a tropical desert biome with accentuated aridity conditions.

The driest season of the year, when water deficiency conditions intensify, occurs between December and March as a consequence of dry northeast winds and the displacement of the ITCZ (Intertropical Convergence Zone) to the south. The rainy season occurs between August and November, as the ITCZ reaches its northernmost position (Eslava, 1993; Mesa *et al.*, 1997).

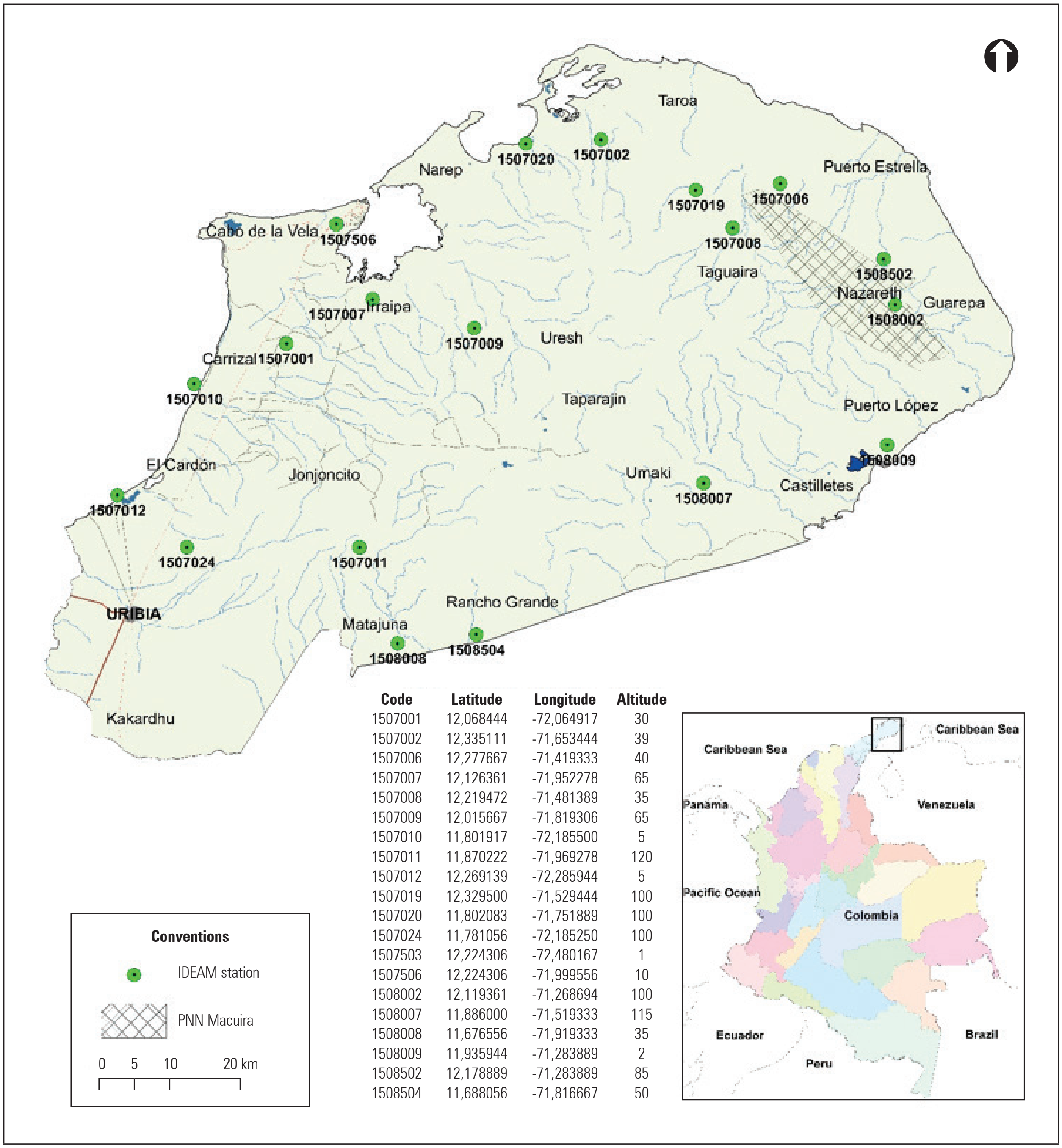
The ET_0 is always greater than the average rainfall, and moisture storage in the soil is minimal or null since the entire available supply is consumed by the high atmospheric demand. The soils are sodium saline, vulnerable to erosion, and have relatively limited fertility. The existing vegetation cover, dominated by desert scrub and sub-xerophytic forests, is subject to high temperatures (Pinilla and Zuluaga, 2014).

Agroclimatic zoning

The zoning was based on an analysis of land use suitability using the land evaluation scheme (ET) published by FAO (1976), the calculation of the probability of the occurrence of water deficit conditions in the soil based on the PDSI (Palmer, 1965), the study carried out by Martínez *et al.* (2016), and the availability and type of plant cover using Corine Land Cover (CLC) (IDEAM, 2012). Then, these analyses were integrated into monthly agroclimatic scenarios (AS). Finally, the AS was used to generate the agroclimatic zoning.

Land use suitability

The land use suitability for the plant species of interest (crops, pastures, and forages) in the agricultural production systems of Uribia was evaluated. The ET methodology was implemented on a general scale (1: 100,000), and physical and chemical soil characteristics were considered, as well as slope and natural drainage conditions, factors that are decisive for the development of agricultural activities. The information source for this evaluation was the general soil survey carried out by IGAC (2009) in the Department



Code	Latitude	Longitude	Altitude
1507001	12,068444	-72,064917	30
1507002	12,335111	-71,653444	39
1507006	12,277667	-71,419333	40
1507007	12,126361	-71,952278	65
1507008	12,219472	-71,481389	35
1507009	12,015667	-71,819306	65
1507010	11,801917	-72,185500	5
1507011	11,870222	-71,969278	120
1507012	12,269139	-72,285944	5
1507019	12,329500	-71,529444	100
1507020	11,802083	-71,751889	100
1507024	11,781056	-72,185250	100
1507503	12,224306	-72,480167	1
1507506	12,224306	-71,999556	10
1508002	12,119361	-71,268694	100
1508007	11,886000	-71,519333	115
1508008	11,676556	-71,919333	35
1508009	11,935944	-71,283889	2
1508502	12,178889	-71,283889	85
1508504	11,688056	-71,816667	50

Figure 1. Location of meteorological stations used for agroclimatic zoning in the municipality of Uribia (PNN: National Natural Park).

of La Guajira, with which the soil offer of land units (UdT) was identified. The edaphic requirements for the development of the agricultural systems were elucidated by consulting secondary information based on the databases of FAO and the Colombian Corporation for Agricultural Research (Agrosavia).

A decision tree was generated in order to determine soil suitability, divided into optimal (A1), moderate (A2), marginal (A3), and unsuitable (N) classes. After the identification of these use suitability classes in the municipality, they were grouped into three categories: suitable soils (classes A1 and A2), soils conditioned to management practices (with severe restrictions depending on the evaluated variables) (classes A2 and A3), and unsuitable soils (classes A3 and N).

Soil humidity conditions

The occurrence frequency of average water-deficit conditions was established, i.e. the prevalent condition in Uribia and the extreme water deficit in the soil associated with climate variability events such as the El Niño phenomenon. The monthly data series of precipitation, average maximum, medium and minimum temperature, relative humidity, and solar brightness was determined for the period 1980 to 2011. The information was gathered from 20 stations belonging to the meteorological observation network of the Colombian Institute of Hydrology, Meteorology, and Environmental Studies (IDEAM) in the study area (Fig. 1).

Based on the methodology of Martínez *et al*. (2016), the PDSI was calculated (Palmer, 1965), and index values were established for the average water deficit in Uribia (between -2.00 and 0.50) and for the extreme water deficit in this region (less than -2.00) (Tab. 1). Frequencies were calculated for the periods January to April (JFMA), the driest period of the year, and August to November (ASON), the rainiest period.

Table 1. PDSI categories that indicate average water deficit and extreme water deficit conditions for agricultural production systems in Uribia.

PDSI category	Value	Soil humidity condition	Index ranges and thresholds
Normal	(-0.50 – 0.50]	Average water deficit	[-2.00 – 0.50)
Incipient	(1.00 – -0.50]		
Reduced	(-2.00 – -1.00]		
Moderate	(-3.00 – -2.00]	Extreme water deficit	< -2.00
Severe	(-4.00 – -3.00]		
Extreme	$\leq$ -4.00		

Based on the frequency of occurrence of the PDSI values from each of the climatological stations, the monthly probability of average water deficit was established for each four-month period, taking into account the entire series. Only PDSI values lower than -0.5 were assessed for extreme water deficit. Then, five levels were established in order to determine the frequency of occurrence of each water deficit condition as follows: very low (0-20%); low (20-40%); medium (40-60%); high (60-80%) and very high (80- 100%).

Land cover

The different land cover types in the municipality were identified based on the thematic layer of land cover for the period 2005-2009 at a scale of 1:100,000, generated with the CLC methodology (IDEAM, 2012). The land cover types were classified into three categories based on their level of limitation for implementing agricultural systems (Tab. 2).

Table 2. Limitation category for conserving water in the soil according to the cover type.

Limitations for agricultural systems	Coverage type
High	Sandy areas
	Sandbanks
	Dune fields
	Rocky outcrop
	Bare and degraded lands
Medium	Herbaceous open land
	Open shrubland
Low	Clean pastures
	Crop mosaic
	Pasture and crop mosaic
	Crop, pasture and natural spaces mosaic
	Pasture mosaic with natural spaces
	Low dense forest
	Low open forest
	Fragmented forest with pastures and crops
	Fragmented forest with secondary vegetation
	Gallery and riparian forests
	Dense herbaceous land
	Dense shrubland
	Secondary or transition vegetation

Monthly agroclimatic scenarios

Agroclimatic scenarios (AS) constitute a spatial monthly representation where use suitability, soil moisture conditions, and land coverage are integrated. The AS were constructed by overlapping with GIS geoprocessing tools, showing the behavior and dynamics of the soil moisture (extreme and average water deficit) during the analyzed periods (JFMA and ASON).

Agroclimatic suitability

The areas with a low water deficit exposure were identified with the AS, that is, the areas that persistently exhibited a low and medium average water deficit and extreme water deficit frequencies of occurrence. The agroclimatic suitability was classified into six categories according to the ranges established in a qualification matrix (Tab. 3).

Finally, four agroclimatic suitability maps for agricultural systems in Uribia were elaborated.

RESULTS AND DISCUSSION

Soil use suitability

Most of the Uribia area exhibited restricted conditions for establishing and developing agricultural systems since more than 80% of the soils exhibited marginal suitability or were not suitable for agricultural uses. Only 3.7% of the soils were suitable (A1), while 15% had moderate suitability (A2), 31.5% were marginal (A3), and most, i.e. 49.8%, were not suitable (Fig. 2).

Humidity condition

Average water deficit

During the dry quarter, JFMA, the probability of occurrence of an average water deficit was mainly medium (40-60%) (Fig. 3: A, B, C, and D). The water deficit probability increased in February and March as a result of intensification of the dry period.

Table 3. Agroclimatic suitability qualification categories for goat production in the municipality of Uribia.

Category	Soil use suitability (FAO)	Coverage limitation	Average/extreme water deficit probability
Areas recommended for goat production, and with potential for the establishment of plant cover	Optimal with low restrictions	Low and medium	Very low, low, medium (<60%)
	Conditioned to management practices		
Areas limited by low or no current vegetation cover, and with potential for the establishment of plant cover	Optimal with low restrictions	High	Very low, low, medium (<60%)
	Conditioned to management practices		
Areas limited by high exposure to water deficit	Optimal with low restrictions	Low and medium	High, very high (>60%)
	Conditioned to management practices		
Areas not recommended for goats	Not recommended (unsuitable)	High	High, very high (>60%)
	Optimal with low restrictions		
	Conditioned to management practices		
	Not recommended (unsuitable)	High	Very low, low, medium (<60%)
Areas for current goat use and with soil limitations for the establishment of plant cover	Not recommended (unsuitable)	Low and medium	Very low, low, medium (<60%)
Areas for current goat use and with low potential for the establishment of plant cover	Not recommended (unsuitable)	Low and medium	Very low, low, medium (>60%)

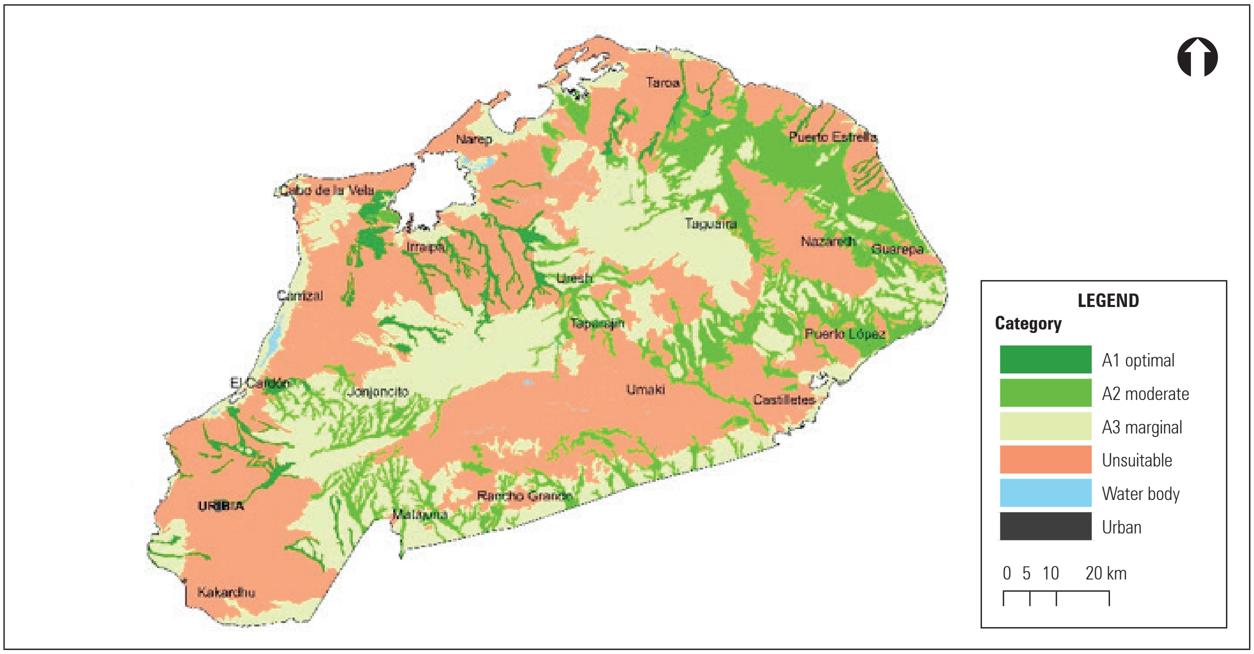


Figure 2. **Soil suitability classification for agricultural uses in the municipality of Uribia.**

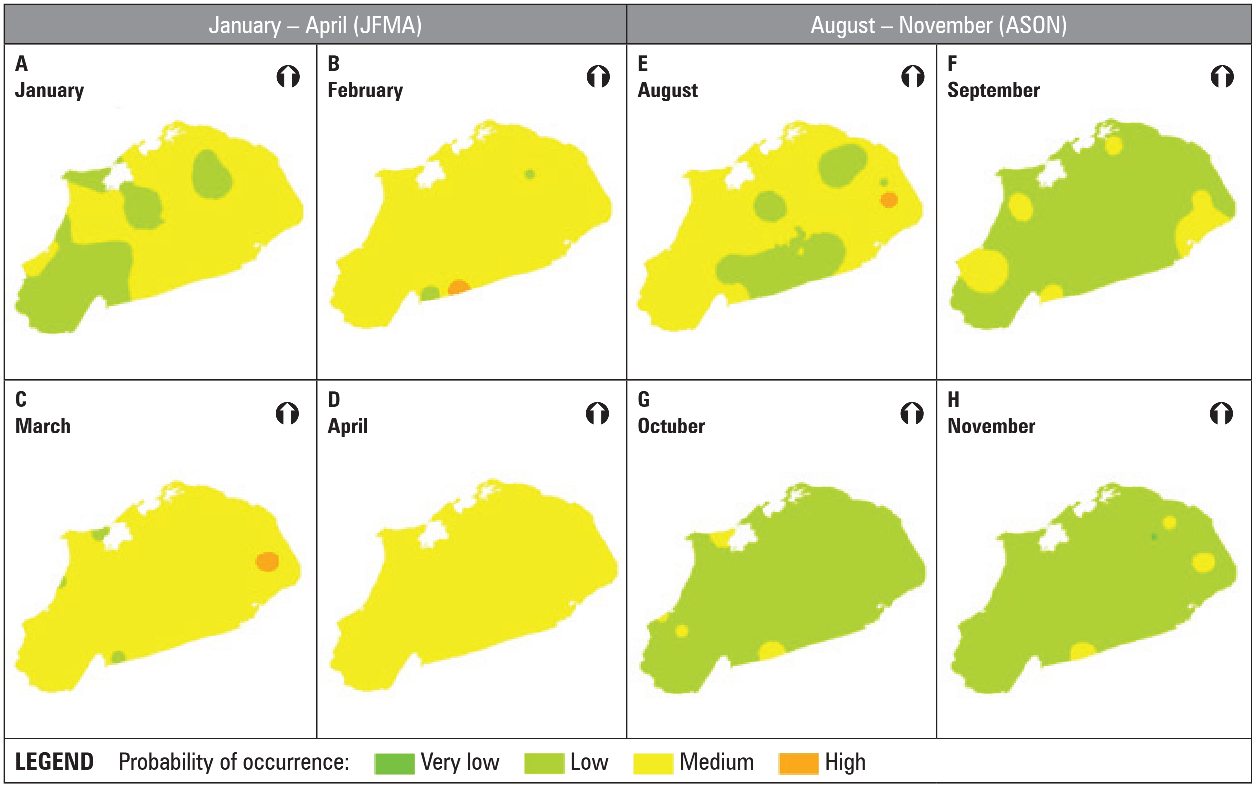


Figure 3. **Monthly frequency of occurrence of average water deficit conditions in the soil of Uribia during the dry (JFMA) and the rainy (ASON) periods.**

By contrast, during the ASON period, low probabilities of the occurrence of an average water deficit occurred in most of the municipality. Furthermore, during the months of September, October, and November, the probabilities did not exceed 40% (Figs. 3: E, F, G, and H).

Extreme water deficit

The frequency of occurrence of an extreme water deficit was mainly average (40%-60%) during the two periods (Fig. 4: A, B, C, and D). In comparison with the January-April period, during the rainy four-month period, a larger area of the municipality was exposed to a high frequency (60%-80%) of extreme water deficit conditions, mainly during October and November (Figs. 4: E, F, G, and H).

Land coverage

Shrub vegetation represents the most abundant cover type (48.4%) in Uribia, followed by the coverage associated with bare and degraded lands (25.6%). Thirty-four categories of land use were identified in the municipality and reclassified according to the limitations (Fig. 5).

Agroclimatic suitability for agricultural systems

Average water deficit condition

Under average water deficit conditions, approximately 171,000 ha (22% of the municipality) were identified as "areas recommended for goat production with potential for the establishment of vegetation cover" in the two evaluated periods (Fig. 6: A and B, and Tab. 4). These areas exhibited fewer limitations for the establishment and management of smaller species (i.e. goats). Moreover, they exhibited dense and continuous coverage (grasslands, shrubs, forests, pastures, and some crops) that provide a viable food supply for goats. They exhibited optimal soils for the system or for effective depth management practices:

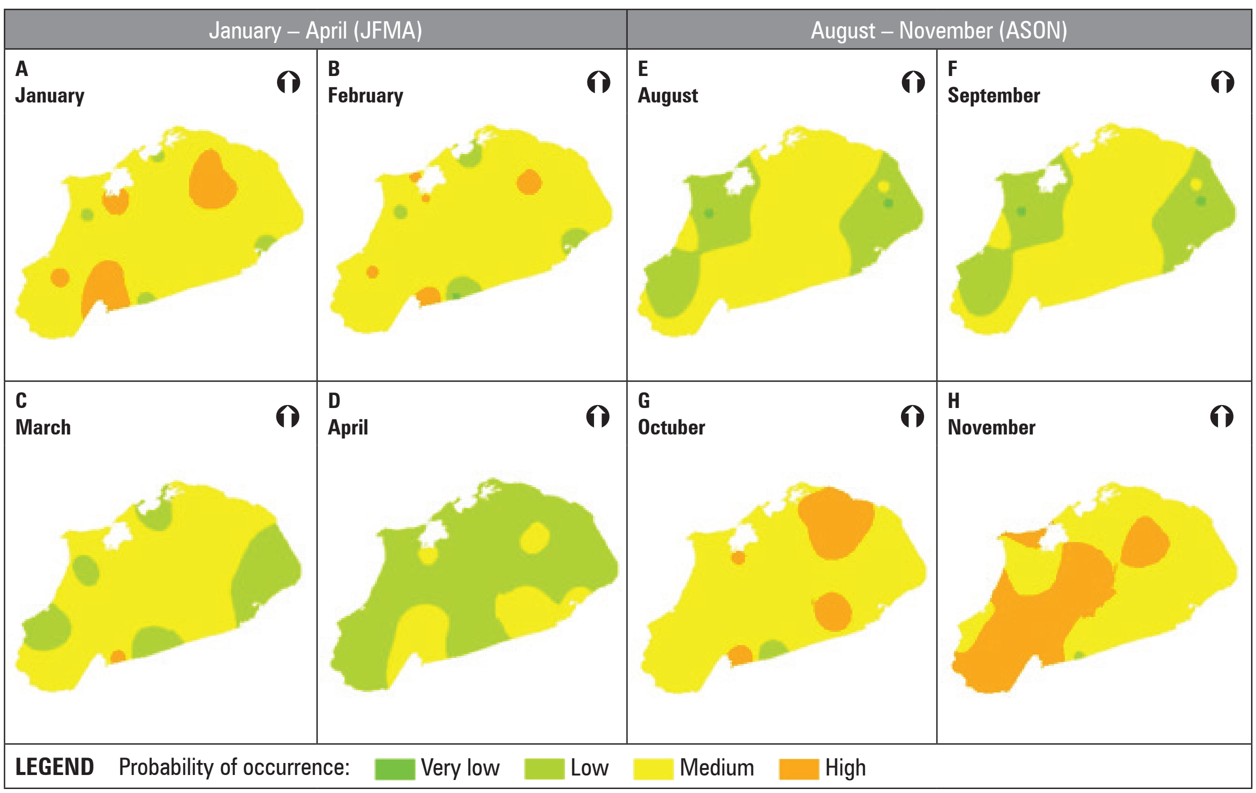


Figure 4. Monthly frequency of occurrence of extreme water deficit conditions in the soil of Uribia during the dry (JFMA) and the rainy (ASON) periods.

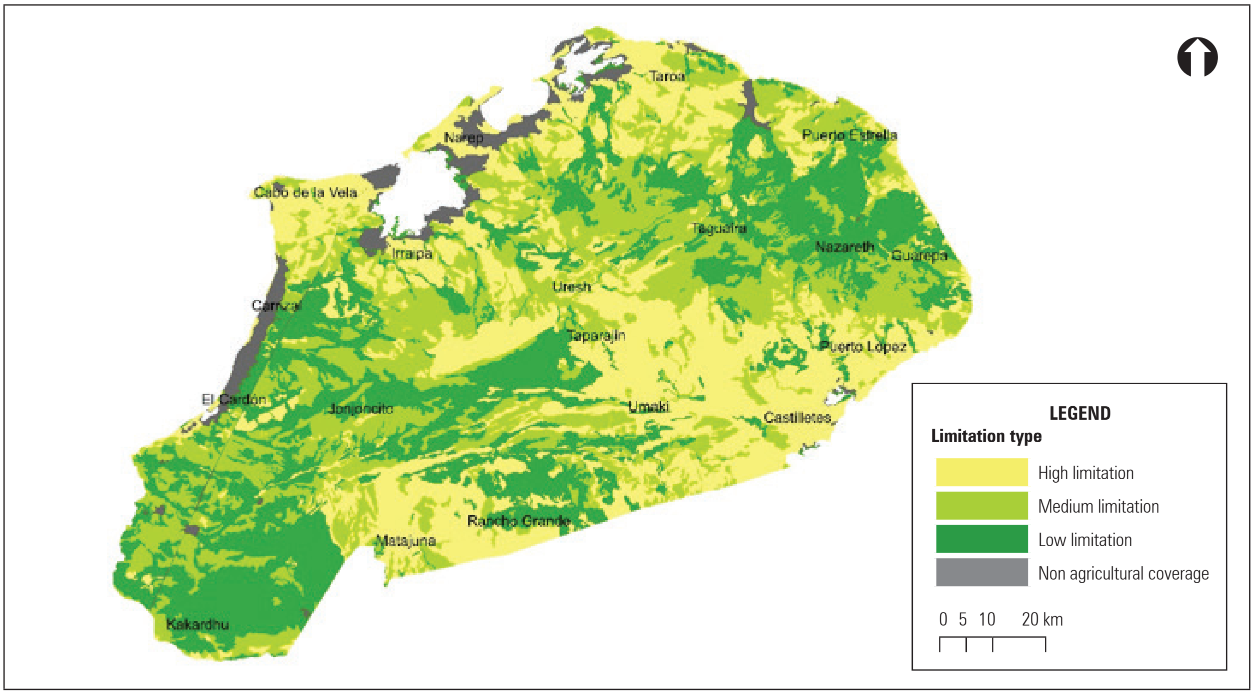


Figure 5. Land cover classification in the municipality of Uribia according to limitations for the conservation of water in the soil.

coarse textures, alkaline pH, compacted horizons, excessive drainage, salinity, and sodium content. The frequency of average water deficit conditions was predominantly low (<40%) although the probabilities were between 40% and 60%, indicating less exposure to water deficits for the species established in these zones. Under the average conditions in the municipality and considering factors such as soil, water deficit, and coverage, these areas have a lower agroclimatic risk for goat grazing and for the establishment of agricultural species that would ensure food security in the region.

Nearly 75,000 ha in each four-month period corresponded to "areas limited by low or no current vegetation coverage and with potential for the establishment of vegetation cover" (Tab. 4). In these areas, there is currently not adequate plant cover for goat grazing. They are bare lands where the frequency of water deficits is less than 60% and where the soil, despite its limitations (at the effective depth level, texture, pH, compaction, drainage, salinity, and sodium), would allow for the establishment of a vegetation cover. The recommendation is to start planting shrubs, grasslands, and adapted forest species that could serve as a food source for goats in the medium term.

Around 21,000 ha scattered throughout the municipality were identified as having the greatest risk, i.e. cataloged as "areas not recommended for goats". There, the establishment of agricultural crops or even minor species is not recommended. The areas known as "areas for current goat use with soil limitations for the establishment of vegetation cover", which covered more than 40% of the municipality, have low and continuous coverage (mainly grasslands and shrubs) that can supply food to animals in the short term.

Extreme water deficit condition

When analyzing "areas recommended for the production of goats and with potential for the establishment of vegetation cover", under extreme water deficit conditions in the two evaluated periods, reductions of 35% (period JFMA) (Fig. 6C and D) and 55% (period ASON) were found, as compared to the 170,000 ha identified as having an average water deficit. During the first four-month period, approximately 130,000 ha were in this category.

Because of the significant reductions in the area with a lower agroclimatic risk, the ASON period was the most critical one since about 90,000 ha were no longer recommended for goat production, becoming "areas limited by high exposure to water deficit", where the high frequency of extreme water deficit conditions limits the establishment of plant species.

In general, the areas with the greatest agroclimatic risk for extreme water deficit conditions totaled between 306,000 and 513,000 ha for the JFMA and ASON periods, respectively (Tab. 4). This increase in the risk area for goat production resulted from the greater impact of extreme water deficit phenomena during the second semester of year. The greatest alteration in precipitation volume and maximum temperature were recorded between June and December as a result of events such as El Niño.

Vegetation growth and development varied depending on the humidity conditions and limitations in the soils for the establishment of vegetation cover. The nutritional status of goats was affected, generating malnutrition and low meat and milk production, which are main products of goats in this area (Roncallo, 2002). Furthermore, malnutrition conditions can be exacerbated by a lack of management practices (Islam *et al.*, 2002).

Goat and sheep production systems have a wide range of adaptation under favorable and extreme weather conditions (Arbiza, 1986; Devendra, 2006, cited by Grajales *et al.*, 2011); frequently, they are established in areas with climatic limitations (dry areas with low rainfall) and areas of low vegetation. However, the success of feeding these species is based on the efficient selection of the most nutritious parts of herbaceous and shrubby vegetation with a lower water requirement because of the ability to concentrate and reduce the volume of urine, evaporation of feces, and reduction of water losses through evaporation, as reflected in a higher digestibility of fiber-rich foods (Grajales *et al.*, 2011). Therefore, it is necessary to generate alternatives for the management of goats and sheep that guarantee a food supply and security in communities since this production system represents 60% of the meat diet of the inhabitants (Márquez, 2006).

Agroclimatic suitability zoning is in line with the proposal by Gupta (1992) and the concept of zooclimatic regions, incorporating factors inherent to the development of a goat-productive system, such as species preference and ecological niches, considering the diversity of biomass sources such as trees, shrubs, or pastures (protein banks). Moreover, the developed zoning incorporates water balance and land cover elements, as does the zoning used in pasture suitability studies, such as those developed following the FAO method (1991), where emphasis is placed on forage

Table 4. Agroclimatic suitability for goats in the municipality of Uribia under two water deficit conditions.

Category	Average water deficit		Extreme water deficit	
	JFMA area ha (%)	ASON area ha (%)	JFMA area ha (%)	ASON area ha (%)
Areas recommended for goat production with potential for the establishment of plant cover	171,440.0 (21.8%)	172257.6 (21.9%)	129,795.5 (16.5%)	77,485.5 (9.8%)
Areas limited by low or no current vegetation cover with potential for plant cover establishment	75,387.7 (9.6%)	76261.7 (9.7%)	57,575.1 (7.3%)	30,157.4 (3.8%)
Areas for current goat use with soil limitations for the establishment of plant cover	324,686.7 (41.2%)	325121.0 (41.3%)	292,251.2 (37.1%)	164,756.9 (20.9%)
Areas limited by high exposure to a water deficit	817.6 (0.1%)	-	42,462.2 (5.4%)	94,772.1 (12.0%)
Areas for current goat use with low potential for the establishment of plant cover	434.3 (0.1%)	-	32,869.8 (4.2%)	160,364.0 (20.4%)
Areas not recommended for sheep and goats	213,147.6 (27.1%)	212273.6 (27.0%)	230,960.2 (29.3%)	258,377.9 (32.8%)
Total area	785,913.9 (100%)			

JFMA: dry period from January to April; ASON: rainy period from August to November

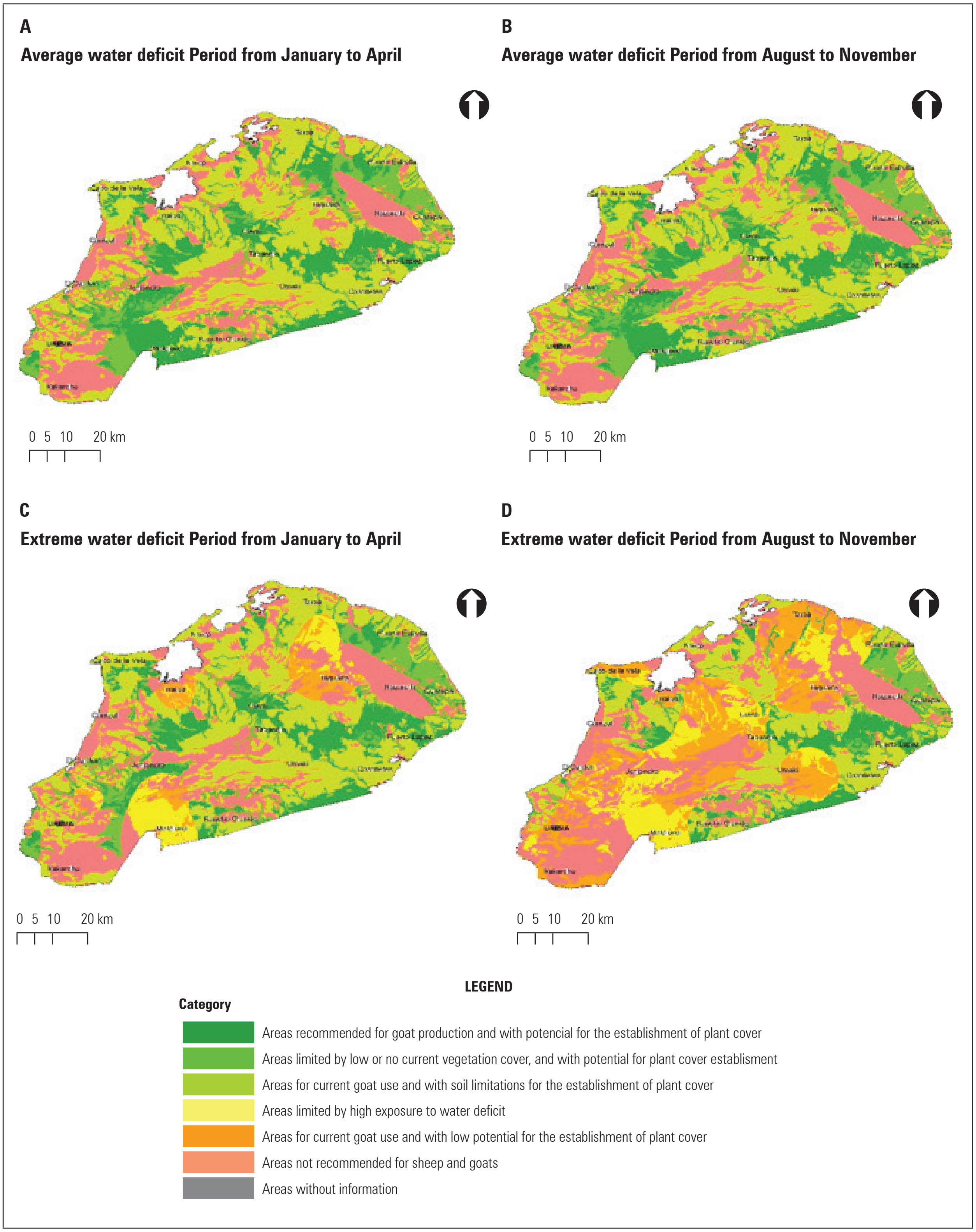


Figure 6. **Agroclimatic suitability for goat production systems under: A) average water deficit conditions in the JFMA period; B) average water deficit conditions in the ASON period; C) extreme water deficit conditions in the JFMA period, and D) extreme water deficit conditions in the ASON period.**

production, water resources, and soil sensitivity to erosion (Amiri *et al.*, 2012; Keno and Suryabhagavan, 2015). Although agroclimatic suitability zoning is a characterization tool, it is more accurate if there is soil, climate, and land cover data on detailed scales. In addition, it must be accompanied by feedback from system information that allows decisions to be made (from producers to rural planners).

CONCLUSIONS

In the municipality of Uribia, under average water-deficit conditions, around 171,000 ha were identified as having the lowest agroclimatic risk, recommended for goat production with the potential to establish plant cover. Furthermore, 75,000 ha were identified as areas with the potential for the establishment of vegetation cover.

Under extreme water deficiency conditions, the recommended areas for goat production varied according to the four-month period (the highest and lowest volume of average rainfall). The largest available area was in the JFMA (January to April) period (approximately 130,000 ha), while for the ASON (August to November) period, the available area was much smaller (approximately 77,000 ha), showing the impact of an extreme water deficit phenomenon in the second semester, where the average rainfall volumes were drastically reduced.

Currently, most of the producers are located in areas with limited soil conditions that are not recommended for this land use because of average water-deficit conditions and low extreme water-deficit conditions that are subject to high exposure to water-deficit conditions and are strongly limited by soils, with little food source availability. Local adaptation strategies need to be developed to mitigate the impact of these conditions, identify alternative areas for mobilizing ethnic communities and minimize animal losses and risks for food security, thereby improving the livelihood of the locals.

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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 the validity of the presented results at risk.

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Bovine manure and rock powder and their influences on the chemical characteristics of a Latossolo soil type (yellow oxisols) under butter kale (*Brassica oleracea* L. var. acephala) cultivation

Estiércol bovino y polvo de roca y sus influencias sobre las características químicas de un tipo de suelo Latossolo (oxisoles amarillos) bajo cultivo de col rizada de mantequilla (*Brassica oleracea* L. var. acephala)

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Butter kale experiment view.

Photo: M.B. Pereira

ABSTRACT

Chemical soil quality is one of the factors more quickly affected by anthropogenic degradation processes and is one of the more important components for the development of agriculture. Thus, this study aimed to evaluate the effects of different doses of cattle manure and rock powder on the chemical characteristics of soil cultivated with butter kale. The treatments were arranged in five randomized blocks in a 4×4 factorial for the different doses of bovine manure (60, 120, 180 and 240 g/plant) combined with doses of rock powder (6, 12, 18 and 24 g/plant). Each block was composed of three plots, 18 m long and 1 m wide. The bed was composed of six portions, and each experimental plot consisted of 14 plants spaced at 0.40×0.40 m. At the end of the experiment, the following were analyzed: pH, organic matter, P, K, Na, Mg, exchangeable acidity, cation exchange capacity (CEC), sum of base and base saturation. The doses of bovine manure and MB-4 provided an increase in pH, organic matter, concentration of phosphorus, potassium, sodium, calcium, magnesium,

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exchangeable acidity, cation exchange capacity, sum of the base and saturation of the soil base. The doses of 240 g of cattle manure and 24 g of rock dust generated an increase in the chemical properties of the soil.

Additional key words: biofertilizers; organic amendments; organomineral fertilizers; nutrient availability; soil suitability.

RESUMEN

La calidad química del suelo es uno de los factores más rápidamente afectados por los procesos de degradación antropogénica, y este es uno de los componentes más importantes para el desarrollo de la agricultura. Por lo tanto, este trabajo tuvo como objetivo evaluar los efectos de las diferentes dosis de estiércol de ganado y polvo de roca en las características químicas del suelo en cultivo de col rizada de mantequilla. Los tratamientos se organizaron en cinco bloques aleatorizados en un factorial de 4×4 en referencia a las diferentes dosis de estiércol bovino (60, 120, 180 y 240 g/planta) combinado con dosis de polvo de roca (6, 12, 18 y 24 g/planta). Cada bloque estuvo constituido por tres parcelas de 18 m de largo y 1 m de ancho, el lecho estuvo compuesto por seis porciones y cada parcela experimental constaba de 14 plantas espaciadas en 0,40×0,40 m. Al final del experimento se analizaron: pH, materia orgánica, P, K, Na, Mg, acidez intercambiable, capacidad de intercambio catiónico (CIC), suma de base y saturación por base. Las dosis de estiércol bovino y MB-4 proporcionaron un aumento en el pH, materia orgánica, concentración de fósforo, potasio, sodio, calcio, magnesio, acidez intercambiable, capacidad de intercambio catiónico, suma de la base y saturación de la base del suelo. Las dosis de 240 g de estiércol de ganado y 24 g de polvo de roca generaron un aumento en las propiedades químicas del suelo.

Palabras clave adicionales: biofertizantes; enmiendas orgánicas; organomineral fertilizers; disponibilidad de nutrientes; aptitud del suelo.

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INTRODUCTION

Growing vegetables is done mostly conventionally. However, alternative systems such as an ecological basis using organic fertilizers such as manure are gaining prominence, especially because of both the quality of food and the environment concern (Vergel *et al.*, 2015). This concern also extends to the forms of cultivation, driving global demand for techniques and technologies that replace the use of agricultural-industrial inputs with high energy, economic and social costs with low-cost organic inputs. The use of organic fertilizers contributes to environmental sustainability and increases in agricultural production (Dick and Gregorich, 2004; Conway and Barbier, 2013; Souza and Resende, 2014; Li *et al*., 2017).

Trying to find solutions to minimize impacts on agricultural production systems which uses techniques of conventional production generating significant losses of arable land, in recent years empirical efforts of practices were carried out in ecological family production systems (Gliessman, 2000; Meirelles, 2004). The use of materials from natural sources in agricultural fertilizer has been highlighted as a way to meet and balance nutritional, chemical and biological soil needs.

The growth of organic production and ecological bases worldwide is a response to the demand from society for a sustainable environment and production practices that generate safer and healthier products for fairer social relations and trade (Lima-Filho and Quevedo-Silva, 2012; Albuquerque Júnior *et al*., 2013; Braga Júnior *et al*., 2014).

Organic fertilizers are an alternative that is easy to acquire and low cost, with several benefits, such as increased water holding capacity and infiltration, increased pH and ability to exchange cations (CEC), providing nutrients (Clemente *et al*., 2012; Silva *et al*., 2013; Dutra *et al*., 2015) that provide higher productivity of various vegetables, including brassica (Candian *et al*., 2015).

Among the materials commonly used in the composition of natural fertilizers, manure and rock powders have been widely used. Manure mainly stands out as a nitrogen source, and rock powder is an excellent alternative of calcium, magnesium, potassium and phosphorus, as well as all essential plant micronutrients (Clemente *et al*., 2012; Silva *et al*., 2013; Dutra *et al*., 2015; Nascimento *et al*., 2015, 2017).

Manure is the preferred source for supplying organic matter to soil cultivated with vegetables. Organic carbon is a power source for the microbial mass in soil; it improves soil physical, chemical and biological properties and is an important option to maintain sustainable agricultural practices (Ceretta *et al*., 2010; Silva *et al*., 2010; Yang *et al*., 2016).

Rock powder is a good fertilization alternative because, depending on availability, it has a low market value. It is the material that remains after breaking rocks, mainly in quarries and mining. Usually, it contains nutrients in varied amounts and quality, depending on the source of the material or rock mother. The material can be used as a nutritional supplement, releasing nutrients over years, and, in some cases, releasing nutrients quickly (Ribeiro *et al*., 2010).

Therefore, the aim of this study was to evaluate the effect of different doses of manure and rock powder on the chemical characteristics of soil cultivated with butter kale.

MATERIALS AND METHODS

This experiment was conducted on Campus III of the experimental area of the Federal University of Paraíba, in the municipality of Bananeiras-PB (CCHSA/UFPB), located at 6°45'4" S and 35°38'0" W.

According to the Koppen classification, climate is considered Type As - Tropical rainy, dry summer, irregular annual rainfall distribution (1,174.7 mm), with a maximum annual temperature of 27°C and minimum of 18.8°C, and annual average of 22°C. The meteorological data for the experimental period are shown in Tab. 1.

The treatments were arranged in five randomized blocks in a 4×4 factorial for the doses of bovine manure (BM) (60, 120, 180 and 240 g/plant) combined

Table 1. Representation of relative humidity, soil temperature, rainfall and average air temperature in the period from October 2014 to February 2015. Data from the weather station, EFSA, PB (2015).

Relative humidity (%)					
	October	November	December	January	February
	87.64	84.39	84.36	82.32	82.12
Soil temperature (°C)					
Layer	October	November	December	January	February
10 cm	22.79	23.67	23.58	24.06	23.12
20 cm	22.94	24	23.62	24.17	25.14
40 cm	22.96	23.78	23.68	23.78	24.65
Rainfall (mm)					
	October	November	December	January	February
	70.9	30.3	27.3	34.9	35.1
Average air temperature (°C)					
	October	November	December	January	February
Maximum	22.31	23.01	23.4	23.87	22.32
Average	21.52	22.46	22.58	22.95	21.56
Minimum	20.81	21.7	22.01	22.31	20.85

with powder rock doses (6, 12, 18 and 24 g/plant). The doses were selected based on the organic fertilization recommendation for leaf cabbage crops (Trani *et al*., 2015). Each block was composed of three plots, 18 m long and 1 m wide. The bed was composed of six portions, and each experimental plot consisted of 14 plants spaced at 0.40 x 0.40 m, for a total of 62,500 plants/ha, 85 portions.

The soil of the prevailing experimental area corresponded to a yellow Oxisol with a medium texture, soft curly relief, deep profile, good drainage, moderate moisture retention capacity and Sandy Clay Loam textual class.

Before conducting the experiment, 25 soil simple samples were collected in the experimental area (0-20 cm). The simple samples were homogenized in containers forming five composite samples specimens; afterwards, the chemical and physical characterizations were made using the methodology suggested by Embrapa (2017). The samples had the following characteristics: sand (g kg^{-1}) = 740; silte (g kg^{-1}) = 120; clay (g kg^{-1}) = 140; clay dispersed in water (g kg^{-1}) = 78; degree of flocculation (%) = 30; dispersion index (%) = 56; soil density (kg dm^{-3}) = 1.61; particle density (kg dm^{-3}) = 2.60; total porosity (m^3 m^{-3}) = 0.49; macroporosity (m^3 m^{-3}) = 0.049; microporosity (m^3 m^{-3}) = 0.442; field humidity capacity to 0.01 to 1.5 MPa (m^3 m^{-3}) = 0.209; wilting point tensions to 0.01 to 1.5 MPa (m^3 m^{-3}) = 0.134; available water (g kg^{-1}) = 0.074; pH in H_2O (1.0:2.5) = 6.50; P (mg dm^{-3}) = 69.5; K^+ ($cmol_c$ dm^{-3}) = 0.20; Ca^{2+} ($cmol_c$ dm^{-3}) = 3.50; Mg^{2+} ($cmol_c$ dm^{-3}) = 2.20; Na^+ ($cmol_c$ dm^{-3}) = 0.20; H^+ + Al^{3+} ($cmol_c$ dm^{-3}) = 1.50; Al^{3+} ($cmol_c$ dm^{-3}) = 0.00; bases sum ($cmol_c$ dm^{-3}) = 6.1; cation exchange capacity in pH7 ($cmol_c$ dm^{-3}) = 7.6; effective acidity (aluminum saturation) (%) = 0.00; V (%) = 80.26 and organic matter (g dm^{-3}) 24.96.

Butter kale seedlings of the *Brassica oleracea* L. var. acephala were produced in the substrate composed of vegetable soil (60%), sand (30%) and bovine manure (10%) and planted in disposable cups. During the production of seedlings, a simple biofertilizer foliar application was done (Silva *et al*., 2012) to supply nutrients.

Conventional soil tillage preparation was used with plowing, harrowing and a subsequent lifting of the beds. Holes were made at 0.20 m in the transplanting seedling stage when the fertilizer was also added using bovine manure and rock powder (MB-4®) (Mineração Barreto SA - Arapiraca, Alagoas-Brasil) (Mibasa, 2007) according to the proposed treatments. The bovine manure was analyzed and had the following characteristics: pH in H_2O (1.0:2.5) = 8.25; P (mg dm^{-3}) = 5.06; K^+ ($cmol_c$ dm^{-3}) = 0.0018; Ca^{2+} ($cmol_c$ dm^{-3}) = 4.00; Mg^{2+} ($cmol_c$ dm^{-3}) = 3.90; Na^+ ($cmol_c$ dm^{-3}) = 1.08; H^+ + Al^{3+} ($cmol_c$ dm^{-3}) = 0.495; Al^{3+} ($cmol_c$ dm^{-3}) = 0.00; Ca^{2+} + Mg^{2+} = 7.90; electrical conductivity (dS m^{-1}) = 5.48; cation exchange capacity ($cmol_c$ dm^{-3}) = 9.47; base saturation (%) = 5.10; base sum = 9.69; and organic matter (g kg^{-1}) = 100.82.

The rock powder was analyzed and had the following characteristics: pH = 6.80; P (mg dm^{-3}) = 42.82; K^+ ($cmol_c$ dm^{-3}) = 0.12; Ca^{2+} ($cmol_c$ dm^{-3}) = 5.65; Mg^{2+} ($cmol_c$ dm^{-3}) = 1.95; Na^+ ($cmol_c$ dm^{-3}) = 0.30; H^+ + Al^{3+} ($cmol_c$ dm^{-3}) = 1.70; Al^{3+} ($cmol_c$ dm^{-3}) = 0.00; Ca^{2+} + Mg^{2+} = 7.60; cation exchange capacity ($cmol_c$ dm^{-3}) = 9.72; base saturation (%) = 97.77; base sum = 74.56 and organic matter (g kg^{-1}) = 43.85. The rock powder came from Neoproterosóica Brasiliane s.I - Granitoids (Santo *et al*., 2002), composed of 55% sand, 43% silt and 2% clay.

After transplantation and fertilization, the beds were covered with straw, *Brachiaria decumbens,* using a 2 cm thick layer in order to enhance the development of biological activities, control spontaneous plants, and maintain soil moisture.

Irrigation was done with a drip system according to the water needs of the butter kale culture, adopting shifts of 9 h irrigation in the initial phase when the crop was established, increasing up to 1 d in the final stage, corresponding to the last 20 d of the crop cycle. Self-cleaning drippers with self-compensating emitters with a flow of 6.0 L h^{-1} were used, 0.40 m spaced between themselves and distributed along the lines. The methodology used for the irrigation calculation (Cleves *et al.*, 2016) was based on Etc = Kc x ETo (crop evapotranspiration = crop coefficient × evapotranspiration of reference (mm d^{-1}).

At the end of the experiment, 60 d after transplantation, the following were measured: pH, organic matter (OM), phosphorus content (P), potassium (K), sodium (Na), calcium (Ca), magnesium (Mg), acid exchangeable (H and Al), cation exchange capacity (CEC), base sum (BS) and base saturation (V). For the soil fertility analysis, soil samples were collected in the 0 to 20 cm layer. The analyses were performed at the Soil Laboratory of the Federal University of Paraiba, Campus III, Bananeiras (CP), with the method suggested by Embrapa (2017).

The data were analyzed with analysis of variance and regression using Sisvar, and the means were compared with the Tukey test, up to 5% probability. The choice of the regression model was based on the significance of the regression coefficients using the t-test at 5% probability, the coefficient of determination (R^2 = SQReg / SQtrat) and a biological behavior study.

RESULTS AND DISCUSSION

It was observed that the pH, organic matter, phosphorus content, potassium, sodium, calcium, magnesium, exchangeable acidity, CEC, amount of base and base saturation presented a significant interaction between the doses of bovine manure (BM) and doses of rock powder (MB-4). The manure doses significantly influenced the variables, except pH and magnesium, and the rock powder doses affected the pH, organic matter, potassium, sodium and sum of base.

There was an increase in pH (Fig. 1A) in the treatments with BM and MB-4 interactions. The pH of the soil treated with the doses of 120 g of BM + 18 g of MB-4 had a higher value (7.07), and the minimum value (6.71) was observed with BM 120 g + 6 g MB-4, meaning the pH increased as the MB-4 amount increased. Comparing the pH values before the experiment, which was 6.50, and after the experiment, the values increased with the fertilization used, but it was observed that the rising pH not harmed the development of butter kale.

The higher pH values were probably due to the buffering of bicarbonates and elemental cations introduced by the bovine manure and rock powder, such as Ca^{2+} and Mg^{2+} (Mkhabela *et al*., 2005). Yang *et al*. (2016), when working with watermelon, observed a higher pH in plants fertilized with 80 t ha^{-1} of bovine manure. Resende *et al*. (2006) also observed an increase in the pH of clayey soil that received doses of alkaline ultramafic rock powder.

For the phosphorus content, the highest value (169.94 mg dm^{-3}) was found in the soil treated with 60 g of BM + 6 g of MB-4, corresponding to the lower doses, while the smaller levels were found with the dose 120 + 18 g MB-4, presenting a decrease of 77.35 mg dm^{-3} from the higher values found with fertilization (Fig. 1B).

It was also found that the phosphorus content had significant differences between the treatments, with higher levels observed in the soil at the end of the experiment (Tab. 1), when the phosphorus presented 69.5 mg dm^{-3}. The increase in phosphorus in the soil may have occurred because of the increase in organic matter, as OM is an important controller of phosphorus synthesis in the soil and the availability of this element for plants depends on its fixation (Harrison, 1987; Andrade *et al*., 2003; Dao, 2004).

The interaction of BM and MB- 4 doses provided a quadratic effect on the potassium content (Fig. 1C), sodium (Fig. 1D) and calcium (Fig. 1E). For these elements, it was observed that the maximum values in the soil was obtained with the 120 g BM dose, leading to a dose reduction from 180 to 240 BM.

The potassium content reached maximum values in the treatment with BM 120 g + 18 g of MB-4, 0.409 mg dm^{-3} (Fig. 1C), twice that was measured at the beginning of the experiment (0.20 mg dm^{-3}). Sodium presented the highest level in the treatment with BM 120 g + 6 g of MB-4, obtaining 0.333 $cmol_c$ dm^{-3} (Fig. 1D). Thus, both the potassium and sodium values obtained with the soil analysis were higher after the completion of the experiment.

The lower sodium levels were observed at doses of BM 240 g and 6 g of MB-4 (Fig. 1D), indicating that doses higher than 4-MB can cause soil salinization, making it unsuitable for cultivation.

For the calcium content, it was found that the doses of 240 g of BM + 12 g of MB-4 provided an increase of 0.40 $cmol_c$ dm^{-3} (Fig. 1E) when compared with the results of the soil analysis performed at the beginning of the experiment. After the experiment, the soil calcium content was 3.8 $cmol_c$ dm^{-3}.

The magnesium content in the soil increased as the BM doses increased, with maximum values of 3.96 $cmol_c$ dm^{-3} in the soil fertilized with 240 g of BM and 12 g of MB-4, leading to a reduction of 1.03 $cmol_c$ dm^{-3} as a result of the application of maximum MB-4 doses. After the completion of the experiment, an increase of 1.76 $cmol_c$ dm^{-3} of magnesium was observed in the soil, as compared to that observed before the experiment, with 2.20 $cmol_c$ dm^{-3} (Fig. 1F).

The increases verified in the soil for the potassium, phosphorus, sodium, calcium and magnesium can be associated with the joint application of bovine manure and MB-4 since the application of bovine manure and rock powder can increase the availability of nutrients in soil because of the greater solubility of rock powder and mineralization of bovine manure

(Camargo *et al*., 2012). As seen in the present study, an increase in nutrient contents in soil has been observed in different species grown under conditions of fertilization with bovine manure and/or rock dust, such as *Raphanus sativus* L. (Tito *et al*., 2019), *Annona muricata* L. (Malta *et al*., 2019), *Phaselous vulgaris* L. (Gotz *et al*., 2016); and *Helianthus annuus* L., Sol Noturno variety (Andrade *et al*., 2015).

The bovine manure and rock powder had a broad-spectrum effect by acting on the chemical, physical and biological mechanisms of the soil. Because bovine manure is a source of organic matter that, when added to soil in proper amounts, promotes mineralization, that is, it is easily released to the soil solution, absorbed by the plants or leachate (Clemente *et al*., 2012; Silva *et al*., 2012, 2013).

The interaction between the doses of BM and MB-4 provided an increasing effect of exchangeable soil acidity on all analyzed treatments (Fig. 2A). However, almost all observed values were lower than the

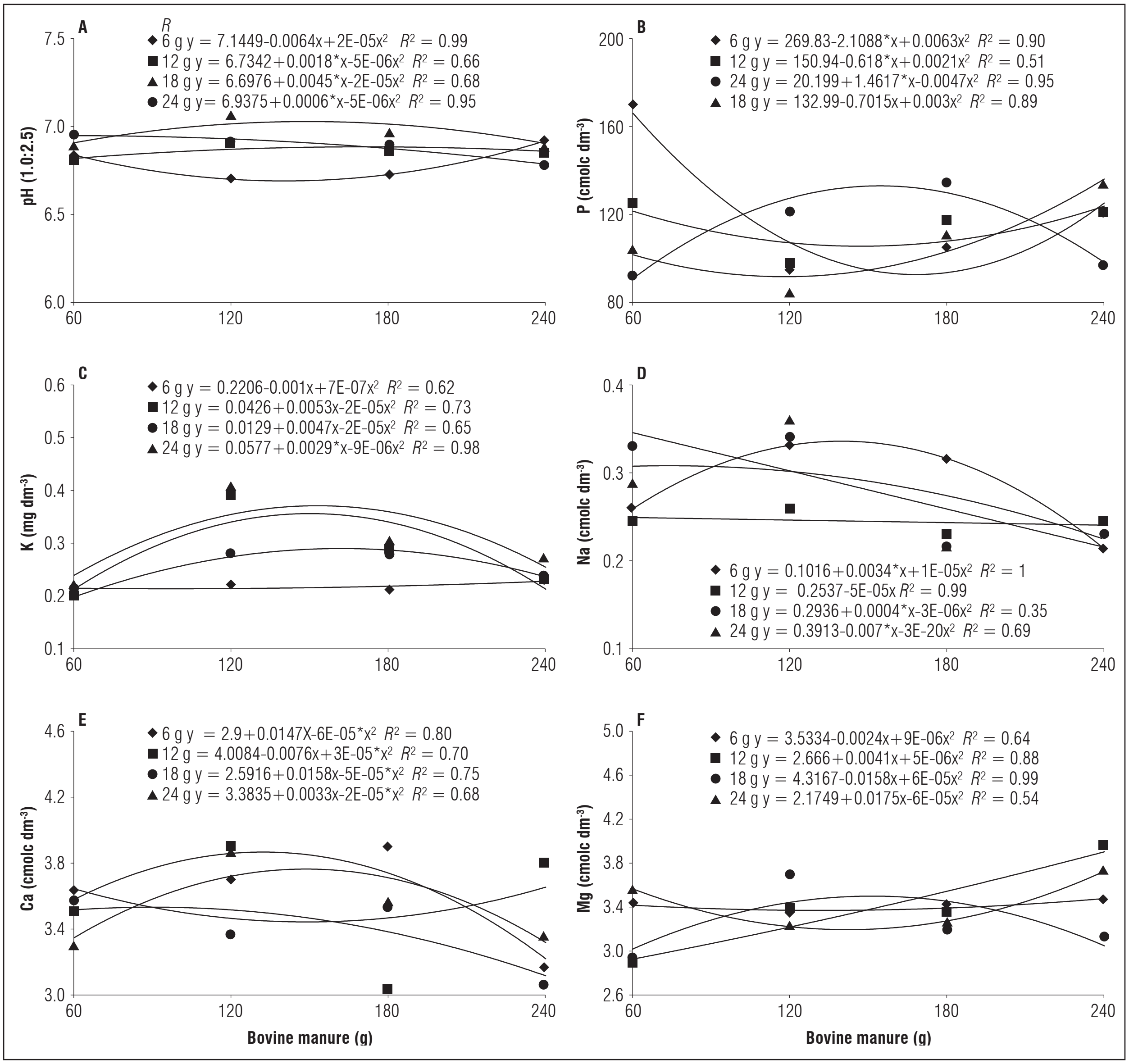

Figure 1. Effect of bovine manure doses and rock powder on the pH (A), phosphorus content (B), potassium (C), sodium (D), calcium (E) and magnesium (F) in yellow Oxisol under butter kale cultivation.

values in the soil analysis before the experiment, in Tab. 1, except for treatments 240 g of BM + 12 g MB-4, 240 g of BM + 18 g MB-4 and 240 g of BM + 24 g of MB-4, which presented slightly higher values for the initial results, respectively, 1.54 and 1.59 $cmol_c$ dm^{-3}.

The effects of organic compounds on the exchangeable soil acidity can be associated with the complexation of Al by organic matter, which promotes the removal of that element from the soil solution and the formation of the dissolved complex by the dissolved organic carbon. The replacement of Al^{3+} with Ca^{2+} in the cationic complex immobilizes Al^{3+} with organic ligands, and BM reduced toxic aluminum from the ground (Hargrove and Thomas, 1981; Cancès *et al*., 2003; Zambrosi *et al*., 2007). Thus, during the decomposition of organic matter, aluminum can be complexed in the soil solution with fulvic acids (Anghinoni and Salet, 2000) or organic acids with a low molecular weight, such as citric, oxalic and tartaric acids.

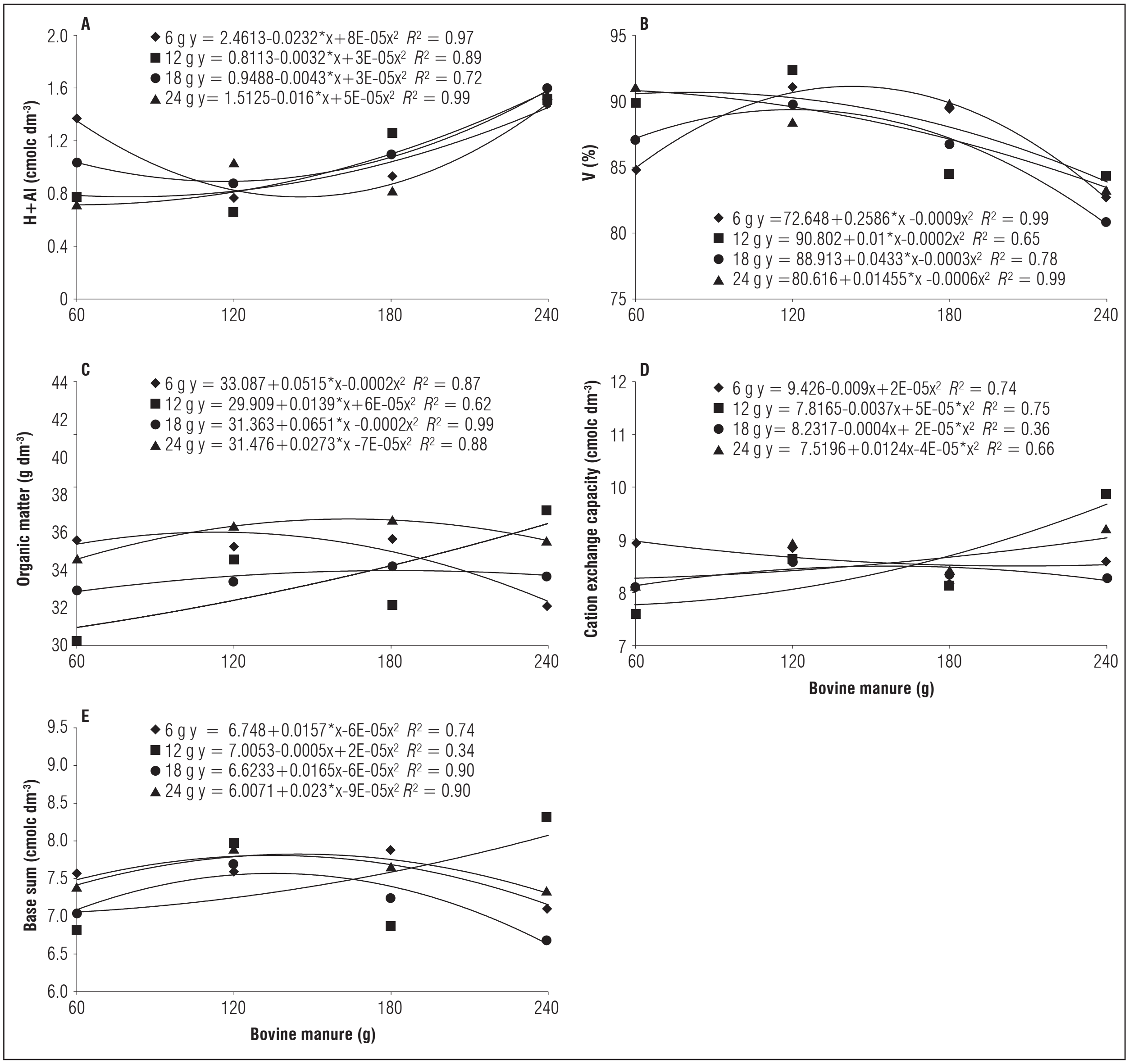

Figure 2. Effect of bovine manure and rock powder on exchangeable acidity (A), base saturation (B), organic matter (C), cation exchange capacity (D) and sum bases (E) in yellow Oxisol under butter kale cultivation.

However, the increase of the exchangeable acidity as a result of the increase of bovine manure can also be associated with the increase of the MB-4 doses or with material mineralization processes or through removing a greater amount of nutrients by cultivated plants (Hanisch *et al*., 2013; Ramos *et al*., 2014).

For the base saturation, it was found that the interaction between the doses of BM and MB-4 gave the highest interaction values, with up to 92% doses of 120 g BM + 12 g of MB-4, with a subsequent reduction of the doses of 240 to 180 g BM (Fig. 2B). This result shows excellent soil fertility indices, especially for the contents of the Ca, Mg, K and Na in the soil. May *et al*. (2007) emphasized that the values of the base saturation, which are for the butter kale between 70 and 80%, are relevant for the productivity of this culture.

The organic matter content (Fig. 2C), cation exchange capacity (Fig. 2D) and values of the base sum (Fig. 2E) presented superior results than those observed in the initial soil characterization. And doses of 240 g BM + 12 g MB-4 increased more than those values.

Increases in organic matter levels, cation exchange capacity and base sum values as a function of fertilization with BM and or MB-4 were also observed by Barcellos *et al*. (2015) when analyzing a typical dystrophic latosol Bruno, Silva *et al*. (2018) and evaluating a culture of *Brachiaria brizantha* cv. Marandú, Batista *et al*. (2017) in a red-yellow latosol under soybean and sorghum cultivation, and by Alovisi *et al*. (2017) when analyzing the availability of nutrients in soil during three incubation periods with rock dust.

The BS, CEC and V parameters were calculated with a base on Ca, Mg, K, and $H^+ + Al^3$. Therefore, as in the treatments with the greatest presence of bovine manure (120 to 240 g), there was greater availability of Ca, Mg, K and a reduction of $H^+ + Al^{3+}$. The BS, CEC and V values had significant changes.

The observed results are similar to those obtained by Silva *et al*. (2012), where the same, testing fertilization with 6 types of rocks in a red-yellow oxisol observed that the pH values increased with the rock powder. For Ca, Mg, P and K, according to these authors, there was also an increased release of these elements, which was related to the mineralogical composition of the studied rocks, where the rock powders reacted when in contact with the soil and thus were released. They also observed that the analyzed rock powders provided an increase in the CEC and V values of the soil.

Ribeiro *et al*. (2010), in a greenhouse, applied different doses of phlogopite powder, ultramafic alcaline and pyroclastic gap in a sample yellow Oxisol dystrophic and found that the rock powders released higher amounts of K and provided P. These rock powders promoted further correction in the soil acidity and increased the base saturation as a result of a possible release of bases contained in the minerals of these rocks.

CONCLUSION

The doses of bovine manure and MB-4 provided an increase in pH, organic matter, concentration of phosphorus, potassium, sodium, calcium, magnesium, exchangeable acidity, cation exchange capacity, sum of the base and saturation of the soil base.

The doses of 240 g of cattle manure and 24 g of rock dust generated an increase in the chemical properties of the soil.

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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Growth and quality of lisianthus [*Eustoma grandiflorum* (Shinn.)] cultivated in rice husk substrates in troughs with leaching recirculation

Crecimiento y calidad de lisianthus [*Eustoma grandiflorum* (Shinn.)] cultivado en sustratos de cascarilla de arroz en canales con recirculación de lixiviados

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Lisianthus production in substrate and recirculation of the nutrient solution.

Photo: D. Höhn

ABSTRACT

This study aimed to evaluate the growth and quality of lisianthus cultivated in different rice husk substrates. The evaluated substrates were: carbonized rice husk (CRH; 100%); raw rice husk (RRH; 100%); CRH (70%) + S10 Beifort® commercial organic compost (30%); and RRH (70%) + S10 Beifort® commercial organic compost (30%). Plant dry mass (DM) production and partitioning, plant leaf area and shoot/root DM ratio were analyzed. Quality parameters of the flower stems were also evaluated (length; diameter and number of flowers and buds). The RRH substrate at 100% reduced plant growth and prevented flower development. This modified dry mass partitioning among the plant organs. The substrates RRH + S10 and CRH provided quality flower stems. Thus, both substrates can be used to produce cut lisianthus flowers in recirculating nutrient systems. However, the RRH + S10 substrate stood out because it increased plant growth. This result is ascribed to the improvement of the physical and chemical properties of substrate RRH + S10 as a result of the presence of both the organic compost, which benefited the water holding capacity, and the RRH, which guaranteed high porosity to the substrate.

Additional key words: soilless cultivation; flower stems; dry mass production and partitioning; quality.

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RESUMEN

El estudio tuvo como objetivo evaluar el crecimiento y la calidad de lisianthus cultivado en diferentes sustratos de cascarilla de arroz. Los sustratos evaluados fueron: cascarilla de arroz carbonizada (CAC; 100%); cascarilla de arroz cruda (CACr; 100%); CAC (70%) + abono orgánico comercial S10 Beifort® (30%); y CACr (70%) + abono orgánico comercial S10 Beifort® (30%). Se analizaron la producción y partición de la materia seca (MS) de la planta, el área de la hoja del planta y la relación MS de la parte aérea/raíces. También fueron evaluados los parámetros de calidad del tallo de la flor (altura del tallo, diámetro y cantidad de flores y capullos). El sustrato 100% CACr redujo el crecimiento de las plantas y el formación de flores. Esto cambió la partición de materia seca entre los órganos de la planta. Los sustratos CACr + S10 y CAC proporcionaron tallos florales de calidad. Por lo tanto, ambos sustratos pueden ser utilizados para producir flores de lisianthus cortadas en el sistema de recirculación de nutrientes. Sin embargo, el sustrato CACr + S10 se destacó por aumentar el crecimiento de las plantas. Este resultado se atribuye la mejora de las propiedades físicas y químicas del sustrato CACr + S10, debido la presencia del abono orgánico, que benefició la capacidad de retención de agua y el CAC, que garantizó una alta porosidad de lo sustrato.

Palabras clave adicionales: cultivo sin suelo; tallos florales; producción y partición de materia seca; calidad.

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INTRODUCTION

The lisianthus (*Eustoma grandiflorum* (Raf.) Shinn.) is an ornamental plant originating from northern Mexico and the southern United States (Kuronuma *et al*., 2018). This species belongs to the Gentianaceae family and is cultivated as cut flowers. The flower was introduced to the Brazilian Market in the late 80s and began to stand out economically in the 90s, arousing farmers' and consumers' interest (Camargo *et al*., 2004; Souza Neto *et al*., 2017).

Nevertheless, lisianthus commercial production in soil has been limited by viruses and pathogens, such as *Fusarium solani*, which attacks the root system and causes stem and root rotting and, as a result, wilting and drying of the plant (Backes *et al*., 2007). The diseases become limiting factors for the crop, leading to the search for new production techniques.

Soilless cultivation using substrates allows for horticultural crop production in inappropriate areas, such as dry regions or soils with low drainage capacity (El-Behairy, 2015). The production of cut lisianthus in substrates can represent an important technological advance since it reduces the occurrence of pests and diseases and optimizes the workforce, by allowing cultivation in high soil beds (Asaduzzaman *et al*., 2015).

In Brazil, soilless flower cultivation is little-explored; most of the growers who use this system usually cultivate in pots with free drainage. However, costs with pots are high since many of them cannot be reused from one cycle to another. In addition, waste of water and fertilizers is very high, which causes environmental problems. Some fruit growers have been using trough crop systems, in which the substrate is placed directly into gutters, eliminating pot costs (Neto and Redig, 2017). However, the trough growing system with nutrient solution recirculation ("closed system") has not been studied for cut flower cultivation in Brazil.

The use of materials available in the producing region for the composition of substrates is very important in order to enable economic viability in flower cultivation with this cultivation system in terms of their availability, cost and asepsis, among other characteristics (Barbosa *et al*., 2019).

The carbonized rice husk or the raw rice husk and mixed with organic compost can be good substrate alternatives for use in southern Brazil, as it is a material with great availability and low cost (Kratz *et al.*, 2013).

According to Klein *et al*. (2012), carbonized rice husk presents great potential for use as a substrate given its physical properties. It presents fast and efficient drainage and high aeration space, providing good oxygenation to roots (Caldeira *et al*., 2013). Another benefit of this material is the lack of propagules of spontaneous plants, nematodes and pathogens as a result of the carbonization process, which eliminates possible organisms (Asaduzzaman *et al*., 2015). However, the rice husk carbonization process demands high labor, can cause air pollution and provides low profits once there is a reduction of 50% in the husk volume. Therefore, raw rice husk may be an alternative material instead of the carbonized rice, as observed for fruits in closed growing systems (Strassburger *et al*., 2011; Peil *et al*., 2014). This material has great availability, easy access and low cost in Brazil's southern region (Zorzeto *et al*., 2016).

In addition, information regarding lisianthus plant growth (dry mass production and partitioning) is incipient. Dry mass production and partitioning analysis expresses a plant's morphophysiological conditions and quantifies the net production derived from the photosynthetic process. It represents the performance of the assimilatory system during a certain period (Benincasa, 2003). Growth is influenced by several genetic and environmental factors (Osei *et al*., 2018). Thus, the knowledge on the growth of a specifically cultivated variety and the effects of the growing medium is very important.

The experiment design used randomized blocks with four treatments (substrates) and six replications of 10 plants. Three control plants were harvested for the evaluations. The border plants were disregarded. The results were subjected to analysis of variance, and the means were compared using the Tukey test, with a probability of 5%.

Therefore, the aim of this study was to evaluate the plant growth and quality of lisianthus cultivated in different substrates based on rice husk, with the purpose of generating information that potentiates production in trough growing systems with leaching recirculation.

MATERIALS AND METHODS

This experiment was carried out in a greenhouse at the UFPEL Campus in the municipality of Capão do Leão, Rio Grande do Sul, Brazil (31°52'S, 52°21'W). The climate of this region is characterized by being sub-tropical, with a hot summer, Cfa according to Köppen's classification (Kuinchtner and Buriol, 2001). The greenhouse environment was managed daily only with natural ventilation, opening the doors and the side windows.

Four substrates were used: carbonized rice husk (CRH); raw rice husk (RRH); CRH (70%) + S10 Beifort® commercial organic compost (30%); and RRH (70%) + S10 (30%). The physical and chemical properties of the substrates were determined in the

Table 1. Physical and chemical properties of raw rice husk (RRH) and carbonized rice husk (CRH) substrates, used alone and mixed with commercial organic compost S10 (Beifort®) at a proportion of 30%, at the beginning and at the end (91 d after setting) of lisianthus (*Eustoma grandiflorum*) cultivation in troughs with leaching recirculation.

Physical properties	Substrate – at the beginning and at the end of the crop-cycle							
	CRH		RRH		CRH+S10		RRH+S10	
	begin	end	begin	end	begin	end	begin	end
Wet density (g L^{-1})	262	437	236	338	279	547	264	449
Dry matter (g/100 g)	60	50	38	43	54	52	51	44
Dry density (g L^{-1})	156	218	90	145	150	286	135	196
Total porosity (m^3 m^{-3})	0.77	0.82	0.72	0.86	0.71	0.81	0.76	0.81
Aeration space (m^3 m^{-3})	0.56	0.17	0.58	0.61	0.57	0.19	0.59	0.23
WEA* (m^3 m^{-3})	0.12	0.22	0.04	0.06	0.03	0.14	0.03	0.12
WHC* a 10 cm (m^3 m^{-3})	0.2	0.64	0.14	0.25	0.14	0.62	0.17	0.59
Chemical properties								
EC* (dS m^{-1})	0.11	0.31	0.07	0.21	0.13	0.55	0.19	0.52
pH value (H_2O)	5.1	5.7	5.3	6.4	5.1	5.8	4.8	5.7

* WEA: water easily available; WHC: water holding capacity; EC: electric conductivity.

LASPP/FEPAGRO (Laboratory of Substrates for Plants / Agriculture Research State Foundation) (Tab. 1).

'White Excalibur'® cultivar was used and the experiment was carried out on October 9th, 2015, when the commercial seedlings presented about three to four leaf pairs.

The cultivation system was composed of four wood troughs (4.5 m long, 0.30 m wide and 0.15 m internal height). The troughs had a slope of 4% and were internally waterproofed with plastic (petroleum based geomembrane) channels to facilitate the flow of the drained nutrient solution to the catchment tanks. A 100-liter catchment tank was placed at the lowest end of each trough. A low-power washing machine pump was installed in each tank in order to propel the nutrient solution through a 20 mm PVC pipe to the highest point of the troughs. From this point, the nutrient solution was supplied to the plants through polyethylene pipes and drippers directed to the plant base. The nutrient solution was supplied for 15 min every hour from 08 a.m. to 07 p.m. The solution drained to the end of the troughs returned to the catchment tank, forming a closed system.

Each trough was divided into six plots, with two rows of plants. Spacing plants at 0.15 m, with ten plants per plot and sixty plants per trough. The nutrient solution recommended for chrysanthemum by Barbosa *et al*. (2008) was adapted and used for the lisianthus cultivation. It was monitored every 2 d by checking electrical conductivity (EC) and pH. The EC was adjusted to 1.5 dS m^{-1}, and pH was maintained between 6.0 and 6.5. The monitoring of EC and pH was carried out with a manual digital conductivimeter and pHmeter. Corrections were performed when the EC suffered variations above or below 20%. The pH was corrected with acids or bases when necessary.

The harvesting point was defined when the stem had two fully open flowers. The stem length was measured with a graduated ruler from cut point until upper portion of the highest flower. The stem diameter was measured in their lower third portion with a manual pachymeter that was also used to measure the flower diameter (distance between the petals borders on opposite sides). The leaf area was determined using leaf area meter equipment (LI-COR, model 3100). The open flowers and floral buds with ornamental potential were counted.

For the dry mass evaluations, the samples were fractionated into root, stem, leaves and flowers (when present). All plant fractions were dried in an oven at 65°C until constant weight and then weighed on a precision balance. As a result, the plant dry mass (DM) production was determined. Dry mass partitioning (in percentage) and the ratio shoot/root DM (g g^{-1}) were calculated from the DM production data.

The four studied substrates were arranged in a randomized block experiment design with six replications composed of 10 plants. Three control plants of each replication were harvested for the evaluations. The border plants were not considered. The results were subjected to analysis of variance, and the means were compared with the Tukey test, at 5% probability.

RESULTS AND DISCUSSION

For plant growth, the highest shoot dry mass was obtained from plants grown in the RRH + S10 substrate, followed by CRH, CRH + S10 and, finally, RRH (Tab. 2).

The accumulated DM production of the plants grown in RRH + S10 presented the following order (g/plant): stem 5.23, leaves 3.00, flowers 2.11 and roots 0.73, totaling 11.07 (Tab. 2). The same order was observed in the CRH and CRH + S10 substrates. Similarly, a study developed by Hernandez *et al*. (2011) with the 'Echo Blue' lisianthus cultivar showed that the stem was the organ with the greatest DM accumulation, followed by leaves.

In contrast, plants cultivated in RRH had higher DM production (g/plant) in the leaves (0.77), followed by stems (0.55) and roots (0.11). Plants cultivated in RRH did not produce flowers. The absence of flowers can be ascribed to the very low total biomass production, only 1.43 g/plant. So, plant development did not occur properly in this substrate and, as a consequence, the plants did not produce flower stems.

For plant leaf area (PLA), the plants cultivated in RRH + S10 presented the highest average (719.53 cm^2), differing from CRH (620.7 cm^2), CRH + S10 (388.27 cm^2) and RRH (96.14 cm^2) (Tab. 2). The PLA of the plants cultivated in RRH + S10 and CRH substrates was close to that of the 'Echo Blue' cultivar, as observed by Hernandez *et al*. (2011).

Table 2. Dry mass production of stem, leaves, flowers, shoot and roots; and plant leaf area of lisianthus (*Eustoma grandiflorum*) plant cultivar 'White Excalibur' grown in different rice husk substrates in troughs with leaching recirculation.

	Dry mass production (g/plant)					Leaf area
Substrate	Stem	Leaf	Flowers	Shoot	Roots	(cm^2/plant)
70% RRH + 30% S10	5.23 a	3.00 a	2.11 a	10.34 a	0.73 a	719.53 a
100% RRH	0.55 d	0.77 d	0.0 d	1.32 d	0.11 c	96.14 d
70 % CRH + 30% S10	2.98 c	1.59 c	1.10 c	5.67 c	0.49 b	388.27 c
100% CRH	4.37 b	2.26 b	1.60 b	8.23 b	0.57 b	620.70 b
CV %	7.96	16.89	13.19	9.76	17.36	8.57

Means followed by the same letter in the column do not differ at the 5% probability according to the Tukey test. RRH: raw rice husk, CRH: carbonized rice husk, S10: organic commercial compost (Beifort®).

The dry mass partitioning into the different plant organs (Tab. 3) showed similarity among the treatments RRH + S10, CRH + S10 and CRH. As expected from the DM production data (Tab. 3), the stems represented the highest fraction of the produced DM, followed by leaves, flowers and roots. On average, in the three substrates, stems comprised 48.4%; leaves, 26.2%; flowers, 18.4%; and roots, 7.0% of the total plant DM.

Plants grown in RRH substrate showed different DM distribution ratios. In this case, the leaves comprised 53.8% of the total plant DM, followed by stems (38.5%) and, finally, by roots (7.7%) (Tab. 3). This modification of the DM partitioning was attributed to the absence of flowers in the plants grown in this substrate.

The DM distribution to the roots did not differ among the substrates. The roots comprised, on average, 7.0% of total DM. Despite the different DM productions (Tab. 2), the ratio shoot/root DM was not affected by the substrates (Tab. 3). On average, this ratio was 13.04 g g^{-1}. The ratio shoot/root DM indicates the root activity in the sense of providing water and mineral nutrients to the shoot in order to support adequate plant growth.

For plant quality (Tab. 4), the RRH + S10 and CRH substrates provided the longest stems, 65.1 cm and 61.8 cm length, respectively, differing from CRH + S10 (46.6 cm) and RRH (only 16.5 cm length).

The plants grown in RRH 100% produced stems that were inappropriate for commercialization, differing significantly from the other treatments (Tab. 4). The first three substrates provided proper stem length regarding the marketable requirement, between 40 and 70 cm (Veiling Holambra, 2019). The plants grown in substrates RRH + S10 and CRH stood out in this characteristic since consumers prefer longer stems. Stems over 60 cm in length get a higher commercial price.

The plants produced in substrates RRH + S10 and CRH (Tab. 4) presented stems length similar to those

Table 3. Dry mass partitioning among the different plant organs and shoot/root dry mass ratio of lisianthus plant (*E. grandiflorum*) cultivar 'White Excalibur' grown in different rice husk substrates in troughs with leaching recirculation.

	Dry mass partitioning (%)				Ratio shoot/root
Substrate	Stem	Leaf	Flower	Roots	(g g^{-1})
70% RRH + 30% S10	47.2 a	27.1 b	19.1 a	6.6 a	14.16 a
100% RRH	38.5 b	53.8 a	0.0 b	7.7 a	12.00 a
70 % CRH + 30% S10	48.4 a	26.0 b	18.4 a	7.0 a	11.57 a
100% CRH	49.6 a	25.7 b	18.2 a	6.5 a	14.44 a
CV %	9.36	13.22	10.91	22.68	20.61

Means followed by the same letter in the column do not differ at the 5% probability according to the Tukey test. RRH: Raw Rice husk, CRH: carbonized rice husk, S10: organic commercial compost (Beifort®).

Table 4. Stem length, stem diameter, number of open flowers, flower diameter and number of floral buds with ornamental potential in lisianthus plants (*Eustoma grandiflorum*) cultivar 'White Excalibur' grown in different rice husk substrates in troughs with leaching recirculation.

Substrates	Stem length (cm)	Stem diameter (cm)	Number of open flowers	Flower diameter (mm)	Number of buds
70% RRH + 30% S10	65.1 a	6.4 a	3.8 a	6.6 a	5.1 a
100% RRH	16.5 c	2.8 c	0.0 c	0.0 c	0.0 c
70 % CRH + 30% S10	46.6 b	5.4 b	2.5 b	5.9 b	3.6 b
100% CRH	61.8 a	6.1 a	3.0 ab	6.2 ab	4.1 ab
CV %	11.17	6.17	27.66	6.32	22.82

Means followed by the same letter in the column do not differ at the 5% probability according to the Tukey test. RRH: Raw Rice husk, CRH: carbonized rice husk, S10: organic commercial compost (Beifort®).

found for 'Mariachi Blue Picotee' and 'Mariachi Blue' cultivars with perlite cultivation (De La Riva-Morales *et al*., 2013), but lower than those found for 'Echo' cultivar in soil cultivation (Camargo *et al*., 2004) and larger than those found for 'Echo Champagne', 'Mariachi Pure White', 'Echo Pink' and 'Avila Blue Rim' cultivars in soil cultivation (Backes *et al*., 2007). One has to take into account the fact that the exposed results are related to different cultivars and growing conditions. Thus, in addition to the plant genetic material, the growing system is a factor with a significant effect on plant development.

The substrates RRH + S10 and CRH provided plants with bigger stem diameters (SD), 6.4 and 6.1 mm, respectively (Tab. 4), followed by CRH + S10 substrate, with 5.4 mm, and RRH (2.8 cm). SD is an important parameter for vertical support of stems. According to Veiling Holambra (2019), *E. grandiflorum* plants need to have, at least, 4.0 mm for SD to ensure adequate support for flowers and proper marketable standards. In this sense, the three mentioned substrates provided stems that met the market requirements, with emphasis on the substrates RRH + S10 and CRH. On the other hand, besides the absence of flowers, the RRH substrate provided inadequate stem diameters, which were much lower than the minimum standard required for commercialization.

For the flowers parameters (Tab. 4), substrates RRH + S10 and CRH presented the highest averages: 3.8 and 3.0 open flowers per stem, flower diameter of 6.6 and 6.2 mm, and 5.1 and 4.1 floral buds, respectively. The substrate CRH + S10 presented lower averages: about 2.5 open flowers, flower diameter of 5.9 mm and 3.6 floral buds. Although this could be unattractive for consumers, stems with two/three buds at the beginning of the floral opening stage are recommended to be harvested. On the other hand, harvesting stems with many open flowers can lead to damage during handling and reduced post-harvest durability.

The substrate had significant effects on *E. grandiflorum* shoot growth and development since RRH + S10 and CRH provided the highest plant growth (Tab. 2) and more adequate stems in terms of market requirements (Tab. 4).

Although the four substrates presented different DM production in all aerial organs of the plants (Tab. 2), the dry mass distribution (Tab. 3) was similar for the three substrates in which plants presented flower development (RRH + S10, CRH and CRH + S10). In this case, on average, the stems comprised 48.4% of the total DM production, which demonstrated that they were the strongest sinks for assimilates. On the other hand, on average, in the three substrates, the flowers comprised only 18.5% of the total DM production, which demonstrated that they were weak sinks for assimilates regardless of the treatment analyzed.

The cultivation in RRH substrate prevented flower development and modified the dry mass distribution among the plant organs (Tab. 3). The leaves were the strongest sinks for assimilates. Thus, the absence of generative growth caused deep changes in plants grown in this substrate.

Therefore, the RRH substrate did not provide quality compatible with lisianthus cultivation for marketable cut flower since the plants did not grow adequately and did not develop generatively, which resulted in flower absence (Tab. 2 and 3). The plants presented a low leaf area and DM production, which were associated with the lower water holding capacity (Tab. 1) and the low adsorption of nutrient ions of the RRH.

On the other hand, the mixture RRH + S10 benefited root growth and, as a consequence, resulted in a higher PLA and DM production in all aerial organs, including flowers (Tab. 2). Plant leaf area is related to plant growth since leaves are responsible for the interception of the solar radiation that influences photoassimilate accumulation and DM production. Therefore, the highest PLA of the plants grown in RRH + S10 contributed to a greater growth and biomass accumulation (Tab. 2), which resulted in a greater generative growth and stem quality (Tab. 3). Possibly, these results are ascribed to the improvement of physical and chemical properties (Tab. 1) of RRH + S10 substrate provided by the organic compost, as compared to RRH alone.

The S10 addition to RRH increased the easily available water, guaranteed a greater water holding capacity and improved mineral nutrient reserve for the plants. This can be confirmed by the higher values of the first two characteristics and the higher EC observed in this substrate at the end of the crop cycle (Tab. 1). On the other hand, the presence of RRH in the mixture guaranteed good aeration of the root environment. In contrast, the addition of S10 to CRH excessively increased the water holding throughout the plant growth cycle (Tab. 1), which possibly damaged plant growth and quality.

In terms of alteration of the substrate properties after the lisianthus cultivation, a considerable increase in the wet density was observed for all substrates (Tab. 1). The substrate DM increased using RRH and decreased using CRH, CRH + S10 and RRH + S10. The dry density of all substrates increased considerably as a result of the materials accommodation during the growing period.

The total porosity of the four evaluated substrates did not change significantly. However, the aeration space was reduced considerably in CRH, CRH + S10 and RRH + S10, which decreased the aeration levels to a range considered low for horticultural substrates used in recipient cultivation; whereas, the RRH did not affect this parameter. The lower aeration space did not alter the easily available water of the four substrates. The water holding capacity of the RRH + S10, CRH and CRH + S10 substrates increased considerably after the crop cycle, whereas it was not affected in RRH (Tab. 1).

The physical changes of the substrates after the crop cycle were related to the rearrangement of particles and material decomposition during the growing period. RRH alone presented a lower amount of small particles to be rearranged and higher decomposition resistance than the other substrates. Both characteristics led to practically no change in the main physical characteristics of this substrate even after 91 d.

For the changes in chemical properties during the crop cycle, the EC of all substrates increased considerably, which was more expressive in the mixtures containing organic than in the pure materials (Tab. 1). Possibly, the addition of S10 promoted the adsorption and release of ions into the substrate solution throughout the growth cycle, increasing EC to 0.42 dS m^{-1} for CRH + S10 and 0.33 dS m^{-1} for RRH + S10 after the lisianthus cultivation. This increase was 0.20 dS m^{-1} for CRH and 0.14 dS m^{-1} for RRH.

The pH of all substrates increased. RRH presented a higher pH after cultivation than the others. All substrate pH increases were related to the frequent nutrient solution supply throughout the crop cycle.

Thus, changes in the physical and chemical properties of all substrates were evident. Therefore, more studies evaluating different substrate compositions in recirculating nutrient system on *E. grandiflorum* production for cut flowers are needed in order to verify which one may be more suitable for plant production.

CONCLUSION

The use of raw rice husk alone as a substrate caused damage to the plant growth and prevented flowering of the *E. grandiflorum* by modifying the dry mass partitioning among the plant organs.

The mixture raw rice husk + S10 organic compost (RRH + S10) and the carbonized rice husk (CRH) can be used as substrates for *E. grandiflorum* cut flower production in troughs with leaching recirculation since both provided standard stems for commercialization, especially the former, which promoted greater plant growth.

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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SCIENTIFIC NOTE

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Pre-depulping and depulping treatments and the emergence of queen palm seeds (*Syagrus romanzoffiana* [Cham.] Glassman)

Tratamiento de pre-despulpado y despulpado sobre la emergencia de semillas de palma reina (*Syagrus romanzoffiana* [Cham.] Glassman)

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Syagrus romanzoffiana **under conditions of Brazilian Cerrado.**

Photo: L.M. Nascimento

ABSTRACT

The propagation of the palm *Syagrus romanzoffiano* is done sexually with seeds, making the process of obtaining new plants slow and difficult, especially on large scales. In addition, seed germination is slow, uneven and susceptible to degradation and loss of vigor because of embryo deterioration, even under laboratory conditions. As a result of the lack of information on efficient depulping methods for queen palm fruits, the present study aimed to establish a depulping methodology that is less aggressive to embryos, maintaining emergence quality. This experiment was carried out in Goiânia, Brazil, using fruits from eight stock plants submitted to three pre-depulping treatments (control, fermentation and drying) and two depulping methods (industrial depulping and concrete-mixer with the addition of gravel). After the different pre-sowing processes, the fresh and dry pyrenes mass, remaining fibers adhered to the pyrene and seedling emergence were evaluated. The pulper removed an average of 45% more pyrene pulp than the concrete mixer. However, these methodologies did not result in differences in the emergence of plants, which was affected only by the pre-depulping treatment, with superiority in the use of fresh fruits. Thus, the pulper was more efficient for

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the removal of pulp and pulp fibers than the concrete mixer. The depulping method did not significantly affect the pyrenes mass. Higher seedlings emergence values were observed for the fresh fruits that were pulped and sown immediately. Fermentation and partial pulp dehydration exert a negative influence on seedling emergence.

Additional keywords: fermentation; seedling production; native species; ornamental perennials; embryo deterioration.

RESUMEN

La propagación de la palma *Syagrus romanzoffiano* se realiza sexualmente a través de semillas, lo que hace que el proceso de obtención de nuevas plantas sea lento y difícil, especialmente a gran escala. Además, la germinación de la semilla es lenta, desigual y susceptible a la degradación y pérdida de vigor debido al deterioro del embrión, incluso en condiciones de laboratorio. Debido a la falta de información sobre métodos eficientes de despulpado de frutos de palma reina, con el presente estudio se buscó establecer una metodología de despulpado menos agresiva para el embrión, manteniendo la calidad de la emergencia de las plántulas. El experimento se llevó a cabo en Goiânia, Brasil, utilizando frutas de ocho plantas de stock sometidas a tres tratamientos de pre-despulpado (control, fermentación y secado) y dos métodos de despulpado (despulpado industrial y mezcladora de cemento más grava). Después de los diferentes procesos previos a la siembra, se evaluaron la masa de pirenos frescos y secos, la pulpa restante adherida al pireno y la emergencia de plántulas. Se observó que el despulpado industrial eliminó un promedio de 45% más de fibra del pireno que la mezcladora de cemento. Sin embargo, estas metodologías no presentaron diferencias en la emergencia de plantas, que se vio afectada solo por el tratamiento pre-despulpado, con superioridad en el uso de frutas frescas. Por lo tanto, se concluye que el despulpado industrial es más eficiente para la eliminación de pulpa y fibras en comparación con el mezclador de cemento. El método de despulpado no afectó significativamente la masa de pirenos. Se observaron valores de emergencia de plántulas más altos para las frutas frescas despulpadas y sembradas inmediatamente. La fermentación y la deshidratación parcial de la pulpa ejercen una influencia negativa en la emergencia de las plántulas.

Palabras clave adicionales: fermentación; producción de plántulas; especies nativas; plantas perennes ornamentales; deterioro del embrión.

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INTRODUCTION

The queen palm (*Syagrus romanzoffiana* [Cham.] Glassman), also known as jerivá, baba-de-boi and pindó, is a native palm of South America, distributed in Brazil, Paraguay, Argentina and Uruguay. In Brazil, this species is found from southern Bahia to the Rio Grande do Sul State in several forest habitats, including in dry areas (Falasca *et al*., 2012; Lorenzi *et al*., 2015; Santos and Salomão, 2017; Bruno *et al*., 2019).

The *S. romanzoffiana* palm is widely cultivated for ornamental purposes because of its beauty and low maintenance (Downer and Hodel, 2001; Zimmermann *et al*., 2011; Santos and Salomão, 2017). It has high fruit production that stands out because of its importance as a natural food source for several wild species of birds and frugivorous mammals during periods of food shortages (Silva *et al*., 2011; Begnini *et al*., 2013). The fruits are globous, smooth, with a yellow or orange epicarp, and a yellow pulp (mesocarp). They are fleshy-fibrous, sweet flavored, can be consumed *in natura* in the form of juices and the pulp oil serves as a vitamin A source because of the contents carotenoids and tocopherols (Messias and Alves, 2009; Goudel, 2012; Coimbra and Jorge, 2012; Lorenzi *et al*., 2015; Santos and Salomão, 2017). It presents a single seed per fruit, protected by a woody endocarp, measuring about 2.4 by 1.6 cm. The seed contains high levels of lipids (more than 50%), making it possible to obtain biodiesel (Vallilo *et al*., 2001; Goudel *et al*., 2013; Moreira *et al*., 2013).

The propagation of Arecaceae plants, such as *S. romanzoffiana*, is done sexually with seeds, which makes the process of obtaining new plants slow and difficult, especially on large scales (Zimmermann *et al*., 2011; Oliveira, 2014; Lorenzi *et al*., 2015; Santos and Salomão, 2017). In addition, seed germination is slow, uneven and susceptible to degradation and loss of vigor as a result of embryo deterioration, even under laboratory conditions, possibly because of the crystallization of endosperm lipids (Dewir *et al*., 2011; Goudel *et al*. 2013; Oliveira *et al*. 2015; Silva-Cardoso *et al*., 2017).

The queen palm seed is small and connected to the endocarp with a recess, thus forming the pyrene. Separation without damage to the seed is practically impossible, so it is common for the whole pyrene to be used for *S. romanzoffiana* propagation (Goudel *et al*., 2013; Garcia, 2015). Numerous studies with palm seeds recommend before the pyrenes planting use some kind of pulp removal (depulping), either manually (Matteucci, 2007; Dias *et al*., 2011; Goudel, 2012; Pinheiro *et al*., 2017; Pernús and Sánchez, 2017; Beltrame *et al*., 2019), scraping with the aid of sieves (Dewir *et al*., 2011; Garcia, 2015; Isoschi *et al*., 2016; Félix *et al*., 2017; Munhoz *et al*., 2017) or mechanically with the aid of pulpers (Oliveira, 2014). The aim of depulping is to reduce the incidence of fungi and bacteria that use the fruit pulp (mesocarp + epicarp) as a substrate for its development and end up reducing the germinative embryo potential (Bovi and Bortoletto, 1998); however, there is no methodology specific to the queen palm.

Because of the lack of information on efficient methods of depulping queen palm fruits, the present study aimed to establish a depulping methodology that is less aggressive to the embryo of this species, maintaining emergence quality.

MATERIAL AND METHODS

This experiment was carried out in the Laboratory of the Horticulture Sector of the Federal University of Goiás, in Goiânia-GO, in 2017. The fruits used in the experiment were from eight stock plants of the queen palm (*Syagrus romanzoffiana* [Cham.] Glassman), 11 years old, located on private property in the municipality of Anápolis-GO. The experiment design was completely randomized in a 2×3 factorial scheme (depulping methods'seed lots: pre-depulping). Four replicates were used, 25 seeds per plot.

Bunches were collected with fruits in stage IV maturation (completely orange fruit), according to the maturity point defined by Garcia (2015). The fruits were then separated into three lots: in the first, the fruits were depulped soon after harvesting the bunches containing fresh fruits. In the second batch, the fruits were kept in a shaded place at room temperature for 7 d (dry in the shade). In the third batch, fruits were placed in a container with water for fermentation in a shaded place, also for 7 d (fermented fruit).

After the pre-depulping treatments, the fruits were submitted to two depulping methods: one used a mechanical pulper, and the second used a concrete-mixer with gravel (gravel number 2: between 2 and 64 mm). An industrial pulper (Bonina 0.25 DF model, Itametal, Brazil) equipped with a sieve with 0.8 mm diameter holes and a concrete-mixer (MAQTRON-M400 model, Joaçaba, Brazil) with a 1.5 kW power engine and 400 L capacity were used.

After the pulp extraction, the material was allowed to dry in a shaded and ventilated place for 4 d. Subsequently, the pulp and remaining fibers were manually separated from the pyrenes (seeds with endocarp attached) with the aid of knives. The pyrenes and fibers were placed in a greenhouse with forced air circulation for 24 h at 65 °C to dry. Afterwards, the material was weighed to determine the remaining dry mass that was adhered to the pyrene in each treatment.

To determine the effect of the pre-depulping treatment on seed germination, samples containing 25 seeds per replicate were prepared in plastic perforated trays using medium sand sterilized in an autoclave for 1 h at 121°C and 1 atm pressure, which were kept in a greenhouse. The sand was moistened with the amount of water equivalent to 60% of the retention capacity at planting time.

After 330 d, the emerged seedlings that reached adequate transplant size (25 cm) were quantified. The data were submitted to analysis of variance, and the means were compared by the Tukey test at 5% probability.

RESULTS AND DISCUSSION

The pulp and fiber removal from the pulper was significantly more efficient than the concrete mixer, with the fresh fruit being the most difficult to depulp with the concrete mixer (Tab. 1).

Table 1. Fresh mass (FM) and dry mass (DM) of fibers remaining on queen palm pyrene at different pre-sowing and depulping processes.

Pre-depulping process	FM (g)		DM (g)	
	Pulper	Concrete-mixer	Pulper	Concrete-mixer
Fresh fruit	0.33±0.09 aB	0.82±0.09 aA	0.19±0.05 aB	0.51±0.06 aA
Fermented fruit	0.36±0.01 aA	0.39±0.07 bA	0.18±0.03 aB	0.27±0.02 bA
Fruit dried in the shade	0.34±0.12 aB	0.50±0.07 bA	0.22±0.04 aB	0.31±0.03 bA
CV (%)	17.53		13.73	

Means with different lowercase letters in the column and uppercase letters in the row indicate significant statistical differences according to the Tukey test ($P \leq 0.05$; $n=4$). CV = Coefficient of variation; ± = standard error.

The depulping objective is to remove the maximum amount of the pulp adhered to the pyrene to reduce possible attack by contaminants and predators on the seed since the average pulp percentage in queen palm fruits corresponds to about 60% of the total fruit weight and has around 65% moisture (Goudel *et al*., 2013), providing a food substrate for these organisms. Although the remaining fibers in the fruits were greater in the treatments with the concrete mixer, Oliveira (2014) found that there was no increase in germination with the pore fiber removal since these fibers do not restrict water absorption in queen palm seeds. In addition, the endocarp itself is permeable to water, allowing seeds to acquire or lose moisture within the structure (Baskin and Baskin, 2014; Oliveira *et al*., 2015; Santos and Salomão, 2017) and offering a physical defense from attack by small rodents (Guimarães *et al*., 2005). For other Arecaceae (*Sabal palmetto* e *Thrinax morrisii*), the fruit pericarp (epicarp, mesocarp and endocarp) presents a certain impermeability to water and, possibly, to oxygen, a limiting factor for seed germination (Dewir *et al*., 2011), making the removal of these impediment layers via depulping and scarification important.

There was no significant interaction for the mass of the pyrenes for the depulping method used, only for the pre-treatment. For the fresh fruits, lower values were observed for the pyrene fresh mass and dry mass than in the fermented fruits and fruits dried in the shade (Fig. 1). Several authors have indicated that the mean values of queen palm pyrenes dry mass vary from 1.21 to 2.40 g (Goudel, 2013; Fleury *et al*., 2015; Garcia, 2015). In the present study, the average dry mass was 1.28 g although the values of the coefficient of variation (CV) were relatively low, indicating high sample homogeneity. Variation in several characteristics is normal in palms of the same species

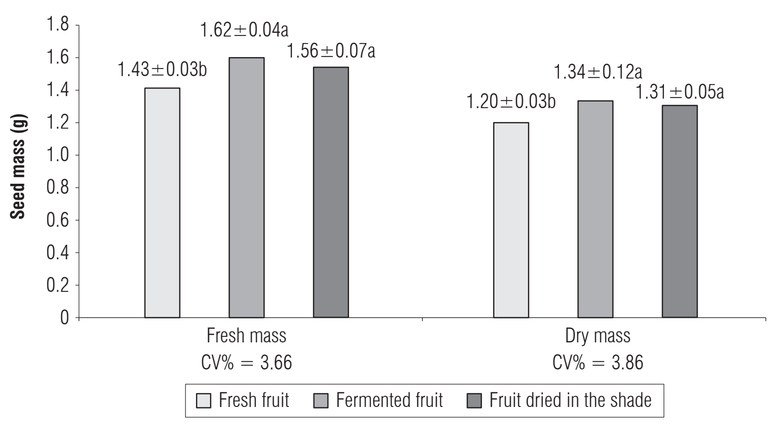

Figure 1. Fresh and dry mass of queen palm pyrenes after different pre-sowing processes. CV = Coefficient of variation; ± = standard error.

as a result of environmental factors and genetic variability (Batista *et al*., 2011a; Mhanhmad *et al*., 2011).

Fruits used in the experiment had an average moisture content of about 62.6%, and the pulp was responsible for 60% of the total moisture content of the fruits. A study on *S. romanzoffiana* showed that the pulp corresponds to about 60% of the total fruit weight and has 66.83% of the moisture (Goudel *et al*., 2013). The high moisture content can cause undesirable changes in the physical, chemical and organoleptic characteristics of fruits and seeds (Moura *et al*., 2010), meaning depulping presents advantage as a seed preservation mechanism. For *Euterpe espiritosantensis*, the presence of pulp impairs final seed quality during storage (Martins *et al*., 2000)

For *S. romanzoffiana,* the highest plant emergence was observed for fresh fruits depulped and immediately sown (62.50%) (Tab. 2). In the non-depulped pyrene, germination was about 35% (Goudel *et al*., 2013). The seeds of freshly harvested ripe queen palm fruits (completely orange) germinate better than stored seeds (Oliveira, 2014), reaching up to 91.25% germination (Garcia, 2015). For *Syagrus oleracea,* the germination rate ranges from 50% to 65% (Diniz and Sá, 1995; Batista *et al*., 2011b). The *Euterpe precatoria* (Arecaceae) fruits sown immediately after harvesting showed the highest germination percentage (Costa *et al*., 2018). The great variability found in the literature was attributed to the fact that the plant is semi-domesticated, with little selection and improvement (Nascente and Peixoto, 2000).

It was observed that fruits dried in a shaded environment presented a low emergence rate when compared to the fresh fruits but were still higher than the fruits with fermented pulp (Tab. 2). The queen palm seed has low longevity and undefined storage behavior (Santos and Salomão, 2017); however, Goudel *et al*. (2013) did not observe negative effects on the viability and vigor of queen palm seedlings, suggesting a certain tolerance to seed water loss.

The pulp fermentation exerted a negative influence on the seedling emergence. The decrease in germination can be attributed to increased susceptibility to deterioration (Oliveira, 2014), possibly because of the increased humidity. For *Archontophoenix alexandrae*, fruit depulping after immersion in water for three days provided the highest germination index of freshly harvested seeds, without resulting in large losses in viability (Teixeira *et al*., 2011). Seed scarification and water immersion over a period of time is efficient at overcoming dormancy and increasing germination rates in Arecaceae (Moussa *et al*., 1998; Dewir *et al*., 2011). It is believed that the fruit immersion for seven days in water caused embryo death, possibly by anaerobiosis, cotyledon fermentation or even temperature increases, as a result of fermentation.

Table 2. Emergence of seedling shoots after different pre-sowing processes.

Pre-depulping process	Emergence (%)
Fresh fruit	62.50±8.66 a
Fermented fruit	1.25±2.50 c
Fruit dried in the shade	26.25±11.09 b
CV (%)	34.73

Means with different letters indicate significant statistical difference according to the Tukey test ($P \leq 0.05$; $n=4$). CV = Coefficient of variation; ± = standard error.

CONCLUSIONS

The electric pulper was more efficient for the removal of pulp and pulp fibers than the concrete mixer, with fresh fruits being the most difficult to pulp with the concrete mixer. However, the depulping method did not significantly affect the mass of the pyrenes. Lower values of fresh mass and dry mass of the pyrene were observed in the fresh fruits than in the fermented fruits and fruits dried in the shade. This fermentation and partial pulp dehydration exerted a negative influence on seedling emergence, different from what was obtained with the use of fresh fruits that were sown immediately, which promoted a higher emergence rate.

Conflict of interest. The manuscript was prepared by the authors, who declare that there are no conflicts of interest that put at risk the validity of the results presented here.

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Acknowledgments

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- The yield measured in dry mass was 10 g^{-1}, but not 10 g^{-1} of dry mass d^{-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.

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