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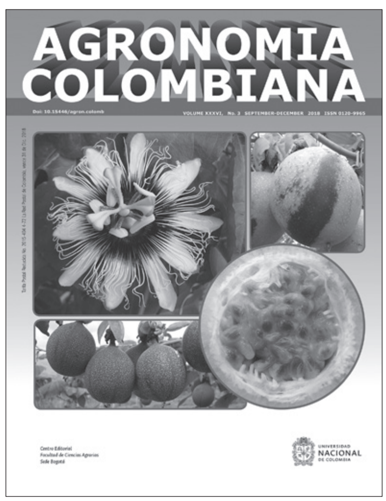

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AGRONOMIA COLOMBIANA

VOLUME XXXVI | No. 3 | SEPTEMBER-DECEMBER 2018 | ISSN (print): 0120-9965 / ISSN (online): 2357-3732

TABLE OF CONTENTS

The Editorial Committee of the Faculty of Agricultural Sciences and the current and former editors wish to express our deepest and most sincere condolences on the passing of Dr. Yoav Bashan, who was part of our journal's Scientific Committee until last September.

Dr. Bashan was an outstanding researcher, leader of the Microbiology and Biotechnology group of the Centro de Investigaciones Biologicas del Noroeste, CIBNOR (La Paz, Mexico) and Associate Professor of Auburn University (Alabama, USA). For three decades, he achieved renown in research areas such as water bioremediation, plant-microorganism interaction, soil microbiology, plant growth-promoting bacteria, desert revegetation and the role of plants in agriculture and the environment.

In this editorial, we would like to thank Dr. Bashan for the support he generously gave to *Agronomia Colombiana*.

Finally, the editor of *Agronomia Colombiana* also wants to inform the readers and future authors that the journal will publish review articles again, maximum one per issue. Before being submitted to peer review, any review article must be approved by the Editor-in-chief and the Editorial Committee. Other details about the publication of review articles can be found in the section 'Requirements for publishing in Agronomia Colombiana'

El Comité Editorial de la Facultad de Ciencias Agrarias y los editores (actual y pasados) desean expresar su profundo pesar por el deceso del Dr. Yoav Bashan, quien hizo parte del Comité Científico de nuestra revista hasta el pasado mes de Septiembre.

El Dr. Bashan fue un destacado investigador, líder del grupo de Microbiología y Biotecnología del Centro de Investigaciones Biológicas del Noroeste (La Paz, México) y profesor titular de la Auburn University (Alabama, USA). Se distinguió durante tres décadas en áreas de investigación como la bio-remediación del agua, interacción planta-microorganismos, microbiología del suelo, bacterias promotoras del crecimiento vegetal, revegetación del desierto y el rol de las plantas en la agricultura y medio ambiente.

En esta editorial se quiere agradecer al Dr. Bashan el apoyo que generosamente ha brindado a *Agronomía Colombiana*.

Finalmente el editor de *Agronomía Colombiana* también quiere informar a los lectores y futuros autores, que la revista volverá a publicar artículos de revisión, máximo uno por número. Cualquier artículo de revisión, antes de ser sometido a revisión por pares, debe ser aprobado por el editor en jefe y el Comité Editorial. Otros detalles de la publicación de artículos de revisión pueden encontrarse en la sección 'Requerimientos para Publicar en *Agronomía Colombiana*'.

Mauricio Parra Quijano
Editor en jefe
Revista Agronomía Colombiana

On-farm conservation of potato landraces in Ecuador

Conservación *in situ* de variedades de papa nativas del Ecuador

Álvaro Monteros-Altamirano[1]*

ABSTRACT

Potato-landrace production systems have not been previously described in Ecuador. Accordingly, three areas of high potato diversity were identified using the passport data of samples collected during the 70s and 80s. Native potato diversity collected at these three locations during 2006-2008 was compared with the diversity at the same places approximately 30 years ago to determine the dynamics in the potato diversity. Additionally, potato-farmers growing landraces were interviewed and invited to local meetings to evaluate the vulnerability of their systems. When the earlier collections were compared with the 2006-2008 collection, many landraces with new names were found. The low number of landraces common to the past and present collections might suggest that the sampling of local landraces was not exhaustive, both during the 1970s and 1980s and during the 2006-2008 collection trips. Mostly elderly people and small-scale farmers are currently maintaining potato landraces. Since farmers cannot live solely on the production of their farms, they look for income alternatives through migration. The vulnerability of the potato conservation varied between the study areas. External conservation interventions performed on-farm, such as diversity fairs or re-introduction of landraces, were highly appreciated by the farmers and could help preserve potato landraces.

Key words: agrobiodiversity, genetic resources, farmers, *in situ*, *Solanum tuberosum*.

RESUMEN

Los sistemas de producción de papas nativas no han sido previamente descritos en el Ecuador. Con este propósito, tres áreas de alta diversidad de papas fueron identificadas usando datos pasaporte de materiales colectados durante los años 70 y 80. Se comparó la diversidad de papas nativas cultivadas durante el 2006 al 2008 en estas tres localidades con la diversidad colectada en los mismos sitios aproximadamente 30 años atrás para determinar las dinámicas en su diversidad. Adicionalmente, los cultivadores de papas del estudio fueron entrevistados e invitados a encuentros locales para evaluar la vulnerabilidad de sus sistemas de producción. Cuando las colectas anteriores fueron comparadas con la colección de este estudio, se hallaron algunas variedades nativas con nuevos nombres. El bajo número de variedades comunes halladas 30 años atrás y durante este estudio puede sugerir que las dos colectas no fueron exhaustivas. Principalmente adultos mayores y agricultores de pequeña escala son los que mantienen todavía las papas nativas. Como sus fincas no logran proveer los ingresos necesarios, los productores buscan ingresos alternativos a través de la migración. La vulnerabilidad de la conservación de papa varía entre las áreas estudiadas. Intervenciones externas para la conservación en-finca, tales como ferias de semillas o re-introducción de variedades nativas de papa, fueron de alto aprecio por los agricultores y puede ayudar a conservarlas.

Palabras clave: agrobiodiversidad, recursos genéticos, agricultores, *in situ*, *Solanum tuberosum*.

Introduction

In South America, there is wide diversity in cultivated and wild potato species. Ecuador is one of the centers of diversity for these species (Hawkes, 1988; Hawkes, 1990). The Ecuadorian biodiversity of potato includes 23 wild species and three cultivated *Solanum tuberosum* (Andigenum group) diploids, triploids and tetraploids (Spooner *et al.*, 1992; Spooner *et al.*, 2007; Spooner *et al.*, 2014). The diversity in cultivated potato is not randomly distributed; spots with high diversity can be identified. These spots or microcenters are small areas in which the diversity of a crop is concentrated (Harlan, 1951). The International Potato Center (CIP) has identified some microcenters of diversity for native potatoes. In Ecuador, the Chimborazo and Carchi provinces are considered such microcenters (CIP, 2017). Areas with high diversity are suitable targets for on-farm conservation of plant genetic resources (Bellon, 2004). On-farm conservation studies on native potatoes have been published in Peru (Brush and Taylor, 1992; Brush *et al.*, 1995; De Haan *et al.*, 2007; De Haan and Juárez, 2010; De Haan *et al.*, 2010) and Bolivia (Terrazas *et al.*, 2005; Terrazas and Cadima, 2008; Iriarte *et al.*, 2009). Initial studies on native potatoes in Ecuador were carried

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out by Monteros *et al.* (2005a), but more studies are required in order to achieve efficient *in situ* conservation of these valuable resources in this country.

Two research sites identified in the study by Monteros *et al.* (2005a) (Carchi and Chimborazo) are identical to the microcenters identified by CIP (2017). The province of Loja was identified as the third research site; this province was recognized as a biodiversity hot spot (Pohle and Gerique, 2008b), but its potato diversity has not been recognized yet. The ethnic background of the farmers who cultivate these potatoes is different in the three research areas.

Most of the population in Carchi is "mestiza", people with a mixed Spanish and Indigenous cultural background. According to Frolich *et al.* (1999) and Espinosa (2006), they were never under Inca influence. Carchi has a relatively small number of indigenous people, 2.8% of the total population in the province according to Chisaguano (2006). Carchi is also the first area where intensive potato monocropping became a common practice during the last 20-30 years. However, this area has changed, currently almost exclusively producing pasture and milk cow grazing (Frolich *et al.*, 1999). Espinosa (2006) highlighted the lack of organization and cooperation among farmers in Carchi.

In Chimborazo, most of the farmers are indigenous people who value their culture (Espinosa, 2006). Chimborazo is considered the capital of the indigenous population, accounting for 38% of the total population in the province (Chisaguano, 2006). From the 17^{th} century on, Chimborazo's countryside has been dominated by the "hacienda" system; the system was based servitude known as "Huasipungo" (Korovkin, 1997). In 1964, these farmers were granted property rights for their small plots of land (Korovkin, 1997). Farmer organization tends to be stronger here than in Carchi (Espinosa, 2006).

Loja, in southern Ecuador, has an indigenous population of about 3.1%, mostly located in the Saraguro canton (Chisaguano, 2006). Saraguro is one of the areas within the Loja province with a higher level of potato crop diversity (Finerman and Sacket, 2003; Pohle and Gerique, 2008b). The Saraguros are a highland Indigenous group who speak Quichua. Since the 19th century, they have kept cattle to supplement their traditional "system of mixed cultivation", featuring maize, beans, potatoes and other tubers. It is assumed that they originally came from the Titicaca region in Bolivia and settled as workers and vassals in the Andean highlands, working for the Incas (Pohle, 2008a). Nowadays, mestizos and indigenous people share the region.

In this study, we used the term landrace as defined by Camacho *et al.* (2006): "A landrace is a dynamic population(s) of a cultivated plant that has historical origin, distinct identity and lacks formal crop improvement. It is also genetically diverse, locally adapted and associated with traditional farming systems". More than 400 landraces of native potatoes have been reported in Ecuador (Cuesta *et al.*, 2005). However, only 20 landraces have been reported to be marketed in the central provinces of Ecuador (Unda *et al.*, 2005). In addition, it is unknown to what extent farmers maintain landraces in the Ecuadorian Andes although it has been suggested that the introduction and use of modern cultivars and the lack of market opportunities are negatively influencing the conservation of landraces (Cuesta *et al.*, 2005). However, there is no systematic inventory on the forces that benefit the conservation of these materials.

This paper describes the state of the conservation of potato landraces in Ecuador. The potato diversity found from 2006 to 2008 at three locations was compared with the diversity at the same places approximately 30 years ago to determine the dynamics in the potato diversity. Potato farmers currently growing landraces were interviewed and invited to local meetings to evaluate the vulnerability of the contemporary system.

Materials and methods

The research areas

To identify the research areas, three databases with the passport data of previous collections in Ecuador from the 1970s and 1980s were used. The data were analyzed with the program DIVA GIS 4.2 (Hijmans *et al.*, 2004). One database was obtained from the International Potato Center (CIP), which contained 459 Ecuadorian accessions, including cultivated and wild species (CIP, 2007); the two remaining databases were obtained from The National Institute for Agricultural Research INIAP (Instituto Nacional de Investigaciones Agropecuarias). The INIAP databases were from the National Program for Root and Tuber Crops-PNRT (692 accessions of cultivated material) and from the National Department of Plant Genetic Resources-DENAREF (187 accessions of cultivated and wild material). Duplicates in the databases were eliminated. These duplicates were determined by identical names and collection sites between the databases; the same was done for the landraces or wild materials. Accessions corresponding to modern cultivars were also eliminated. In addition, landraces with identical names at the canton level were eliminated because the authors assumed they were the same material. In total, 443 accessions of landraces were included in the new database.

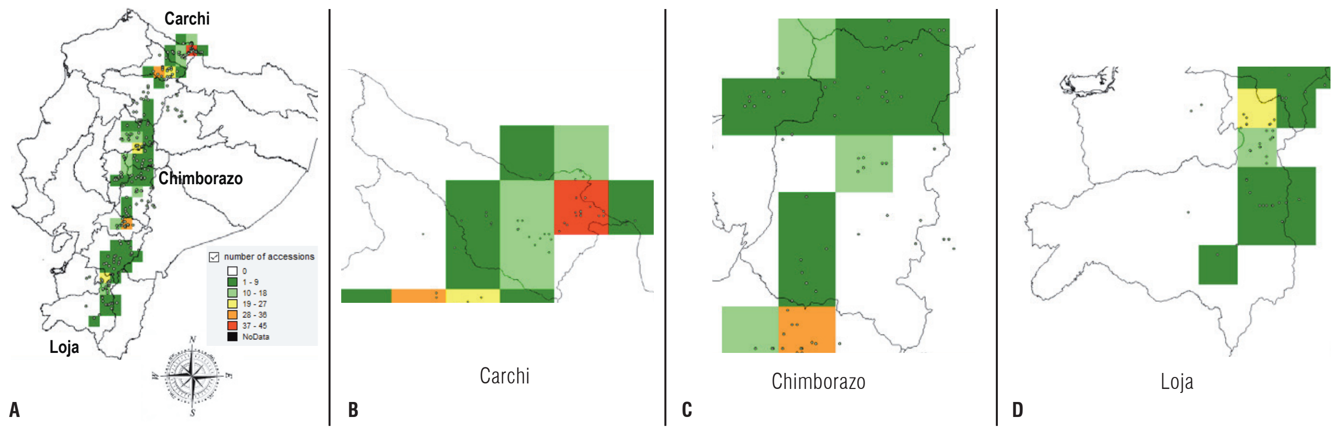

FIGURE 1. Distribution of cultivated native potatoes in the Andean region of Ecuador using DIVA GIS 7.5 (passport data from collections during the 1970s and 1980s). A) Potato landraces distribution in Ecuador. The research areas selected for this study included: B) Province of Carchi (north of the country), C) Province of Chimborazo (central part of the country) and D) Province of Loja (south of the country).

Three research sites were selected. DIVA GIS 7.5 generated maps with colored cells indicating the number of landraces present (Fig. 1), which were used as the first selection criterion. Since there were several areas with high numbers of landraces, the geographical location (north, center and south) was considered as well. The three research sites that were selected were located in the provinces of Carchi, Chimborazo, and Loja (Fig. 1).

Collection of the landraces in the research areas

The earlier potato collection missions in the 1970s and 1980s yielded over 400 accessions of Ecuadorian potato landraces. These samples included 82 accessions from Carchi, 35 from Chimborazo and 41 from Loja. However, over the years, accessions were lost and only 91 Ecuadorian potato landraces were still maintained *ex situ* at the time the first collection activities for this study were performed in 2006.

As done in the earlier collections, we explored the cantons Espejo, Mira, El Angel, Huaca, Montufar, San Gabriel and Tulcan in Carchi; Chunchi, Colta, Guamote, Guano, Penipe and Riobamba in Chimborazo (the canton Alausi was included in this collection, but not in the 1970s-1980s collection), and Gonzanama, Loja and Saraguro in Loja. The collections followed the methodology currently used by INIAP and other gene banks (Castillo and Herman, 1995). The farmers were informed of the purpose of this study and they agreed to provide the materials to the collectors. As there was no information on the individual farmers that were visited in the collection missions in the 1970s and 1980s, information was gathered in every microcenter for farmers holding “old potato landraces”, with the assumption that this snowball technique would deliver some information about the current holders of landraces. The search of landraces was not restricted to those already reported, but for all available old landraces. After collecting a specific landrace, any other landrace with the same name from another farmer in the same canton was discarded. Landraces were only collected when the morphological appearance was (slightly) different from the synonym landrace. Every collection was a sample of five to ten tubers. After every collection trip, the potato samples were taken to the INIAP-Santa Catalina Experimental Station in Quito for propagation and evaluation.

Survey

To collect information about on-farm potato conservation in the three research areas, a questionnaire with 32 questions was prepared (Appendix 1). Fifty (50) farmers were selected in each research area. Initially, all farmers that provided germplasm were interviewed. Then, these farmers were asked to suggest other potato farmers in the area that were currently growing potato landraces or had been growing them in the past. This way, the number of farmers needed to meet the required total of 50 farmers per research area was achieved. At the selected farms, the interviews were done with either men or women, based on availability. This fieldwork was carried out from March to August of 2008.

Farmer meetings

Three one-day long farmer meetings were organized in each research area: one in Tenta-Loja (November, 2009), another in San Gabriel-Carchi (December, 2009), and the last one in Pisicaz-Chimborazo (February, 2010). These meetings had three objectives: 1. to provide farmers with feedback from the surveys, 2. to clarify some of the issues that arose from the interviews, 3. to return landraces collected in each study area to the farmers. All of the farmers involved in the collection and survey processes were invited.

Data analysis

All of the information from the surveys was processed in Excel databases and exported to SPSS 15.0 for analysis (SPSS Inc., USA). Descriptive statistics and bi-variate correlations (Pearson, two tailed) were carried out.

Results and discussion

The collecting missions

The snowball technique used to find farmers conserving potato landraces was effective. The farmers in each location pointed out other farmers with specific landraces. After scanning all of the potential farmers in the areas with landraces, except in Chimborazo where more farmers had landraces in their fields, different landraces were traced for collection. During the first trip to Carchi, 14 accessions of potato landraces were collected in the Montufar canton. A second trip later that year added another 38 landraces. For Chimborazo, INIAP-CIP conducted a collection mission in early 2006 to two cantons: Colta and Guamote. At that time, 46 landraces were collected. A complementary collection carried out in 2008 in other cantons (Guano, Penipe, Riobamba, Alausi and Chunchi) resulted in 16 new landraces. In Loja, during January of 2008, 60 potato landraces were collected. A total of 174 landrace accessions were collected from 17, 28 and 30 farmers in Carchi, Chimborazo and Loja, respectively. Farmers growing potato landraces were scarcer in Carchi and dispersed in Loja (and consequently more difficult to find), while in Chimborazo most of the farmers (including the indigenous communities) kept old landraces in their fields.

Additionally, one diversity fair was organized in Chimborazo (Colta canton) in 2008 (Project INNOVANDES, CIP-INIAP-FAO) to celebrate the International Year of the Potato. This fair, which aimed to create awareness and bring potato growers together to exchange material, resulted in 35 additional landraces based on names, morphological characteristics and origin.

All these potato landraces were integrated into the potato collection at the Ecuadorian genebank at INIAP (Appendix 2). In total, this collection provided 209 accessions of landraces from the research areas. They constitute almost 50% of the newly assembled Ecuadorian potato collection, comprising about 450 accessions.

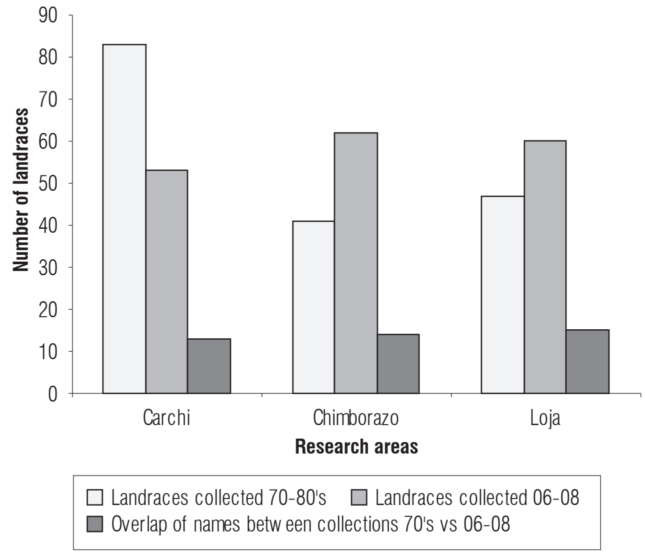

FIGURE 2. Number of landraces collected in the three research areas. The number of different landraces, as judged by name, collected during the 1970s and 1980s and collected during the 2006-2008 period are shown (landraces collected at the diversity fair are not included).

Figure 2 shows the number of landraces collected at each microcenter, based on their names. The names of the landraces collected during the 1970s and 1980s were compared with those collected in the present study (Fig. 2, Appendix 2), and only 13 names were similar between the 2 collection periods for the Carchi research area. In Chimborazo and Loja, only 14 and 15 names were similar, respectively.

Characteristics of the interviewed farmers

The characteristics of the interviewed farmers are shown in Table 1. Men and women growing potato landraces were interviewed according to their availability, which resulted in fairly equal representation in Chimborazo and Loja. Only the survey in Carchi had over-representation by men. However, the men category included six cases in which husbands and wives answered the questions together, but the women preferred their husbands' names to be included in the survey format. According to Table 1, most of the farmers were over the age of 50, with an average age of 53. The race distribution (mestizos and indigenous) differed between the regions. The data in Table 1 also showed that the farmers with landholdings of less than 3 ha (19 farmers in Carchi, 38 in Chimborazo and 31 in Loja) were mainly growing potato landraces. The level of education of the respondents was generally poor. The statistical test (Pearson correlation, two tailed) showed hardly any significant correlations between the descriptors. The only low correlation was between age and education (-0.319). The older people were less educated than the younger generations.

TABLE 1. Characteristics of the 50 respondents to the questionnaire at each of the three research sites in Ecuador. The respondents grew potato landraces.

Descriptor	Characteristics	Carchi	Chimborazo	Loja
Gender	Men	35	23	23
	women	15	27	27
Age	<30 years	2	1	5
	30-40 years	7	9	8
	41-50 years	10	16	12
	>50 years	31	24	25
	Min	25	27	23
	Max	88	70	82
	Mean	58	51	51
	SD	16.1	10.7	15.2
Race	Mestizos	50	10	35
	Indigenous	0	40	15
Farm size (ha)	≤3 ha	19	38	31
	4-10 ha	17	10	4
	≥10 ha	6	1	1
	Min	0.2	0.1	0.03
	Max	70	40	50
	Mean	7.3	3.3	3.0
	SD	12.2	5.9	10.7
Education	No education	3	23	4
	Primary school	42	24	38
	Secondary	3	3	4
	College	2	0	4

In addition to cropping activities, 46% of the farmers in Carchi, 10% in Chimborazo and 64% in Loja kept livestock (cattle and minor animals), or performed house-keeping activities. Other off-farm income-generating activities included paid labor in agriculture or non-agricultural activities, as stated by 20% of the farmers in Carchi, 14% in Chimborazo and 35% in Loja. Figure 3 shows how the farmers valued their activities based on income generation. In Chimborazo, agriculture was the most important activity (86%), whereas in the other regions, the other activities (such as construction or other paid labor) played an important role as well. Most of the potato farmers mentioned having one or more family members who migrated to find a job outside agriculture (53% Carchi, 64% Chimborazo and 52% in Loja).

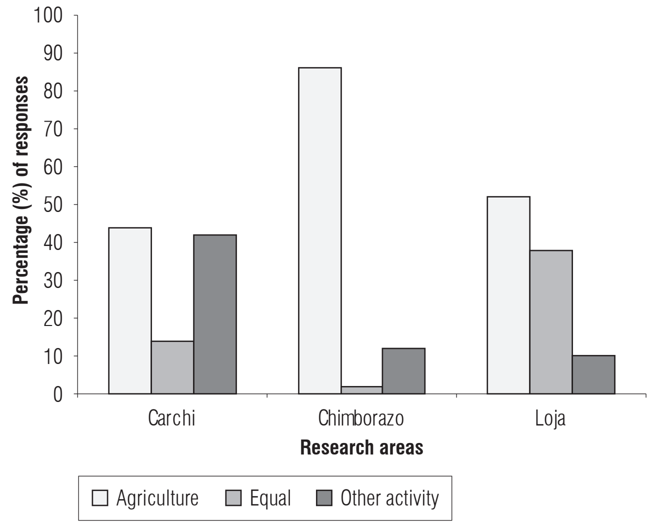

FIGURE 3. Percentage of farmer responses regarding what work (agriculture or other) they considered more important for income generation.

Potatoes in the farming system

The farmers rotated potatoes with other crops. In Carchi, an individual farm can produce up to five different crops, including potatoes, as seen in Chimborazo with 8 crops and Loja with 7 crops. In total, besides potato, 16 crops or crop groups were mentioned: wheat (*Triticum aestivum*), barley (*Hordeum vulgare*), grass (various), faba bean (*Vicia faba*), carrot (*Daucus carota*), peas (*Pisum sativum*), maize (*Zea mays*), other vegetables (various), ulluco (*Ullucus tuberosus*), mashua (*Tropaeolum tuberosum*), oca (*Oxalis tuberosa*), chocho (*Lupinus mutabilis*), fruits (various), quinoa (*Chenopodium quinoa*), bean (*Phaseolus vulgaris*) and other minor crops. In Carchi, the potato farmers included rotations with all of the crops mentioned above except oca, chocho, fruits, quinoa and beans. In Chimborazo, beans were not considered for rotation because this is a crop more suitable at lower altitudes. In Loja, chocho and quinoa were missing from the potato rotations. The questionnaires addressed only the number of major crops that were present in the rotation with potatoes and not all crops present, e.g. medicinal plants or diversity within the other crops were not surveyed.

Most of the farmers in the research areas grew both landraces and commercial cultivars and managed these groups similarly (64% in Carchi, 58% in Chimborazo and 60% in Loja). However, the landraces were grown in smaller plots or in-home gardens, whereas the commercial cultivars were grown in larger plots (field observation). Some farmers grew early sprouting potatoes (*S. tuberosum* andigena group, mainly diploids), referred to as "chauchas", along with other triploids and tetraploids in the same fields, but preferred to grow the landraces in separate rows.

After harvest, the commercial cultivars were sold immediately, and the landraces, which are not usually sold in the market, were stored for consumption (in the kitchen or a nearby storage room), distributed among the family, exchanged with the neighbors or saved as seeds for the next cycle. The farmers in Carchi mentioned exchanging seeds in 46% of the cases, with 23% in Chimborazo and 60% in Loja. However, the farmers usually did not know who else in the community maintained the less common landraces (Carchi 75%, Chimborazo 68% and Loja 53%).

Labor allocation

In the potato farming system in Ecuador, there is a division of labor among family members. The different activities in the potato growing cycle and the different labor divisions are summarized in Table 2.

TABLE 2. Labor division during the potato planting cycle by percentage in the three research areas of potato diversity in Ecuador: land preparation, daily cropping activities and harvest.

Question	Answers	Carchi	Chimborazo	Loja
Who prepares the land?	Farmer (man)	62	22	44
	Farmer (woman)	0	14	14
	Both	9	60	24
	Other	29	4	18
Who does the daily activities?	Farmer (man)	77	30	32
	Farmer (woman)	0	20	26
	Both	6	46	38
	Other	17	4	4
Who harvests the potatoes?	Farmer (man)	32	8	16
	Farmer (woman)	0	0	14
	Both	36	20	52
	Family	0	52	0
	Other	32	20	18

The labor allocation for land preparation was different among the research areas. In Carchi, the men (62%) mainly carried out soil preparation. In Chimborazo, this activity was shared by men and women (60%), and, in Loja, 44% of the respondents answered that only the men prepare the land, but in 24% of the cases, both men and women carried out this activity together. The potato cropping activities (daily activities after planting and before harvesting) were mainly taken care of by the men (77%) in Carchi. This was different in Chimborazo where 46% answered that the activity was shared by men and women. In Loja, 38% answered that men and women together took care of the daily activities, with 32% for only men and 26% for only women. The harvest was, in most cases, a family activity performed by men and women together, as pointed out by the respondents in Chimborazo.

Reasons to maintain potato landraces

Since most potato landraces were not marketed, the farmers grew these potatoes for other reasons. During the meetings, the farmers mentioned culinary characteristics such as good taste and softness after cooking. In Chimborazo, some of the landraces are reported as good for "Cariucho", which is a typical dish with indigenous farmers, made from faba bean, oca, melloco and potatoes boiled together. Other advantageous characteristics include: drought, frost and late blight resistance. Medicinal uses of certain landraces were also mentioned, such as the use of *Puña* to cure headaches or *Chaucha amarilla* to cure arthritis. These and other attributes have kept these potatoes from disappearing.

Potato landraces in farmer's fields

Both the collections during the 1970s and 1980s and these collections (2006-2008) managed to gather a significant number of accessions (158 and 174, respectively). Apparently, the farmers have continued to maintain local landraces. When the earlier collections were compared to the results of later collection trips, many new landraces were found based on the recorded names. There was only a small name overlap between the materials collected in both periods (Fig. 2). This emergence of new landrace names is remarkable and contrasts with the general trend of landraces disappearing and diversity decreasing. However, in Carchi, a decrease of landraces from the past to the present was observed, suggesting genetic erosion. The low number of landraces common to the past and present collections may indicate that the sampling of local landraces was far from exhaustive, both during the 1970s and 1980s and during the reported collection trips. This is further supported by the fact that the diversity fair in Chimborazo resulted in many new landraces. Monteros-Altamirano *et al.* (2017) applied SSR markers to most of the accessions collected in this study, finding significant genetic diversity in the three areas.

A change in the landraces being grown could be explained by the exchange of landraces among farmers, with the associated name changes. Exchanging potatoes is very common in the Andes (Brush *et al.*, 1981; Zimmerer, 1991). However, information does not necessarily travel with the seed lot, producing name inconsistencies (Nuijten and Almekinders, 2008). The movement of potato seed lots may either be inter-regional or intra-regional. The fact that some farmers did not know where the rare landraces were or who in the community held them suggests that this movement has been mainly conducted on an individual basis and not in any organized way. Again, the results presented by Monteros-Altamirano *et al.* (2017) suggested the exchange of potato landraces among the three areas; nevertheless, each area maintained genetic differentiation from the others.

Who maintains the diversity?

This study shows that the majority of the farmers growing native potatoes were relatively old (Tab. 1). This was similar in all three research areas. This group might be expected to be more knowledgeable on potato landraces than younger farmers. With the current educational system, younger generations become better qualified and eventually migrate to cities looking for more rewarding jobs and leaving behind agriculture, these potatoes and their ancestral knowledge. The farmers conserving the potato landraces had mainly small farms (Tab. 1) and were generally associated with low incomes and even poverty. These small farmers maintained the local landraces for food security and/or cultural heritage, but there were no market opportunities.

Potato production in Ecuador is an activity that is shared between men and women. Men carry out the land preparation - which is very intensive labor when done using animals or hand tools - and fungicide application, if any. Women participate in most of the potato cropping activities. In addition, the harvest is an activity performed by both men and women and a family task in Chimborazo. Family involvement in potato cropping is probably advantageous to the conservation of potatoes. However, migration, especially by men, accounts for the feminization of agriculture as observed by Lastarria-Cornhiel (2006).

How and why are the farmers maintaining the potato diversity?

In Ecuador, cultivated potatoes are part of the broader crop diversity seen in farmers' fields and an important element in crop rotation. Potato landraces coexist with commercial potatoes, as is the case in Peru (Brush *et al.*, 1995). The fact that potatoes and other crops co-exist on farms, again as seen in Peru (De Haan and Juárez 2010; De Haan *et al.*, 2010), indirectly supports the survival of potato landraces. The income from marketable crops (potatoes or others) subsidizes the maintenance of non-commercial potato landraces. In addition, it can be inferred that the income from activities outside the farms is important to the families currently maintaining potato diversity (Fig. 3).

Farmers empirically know the nutritional or medicinal value of their potatoes and, so, maintain landraces from generation to generation as a cultural heritage.

Conclusions

Carchi was the most vulnerable area for the conservation of potato landraces. Frolich *et al.* (1999) stated that ancient landraces are no longer found in this area. The farmers holding landraces were scattered throughout the province, i.e. not organized. Mostly elderly people maintained the landraces, and the new generation demonstrated a lack of interest in cropping potato landraces. The potato conservation in Chimborazo appeared more sustainable than in the other areas. Even though old people were currently in charge of the potato landraces, the farmers saw agriculture as the most important source of income. The number of indigenous farmers keeping potato landraces in their fields was higher in this province than in the other areas. Apparently, they were more culturally attached to their land and viewed agriculture as a family activity (Tab. 2). The farmers in Loja have conserved potatoes for a long time, but some aspects could make conservation vulnerable. Similar to Carchi, the farmers holding landraces were scattered throughout the province, i.e. not organized, with conservation mainly done on an individual basis.

Externally driven on-farm conservation activities, such as the diversity fair or re-introduction of landraces, were highly appreciated by the farmers. The diversity fair organized in Chimborazo was effective at raising local awareness on the richness of local crop genetic diversity, as observed in other cases (Almekinders, 2001), and promoting landrace exchanges between farmers. Diversity fairs should be organized in the other areas to support on-farm conservation. The creation of communal potato conservation gardens would also help make landraces more available to farmers and raise local awareness. The newly assembled potato collection at INIAP will complement the *ex situ* – on-farm conservation activities at the national level. Finally, the younger generation of farmers should be motivated to maintain local landraces through education in agrobiodiversity (INIAP-DENAREF, 2009). The creation of market opportunities for the landraces would support both their conservation and use (Monteros *et al.*, 2005b; Devaux *et al.*, 2009).

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APPENDIX 1. Questionnaire

1. Farmer's name:
2. Age:
3. Race: 1 = mestizo, 2 = indigenous
4. Education level: 0 = none, 1 = primary, 2 = secondary, 3 = university
5. Do you have another job besides agriculture? 0 = only agriculture, 1 = raise minor animals, 2 = cattle, 3 = housekeeping, 4 = paid labor, 5 = other
6. Which one is more important? 1 = agriculture, 2 = equal, 3 = other activities
7. Province: 1 = Carchi, 2 = Chimborazo, 3 = Loja
8. Canton:
9. Parish:
10. Locality:
11. Community:
12. Size of the farm (ha):
13. Observations:
14. Date:
15. How many members of the family are men?
16. How many members of the family are women?
17. How many members of the family are working directly in agriculture?
18. How many members of the family have migrated to look for a job different than agriculture?
19. Who prepares the land? 1 = men, 2 = women, 3 = men + women, 4 = hired labor, 5 = tractor, 6 = sharecropper, 7 = men + hired labor, 8 = men + women + hired labor
20. Who takes care of the crop daily? 1 = men, 2 = women, 3 = men + women, 4 = hired labor, 5 = tractor, 6 = sharecropper, 7 = men + hired labor, 8 = men + women + hired labor
21. Who applies fungicides? 0 = not applied, 1 = men, 2 = women, 3 = men + women, 4 = hired labor, 5 = tractor, 6 = sharecropper, 7 = men + hired labor, 8 = men + women + hired labor
22. Who harvests? 1 = men, 2 = women, 3 = men + women, 4 = hired labor, 5 = tractor, 6 = sharecropper, 7 = men + hired labor, 8 = men + women + hired labor, 9 = all family
23. Who sells? 0 = do not sell, self-consumption, 1 = men, 2 = women, 3 = men + women, 4 = hired labor, 5 = tractor, 6 = sharecropper 7 = men + hired labor, 8 = men + women + hired labor
24. Invisible work for women:
25. Crops in the farm:
26. Is there any difference between the management of commercial potatoes and native ones? 1 = Yes, 2 = No
27. Do you grow the landraces mixed or separated? 1 = mixed, 2 = separated
28. If you lose your landrace, do you try to recover it? 1 = Yes, 2 = No
29. If you sell these landraces, where do you do it? 1 = local market, 2 = other
30. Do you exchange seeds with the neighbors? 1 = Yes, 2 = No
31. Do you know anybody that still has these local potato landraces? 1 = Yes, 2 = No
32. Do you believe if you grow potatoes together, they hybridize? 1 = Yes, 2 = No

APPENDIX 2. Potatoes collected in the three research areas

Collection #	Landrace name	Collection #	Landrace name	Collection #	Landrace name
Landraces' names found both during the 70-80's and the 2006-08 collecting missions					
CARCHI		**CHIMBORAZO**		**LOJA**	
JS-28	Botella (blanca)	FM FH RA 005	Cacho	MOPG-009	Bodeguera Blanca
JS-33	Carriza	AMA-301	Chaucha blanca	MPG-029	Chaucha amarilla
AXC-008	Chaucha amarilla	MLL-01	Chaucha colorada	MPG-022	Chaucha amarilla alargada
AC-037	Chaucha borrega o Azul		Chaucha roja	MPG-024	Chaucha amarilla redonda
AXC-015	Chaucha botella	AMA-309	Curipamba	MPG-027	Chaucha Blanca
AXC-007	Chaucha negra	AMA-310	Ilusión blanca	MPG-026	Chaucha negra
AXC-001	Chaucha ratona	FM FH RA 002	Norteña negra	MPG-028	Chaucha roja
JS-35	Curipamba	FM FH RA 003	Pera	MPG-041	Escaleña
AC-041	Mambera	FM FH RA 002	Puña negra	MPG-044	Guano de cuchi
AXC-017	Pamba roja. (Tableada roja).	AMA-303	Tabla	MPG-018	Guata morada
AXC-029	Rosada	FM FH RA 004	Tulca	MPG-017	Guata roja. Guata colorada. Papa cuy
AC-034	Sabanera	FM FY RA 004	Uchu rumi	MPG-033	Negra
JS-25	Violeta	FM FY RA IV 001	Uvilla blanca	MPG-038	Papa de chacra
		FM FY RA 003	Uvilla negra	MOPG-012	Perra dormida
				MOPG-007	Suscaleña blanca
Landraces collected during 2006-2008					
CARCHI		**CHIMBORAZO**		**LOJA**	
JS-29	Alpargata	FM RA 002	Alpargate	ARX-2	Alpargate
JS-36	Cardenilla	MLL-02	Alpargate	MG-004	Bodeguera blanca (ojo blanco)
AC-036	Carriza	FM RA FH 002	Cacho blanco	MG-003	Bodeguera blanca (ojo morado)
AXC-014	Chaucha amarilla	FM FY RA 011	Cacho negro	MG-001	Bodeguera negra (Probable Ambateña)
AXC-012	Chaucha blanca	AMA-300	Camotilla	MOPG-015	Bolona
AC-038	Chaucha botella	FM FH RA 006	Cañareja	MPG-032	Bolona
JS-23	Chaucha negra	FM FY RA IV 004	Cayamarco	MPG-019	Bolona amarilla
AXC-028	Chaucha ratona	FM FY RA 010	Chapituna	MPG-031	Bolona negra
JS-3	Coneja blanca	FM FY RA IV 002	Chaucha amarilla	MPG-020	Carriza
AXC-016	Curipamba		Chaucha blanca	MOPG-001	Chaucha amarilla alargada
AXC-023	Curipamba	AMA-302	Chaucha negra "pera"	MOPG-005	Chaucha amarilla redonda/bolonga
AXC-022	Gualcalá	FM FY RA 005	Chihuila blanca	MG-010	Chaucha negra
AXC-027	Guata= Capiro	FM FY RA 006	Chihuila negra	MOPG-013	Chaucha roja
AC-43	Huevo de indio	FM RA FH 001	Chilca	MPG-023	Chaucha roja
AXC-019	Leona	FM RA FH 002	Coneja	MPG-035	Chola antingua
JS-1	Leona blanca 1	FM FY RA 008	Cornos	MG-005	Churona rosada
JS-34	Leona del Carchi	FM FY RA IV 005	Cuchi chupa	MOPG-003	Colorada
JS-26	Leona negra	FM FY RA 009	Cuchi dzili	MPG-042	Colorada antigua 1
AXC-002	Mampuera	FM FH RA 006	Fayre	MPG-043	Colorada antigua 2
JS-24	Manpuera	AMA-307	Gachu papa	MOPG-004	Colorada chaucha
AXC-009	Morasurco	FM FY 003	Guancala	MOPG-016	Cuchicaca "papa de chacra"
AXC-026	Negra conocida como Morasurco	FM FH RA 001	Guantiva	MOPG-006	Unknown
JS-30	Osito	FM FY RA 003	Huarmi papa	MOPG-011	Guacalá blanca
AXC-018	Pamba pintada. (Tableada pintada).	AMA-308	Jobaleña	MOPG-014	Guacalá roja

Continúa

Collection #	Landrace name	Collection #	Landrace name	Collection #	Landrace name
Landraces collected during 2006-2008					
CARCHI		**CHIMBORAZO**		**LOJA**	
AXC-020	Parda mejorada	FM FY RA 007	Leona negra	MG-007	Guata amarilla
AC-042	Parda pastusa	FM FY RA 008	Leona roja	MG-012	Guata blanca ojona
AXC-021	Parda suprema	FM FY RA 001	Limeña	MOPG-010	Guata roja
JS-31	Puña	FM FY RA 005	Loro papa	MG-016	María Esperanza
AXC-013	Pura sangre	FM FY 002	Mamey	ARX-1	María Esperanza
AC-040	Rabo de gato	FM FY 001	Mami	MPG-040	Negra ojona
AC-039	Ratona amarilla	FM FY RA IV 003	Manuela	MG-011	Negra ojona
JS-32	Roja plancha	AMA-306	Mishi maqui “uña gato”	MOPG-002	Negra, carrizo o catalina
AXC-030	Roja plancha	FM FY RA 004	Moronga	MPG-021	Papa chacra
JS-27	Rosada	FM FH RA 001	Norte roja	MPG-034	Papa curra (como gusanito)
AXC-003	Sulipamba	FM RA FH 003	Noteña	MG-009	Papa de chacra
AXC-004	Super violeta		Papa yerac	MPG-025	Papa huinga
AXC-011	Uva	FM FY RA 006	Pargate	ARX-3	Quiteña
JS-2	Uva	FM FY RA 001	Pudzu uvilla	MPG-030	Roja
AXC-025	Violeta común	FM FH RA 004	Puña	MG-013	Semibolona 1
		MLL-04	Puña	MG-014	Semibolona 2
		FM FY RA 007	Tsujtsuj	ARX-4	Suscaleña blanca
		AMA-304	Turca	MG-006	Suscaleña colorada
		AMA-305	Turca “tablona”	MOPG-008	Suscaleña negra
		FM RA FH 001	Uvilla	MG-015	Wicupa amarilla
		MAP-001	Uvilla	MPG-037	Wicupa colorada
		FM RA 001	Uvilla amarilla		
		MLL-03	Uvilla original		
		FM FH RA 005	Yana pera		
Landraces collected during the diversity fair (CHIMBORAZO)					
XCFM-11	Caperucita	XCFM-4	Huagrasinga	XCFM-7	Rapuña
XCFM-9	Capulí	AMFY-3	Huancala	AMFY-20	Tabaquera blanca
XCFM-1	Castillo	AMFY-1	Manuela 1	AMFY-19	Tabaquera colorada
XCFM-18	Chaucha manzana	AMFY-2	Manuela 2	XCFM-8	Tanda
AMFY-16	Chaucha ratona	XCFM-17	Marta	AMFY-9	Tsujsuj morado
AMFY-6	Chihuila roja	XCFM-12	Morosel	AMFY-8	Tulca blanca
XCFM-6	Chugsho	AMFY-15	Norteña Antigua	XCFM-19	Tulca hembra
AMFY-4	Chuquillinga	AMFY-13	Papa puya	XCFM-3	Unknown
XCFM-2	Cuerno blanco	AMFY-5	Papa table	AMFY-12	Ascho Chaqui (pata perro)
AMFY-18	Curiquinga	XCFM-10	Pera amarilla	AMFY-10	Yanatabla
AMFY-17	Frayla	XCFM-5	Puca table	AMFY-7	Chaucha crespa
XCFM-13	Freila	AMFY-11	Rapuña		

Fertility recovery of anther-derived haploid plants in Cape gooseberry (*Physalis peruviana* L.)

Restauración de la fertilidad de plantas haploides derivadas de anteras en uchuva (*Physalis peruviana* L.)

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ABSTRACT

The cape gooseberry (*Physalis peruviana* L.) is one of the most important Colombian exotic fruits. Chromosome doubling of anther-derived plants is a key factor in the application of double haploid technology for the genetic improvement of crops. In the present study, axillary buds from four haploid cape gooseberry genotypes were used to evaluate artificial chromosome doubling induced by colchicine and its effects on ploidy level and pollen fertility. Three concentrations of colchicine (5, 10 and 15 mM) and three exposure times (2, 4 and 6 h) were used to determine the best treatment for the generation of fertile plants from axillary buds of haploid genotypes. The colchicine increased both the number of chromosomes, from 36 to 129, and the average chloroplasts in stomata guard cell, from 4.5 to 23.8. The optimal chromosome doubling of the haploids was obtained with the 5 mM colchicine solution and 2 h exposure time. This protocol produced chromosome doubling in over 60% of the regenerants of the four haploid genotypes, with a high level of fertility. Morphologically, the fertile mixoploid plants showed variation in the vegetative, flowering and fruit characteristics, as compared to the haploid plants.

Key words: colchicine, chloroplasts, chromosomes, mixoploidy.

RESUMEN

La uchuva (*Physalis peruviana* L.) es una de las frutas exóticas de mayor importancia para Colombia. La duplicación cromosómica de plantas derivadas de cultivo de anteras es un factor clave en la aplicación de la tecnología doble haploide para el mejoramiento genético de los cultivos. En el presente estudio, se evaluó la duplicación cromosómica artificial inducida por colchicina en yemas axilares de cuatro genotipos haploides de uchuva y su efecto en el nivel de ploidía y la fertilidad polínica. Se emplearon tres concentraciones de colchicina (5, 10, 15 mM) y tres tiempos de exposición (2, 4 y 6 h) con el fin de determinar el mejor tratamiento para la generación de plantas fértiles a partir de yemas axilares de genotipos haploides. La colchicina incrementó el número de cromosomas entre 34 hasta 129 y el promedio de cloroplastos por células guarda entre 4.5 hasta 23.8. El doblamiento cromosómico óptimo de plantas haploides se obtuvo con la solución de colchicina 5 mM por 2 h. Este protocolo produjo doblamiento cromosómico en 60% de las plantas regeneradas obtenidas en los cuatro genotipos haploides, presentando un alto nivel de fertilidad. Morfológicamente, las plantas mixoploides fértiles presentaron variación en características vegetativas, de floración y en fruto, comparado con las plantas haploides.

Palabras clave: colchicina, cloroplastos, cromosomas, mixoploidía.

Introduction

The cape gooseberry (*Physalis peruviana* L.) is also known as goldenberry, uchuva, uvilla, and aguaymanto, among many other names. It is an exotic fruit of the Solanaceae family that is distributed worldwide, with the greatest diversity in South America (Olmstead, 2013). *P. peruviana* is a tetraploid species ($2n = 4x = 48$) although genotypes with $2n = 2x = 24$ have been reported, suggesting that the basic chromosome number is $x = 12$ (Rodríguez and Bueno, 2006; Liberato *et al*., 2015).

The cape gooseberry fruit is quite well regarded because of its nutritional value, which has high concentrations of vitamins A, C and B complex, along with phosphorus, calcium, and iron. Additionally, bioactive compounds, such as withanolides, phenolics, and ethanol derived substances, that are found in extracts from the leaves provide antifungal, antioxidant, antitumor and anti-inflammatory health benefits (Ligarreto *et al*., 2005; Wu *et al*., 2006). The cape gooseberry has gained commercial significance in Colombia, where it was widely introduced into cultivation in 1985, mainly for the domestic market as fresh fruit.

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Colombia, one of the major producers of this fruit together with India, Vietnam, and South Africa, is the top exporter of this crop (Patiño *et al.*, 2014).

Anther culture is an effective alternative technique for producing doubled haploid (DH) lines in cultivated crops (DH technology). The production of haploid plants from heterozygous genotypes, followed by chromosome doubling, allows for the fixation of traits without multiple selfing generations; this shortens the breeding time and facilitates the development of new varieties (Murovec and Bohanec, 2012).

In cape gooseberry breeding programs, the anther culture methodology was employed as a complement to conventional methods for homozygous line production (Suescún *et al.*, 2011). However, in one study, only 28% of anther-derived plants presented spontaneous chromosome doubling (Sánchez, 2014). This is an important factor to consider in the application of anther culture technology because breeding programs require a large number of homozygous DH plants with a high level of fertility; therefore, it is necessary to induce chromosome doubling in the cape gooseberry.

Several doubling agents have been used to induce chromosome doubling in species from the Solanaceae family, including oryzalin (Chauvin *et al.*, 2003) and trifluralin (Amiri *et al.*, 2010). However, the most commonly used chemical agent to induce chromosome doubling is colchicine (Murashige and Nakano, 1966; Ojiewo *et al.*, 2006; Amiri *et al.*, 2010), which disrupts mitosis by inhibiting the formation of spindle fibers, resulting in chromosome doubling (Seguí-Simarro and Nuez, 2008). There are no reported protocols for colchicine-mediated doubled haploids in the cape gooseberry although Wenzel (1973), Díaz *et al.* (2008) and Franco (2012) reported high levels of autopolyploidy after an *in vivo* treatment of tetraploid plants. In the present research, four haploid cape gooseberry genotypes were used to evaluate artificial chromosome doubling induced by a colchicine treatment and the effects on the number of chloroplasts per guard cell, number of chromosomes and fertility with the purpose of developing an efficient and reliable artificial chromosome doubling protocol for anther-derived cape gooseberry haploids.

Materials and methods

Plant material and growth conditions

Haploid cape gooseberry plants were produced with the anther culture technique. Four haploid genotypes: 16U490, 16U520, 16U793 and 16U802 were selected based on phenotypic observations in a greenhouse. They were sterile and exhibited a lower plant vigor than anther-donor plants. *In vitro* plants of the four genotypes were subcultured every four weeks in an MS medium (Murashige and Skoog, 1962) supplemented with 50 g L^{-1} sucrose, 0.1 mg L^{-1} indole-3-acetic acid (IAA, 3-IAA) and 0.5% (w/v) phytagel at pH 5.8 and incubated under light condition using a 16 h photoperiod at 21 ± 2°C.

Colchicine treatment and plant regeneration

Axillary buds from the haploid genotypes were excised and immersed for 2, 4 or 6 h in the dark, in an aqueous solution of colchicine at 5, 10 and 15 mM concentrations. The solution was dissolved in 2% dimethyl sulfoxide (DMSO) and sterilized with filtration using a 0.22 µm MILLEX® (Merck KGaA, Germany) membrane. Then, the removed buds were rinsed for 2 min, using three 10 mL changes of deionized sterilized water.

Fifteen axillary buds of every haploid genotype were used in each treatment. The same number of plants of each haploid genotype were grown in DMSO without a colchicine treatment, serving as controls. The control explants were maintained in a Murashige and Skoog (MS) medium, while the colchicine-treated buds were maintained in an MS medium supplemented with 0.1 mg L^{-1} of indole butyric acid (IBA) and sub-cultured once. In this step, the survival rate of the treated buds was evaluated.

Shoots that developed were transferred to Canadian peat and maintained in a moist chamber at a temperature of 24°C with a high relative humidity of 80% and normal light for 2 weeks; the shoots were then placed under shading for 2 more weeks under greenhouse conditions. The plants were transplanted to a soil-rice hull mixture (3:1), then irrigated and weeded as needed until they produced 4 to 5 flowers at a developmental stage that was suitable for evaluating the pollen fertility.

Chromosome and chloroplast number evaluation

The chromosome numbers (chrs) of the regenerated plants were determined with chromosome counting using root-tip meristem squashes, and the chloroplast number (chls) was determined with stomatal guard cell observations. For the squashes, root samples 1 cm in length were collected in the early morning from plants in the growth room. Each sample was placed in 0.25% colchicine solution dissolved in 2% DMSO for 3 h at room temperature. After rinsing 2-3 times with tap water, the root samples were transferred to deionized distilled water in a temperature-controlled water bath at 37°C for 1 h. Then, the roots were fixed in 3:1

ethanol-acetic acid and stored at 4ºC for 12 h, hydrolyzed in 1N HCl for 25 min and rinsed again 4 to 5 times with tap water. The root tips were excised and squashed in 2% propionic orcein stain. The slides prepared for chromosome counting at the metaphase stage were examined under a Zeiss Axiostar-plus microscope (ZEISS, Germany), and the chromosome counts were done on about 25 nuclei.

In order to count the chloroplast number, the epidermis was peeled from the abaxial surface of the leaves and placed in a drop of 1:1 KI-I solution, previously dissolved in 70% ethanol and covered (but no squashed) with a coverslip. The total number of chloroplasts per guard cells was counted with a Zeiss Axiostar-plus microscope, observing at least 25 guard cells.

Pollen fertility

To check fertility, the pollen viability and *in vitro* pollen germination were determined in the anther-donor, haploid and colchicine-treated plants. First, the pollen was collected from 5 flowers per plant during anthesis, distributed on a glass slide with a drop of acetocarmine and covered with a coverslip. The pollen grains were observed through an optical microscope (Zeiss Axiostar-plus microscope x100 magnification) and subsequently classified as fertile or not fertile. The pollen grains with aberrant morphology, little staining and reduced and/or absent protoplasm were considered sterile, while those that displayed intact cell walls and deeply stained protoplasm were considered fertile. This evaluation was performed on 100 pollen grains per plant.

The *in vitro* germination was assessed with the hanging drop method. The pollen germination and pollen tube growth were determined by placing a small drop of germination media on a cover glass, and pollen grains were placed on the drops with a clean dissecting needle. The pollen was incubated under dark conditions at 25°C in a culture medium containing 0.1% sucrose, 0.1% boric acid (H_3BO_3), and 2 ml Tween 80 for 6 h at 21°C (Grisales *et al.*, 2010). After this period, 200 pollen grains were counted in a random space with a Zeiss Axiostar Plus microscope to estimate the mean number of germinated and non-germinated pollen grains. For purposes of analysis, pollen grains whose tubes reached the same or greater length than their own diameter were considered vital or germinated (Báez *et al.*, 2002).

Data analysis

The experiment design was completely randomized in a factorial arrangement of 4 genotypes × 3 colchicine concentrations × 3 exposition times, for a total of 36 treatments with 3 replicates. Each replicate was represented by a glass container with 4 buds. Analysis of variance (ANOVA) and Pearson correlation were carried out using the SAS software (Statistical Analysis System version 9.1.3, Cary, NC, USA). *P*-values of less than 0.05 were considered statistically significant.

Results and discussion

Survival rate and plant regeneration

The survival rate of the treated buds gradually decreased when treated with a high concentration of colchicine and a prolonged immersion period (Tab. 1). The regeneration rate of buds treated with colchicine at a concentration of 5-10 mM was above 40%, while a 33% survival rate was obtained with the 15 mM colchicine at 6 h for genotype 16U802. The colchicine delayed bud development and growth in the first month after treatment. However, after the subculture step, the treated buds grew normally. The treatment with 15 mM of colchicine significantly decreased the plant regeneration frequency. In addition, the highest plant regeneration frequency was achieved using the colchicine treatments of 5 and 10 mM for 2 h. Significant differences were detected in the plant survival rate as a result of the colchicine concentrations ($P \leq 0.0001$) and exposition time ($P = 0.0257$), while no significant differences between the genotypes ($P = 0.2481$) were observed.

TABLE 1. Effects of colchicine concentration and treatment duration on the survival rate of treated cape gooseberry plants.

Colchicine concentration (mM)	Exposition time (h)	Survival rate (%)			
		16U490	16U520	16U793	16U802
Control (DMSO)	2	92	83	100	92
	4	92	83	92	83
	6	83	83	83	83
5	2	83	83	100	67
	4	67	75	92	50
	6	58	75	75	42
10	2	67	58	50	50
	4	50	42	42	50
	6	50	42	42	42
15	2	67	58	50	50
	4	50	50	50	42
	6	50	42	42	33

Chromosome number

The anther-donor plants were tetraploid and showed $2n = 4x = 48$ chromosomes (Fig. 1A), while the haploid plants contained gametic chromosome complement and presented $2n = 2x = 24$ chromosomes (Fig. 1B). The regenerated plants from the treated buds presented an increase in chromosome number and a high frequency of chimeras, with different ploidy levels in the same tissue (mixoploid plants). Mixoploid plants were obtained in all of the colchicine treatments; however, they presented a dominant frequency of chromosomic numbers of 24, 36, 42 and 48 (Tab. 2).

In addition, aneuploid cells and cells with between 39 and 129 chromosomes were found in the mixoploid plants (Fig. 1C-H). The occurrence of aneuploid cells was significantly higher after the colchicine treatment with 15 mM for 2 h (13.6%), mostly in genotype 16U793. High numbers of chromosomes per cell, 102 and 129, (Figs. 1G-H) were

TABLE 2. Comparison of traits in anther-donor, haploid and mixoploid genotypes of cape gooseberry.

Genotypes	Chromosome number (2n)	Chloroplast number range	Mean chloroplast number	Pollen viability	Pollen germination	Fertility
Anther-donor	$4x = 48$	7 - 10	9.3	98.8	36.2	Fertile
Haploid	$2x = 24$	4 - 8	5.2	0.0	0.0	Sterile
Mixoploid*24	24*	4 - 12	4.5 - 9.0	0.0	0.0	Sterile
Mixoploid*36	36*	4 - 12	7.7 - 9.5	0.0	0.0	Sterile
Mixoploid*42	42*	4 - 12	8.7 - 9.6	0.0	0.0	Sterile
Mixoploid*48	48*	7 - 32	8.8 - 23.8	52 - 94.6	21.1 - 39.2	Fertile

*Larger number of cells with the indicated chromosome number.

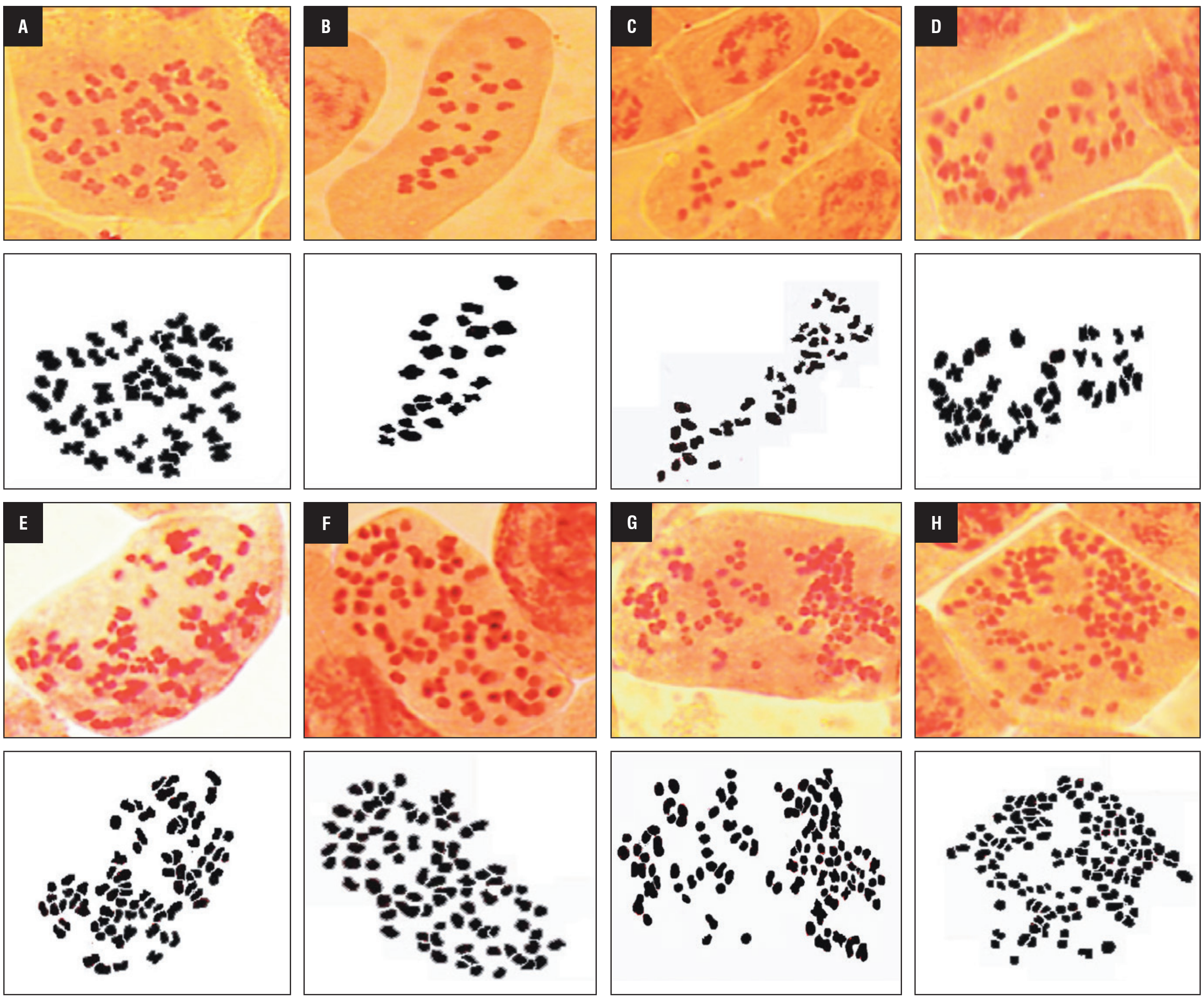

FIGURE 1. Somatic chromosomes in root-tip cells of cape gooseberry. A) Chromosomes of anther-donor genotypes with 48 chromosomes (chrs), B) haploid genotypes with 24 chrs and C) mixoploid genotypes with 39 chrs, D) 42 chrs, E) 78 chrs, F) 84 chrs, G) 102 chrs and H) 129 chrs.

obtained with the treatment with 15 mM colchicine for 2 h and 4 h, respectively; however, this treatment significantly decreased the frequency of regenerated plants (Tab. 1).

Chloroplast number

In the haploid plants, the number of chloroplasts (chls) ranged from 4 to 8, with an average of 5.2, while the tetraploid anther-donor plants had 7 to 10 chloroplasts. The mixoploid plants contained 4 to 32 chloroplasts per stomatal guard cell, with a mean that ranged from 4.5 to 23.8 (Tab. 2, Fig. 2). The chloroplast number was highest in one regenerated plant of genotype 16U793 (23.8 ± 3.3), followed by genotype 16U802 (18.4 ± 2.6), in the 15mM colchicine treatment with 2 h and 4 h, respectively.

The mixoploid plants, with a dominant chromosome number of 24, 36 and 42, had 4 to 12 chloroplasts in the guard cell, with an average of less than 9.6, while the mixoploid plants with 48 chromosomes contained between 7 to 32 chloroplasts, with an average that ranged from 8.8 to 23.8. The Pearson correlation analysis showed a significant correlation (r = 0.61, $P \leq 0.0001$) between the dominant chromosome number and the mean of the chloroplast number. This result suggests that the number of chloroplasts can be a useful marker for inferring the ploidy level.

Pollen fertility

The four haploid genotypes presented non-viable pollen grains with abnormal shapes and sizes that were lightly stained (Fig. 3A) and did not show *in vitro* germination as expected. Likewise, the mixoploid plants with 24, 36 and 42 chromosomes had non-viable pollen. In contrast, the mixoploid plants with 48 chromosomes were fertile, with pollen viability ranging from 52.04 to 94.6% (Fig. 3B) and had an *in vitro* germination percentage from 21.1 to 39.2% (Fig. 3C). The maximum germination percentage of ~39% was reached with 10 mM colchicine for 4 h, followed by 10 mM colchicine for 6 h. The chromosome number correlated positively with the pollen viability (r = 0.72) and pollen germination (r = 0.73).

The treatments of 5, 10 and 15 mM for 2 h produced the highest percentage of mixoploid fertile plants based on regeneration rates of 19, 13 and 16% for fertile plants, respectively. The third treatment (15 mM - 2 h) produced 70% partially fertile plants (defined as plants producing fertile and sterile flowers on different branches or consecutive nodes), while the 5 mM and 10 mM treatments for 2 h produced around 60% plants that were predominantly fertile (defined as plants having only fertile flowers). Consequently, the latter treatments were judged to be the optimal

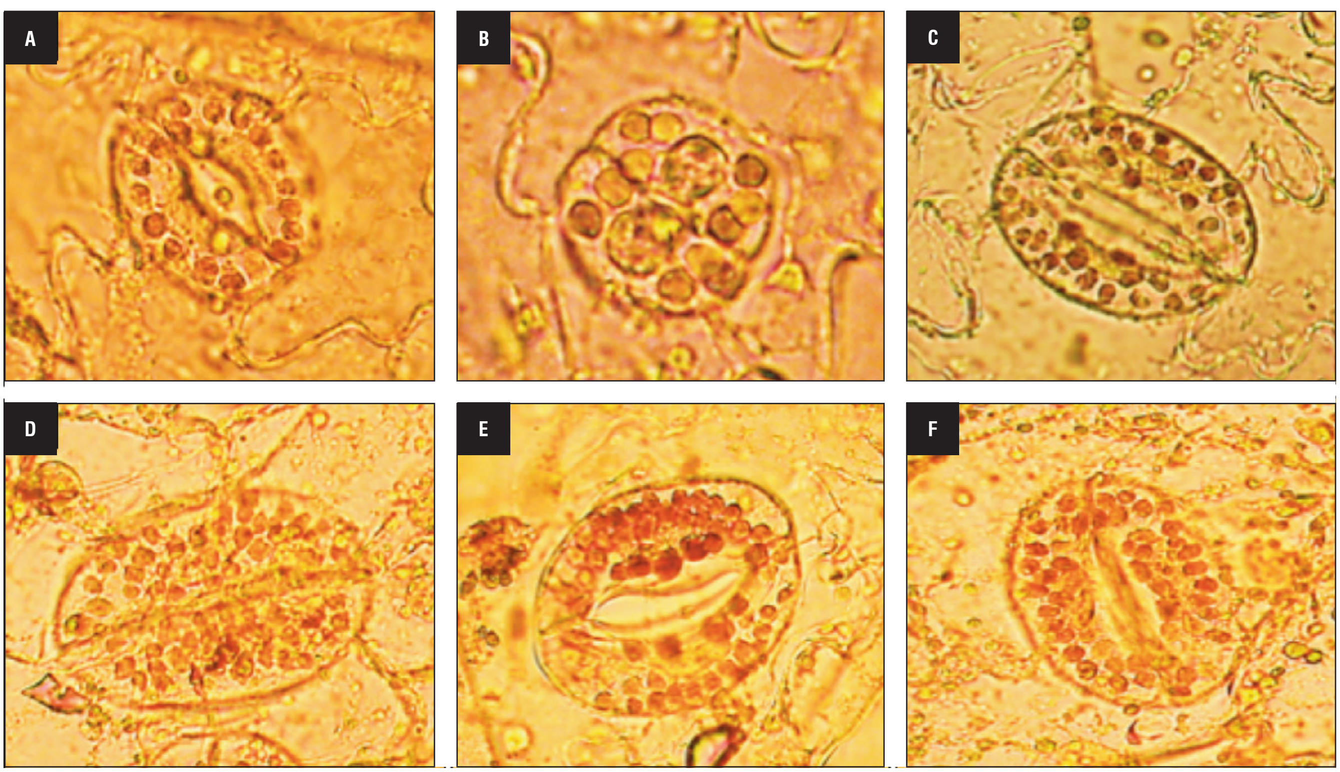

FIGURE 2. Chloroplast number per cape gooseberry stomatal guard cell. Chloroplast of anther donor A) Chloroplast of anther donor, B) haploid and C) mixoploid plants with 18 chls, D) 27 chls, E) 29 chls and F) 32 chls.

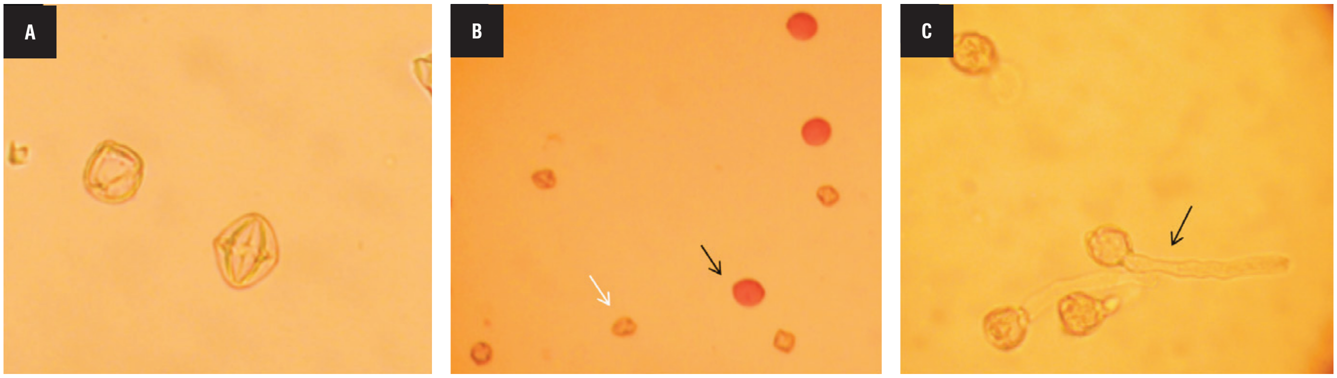

FIGURE 3. Pollen grain fertility of haploid and mixoploid cape gooseberry. A) Non-viable pollen grains of haploid genotypes, B) Viable and non-viable pollen grains of mixoploid plants, C) *In vitro* pollen germination of mixoploid plants (40x magnification).

treatments. The mixoploid fertile plants reached 19% for 16U490, 16% for 16U520, 28% for 16U793 and 37% for the 16U802 genotype, which is indicative of genotype-specific response effects.

Morphological observations

Visual differences in the morphological characteristics, such as leaf size, flower bud shape, anther number, protruding pistil and fruit shape, were identified among the haploid and mixoploid plants with 48 chromosomes (Mixoploid*48). The leaf size of the haploid plants was smaller than in the mixoploid plants (Fig. 4A). The haploid flower buds were thinner and more elongated in shape than the mixoploid flower buds, which were rounded (Fig. 4B-C). The mixoploid flowers were bigger and sometimes presented six anthers, while the haploid plants always had five (Fig. 4D-E). The haploid plants did not produce seeds, but they formed small fruits in unfertilized flowers with an obcordate shape (Fig. 4F); while the fertile mixoploid plants produced normal ovoid-shaped fruit containing abundant seeds (Fig. 4G).

Haploid (Left side) and mixoploid leaf (Right side) (A). Haploid (B) and (C) mixoploid bud flower. Haploid (D) and mixoploid flower (E). Haploid (F1) and haploid fruit longitudinal cut (F2). Mixoploid (G1) and mixoploid fruit longitudinal cut (G2).

The applicability of a doubled haploid (DH) protocol is of great interest for cape gooseberry breeding programs; however, anther cultures provide a low frequency of spontaneous double haploid, as compared to haploid frequency. The regeneration of fertile DH plants from colchicine treated buds is a critical factor in breeding programs for the commercial production of this crop. The regeneration rate fluctuated from 33 to 100%, mainly because of high mortality after the colchicine treatment and different responses of the genotypes treated with the same colchicine concentration. The anti-microtubule colchicine not only led to mitotic restitution in the meristem, but also had severe effects on the viability of the material. This explains the high mortality in some treatments, as reported by Nattaporn and Sompong (2012) in orchids.

In this study, the colchicine application resulted in higher rates of chromosome doubling in all of the genotypes, especially in the 15 mM for 2 h treatment; however, different ploidy levels in the cells of the regenerated plants were also observed. These results are in accordance with those previously reported in cape gooseberry seedling treatments by Díaz *et al.* (2008) and in other plants, such as tobacco (Bürün and Emiroğlu, 2008) and *Brassica* spp. (Smýkalová *et al.*, 2006). The chromosome doubling rate increased more significantly in the 16U793 genotype, suggesting genotypic dependence as described previously in wheat (Zamani *et al.*, 2003) and *Brassica* spp. (Weber *et al.*, 2005).

The chloroplast number in the stomatal guard cells of the haploid genotypes ranged from 4 to 8 chloroplasts, a lower value than in the anther-donor plants. Moreover, the mixoploid plants with 48 chromosomes presented more than 7 chloroplasts, the same as the tetraploid anther-donor plants. Additionally, a significant correlation was found between the number of chromosomes and chloroplast, suggesting that the chloroplast number can be an indirect and efficient indicator of ploidy in cape gooseberry plants. This correlation was also observed in tomato (Koornneef *et al.*, 1989), pepper (Qin and Rotino, 1995) and sweet potato plants (Delgado-Paredes *et al.*, 2017).

Furthermore, chromosome numbers and nuclear DNA contents in plant cell nuclei have been highly correlated in cape gooseberry plants and can be used as an index to estimate ploidy levels in cape gooseberry plants. Liberato

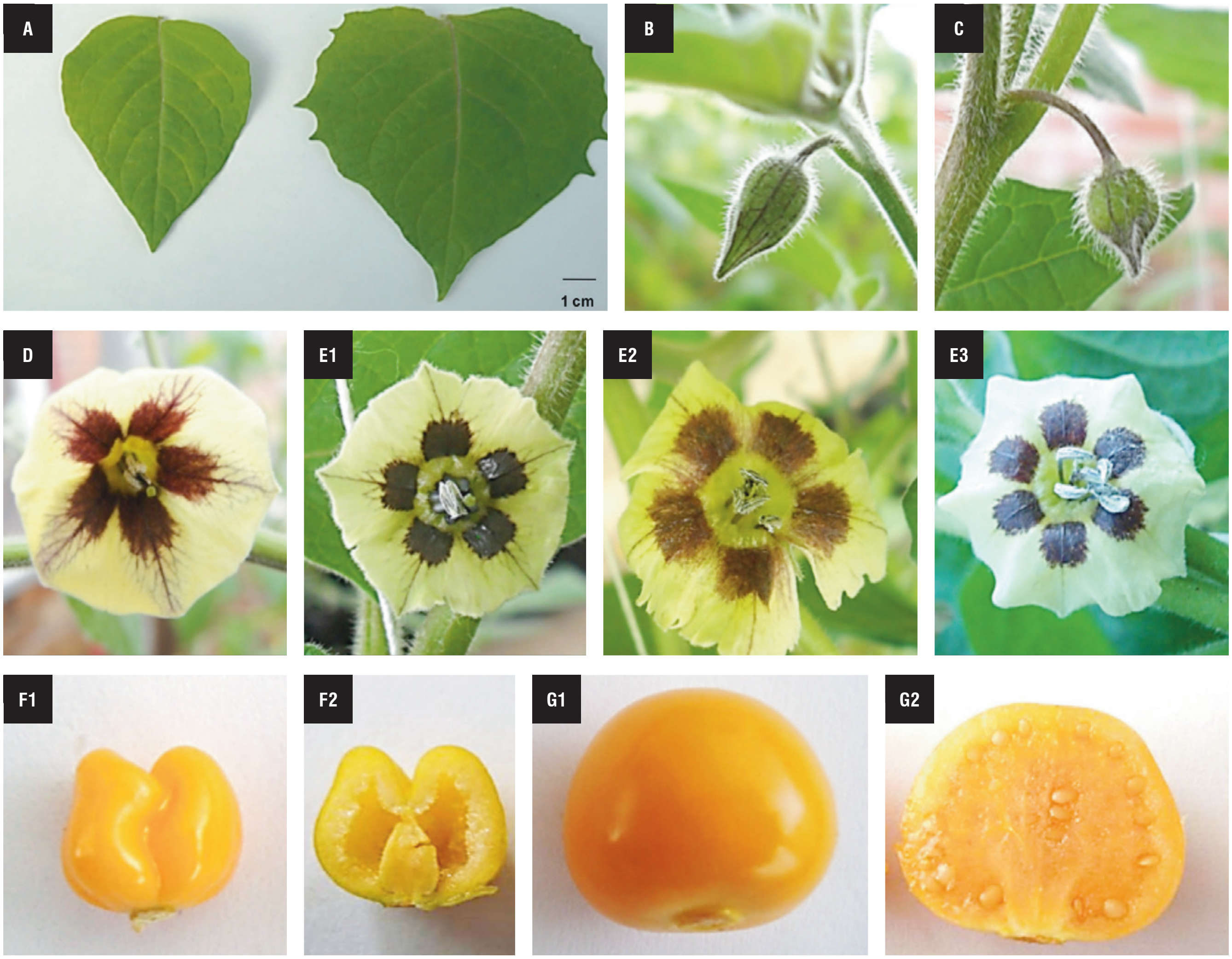

FIGURE 4. Morphological characteristics of haploid and mixoploid cape gooseberry. A) Haploid (Left side) and mixoploid leaf (Right side). B) Haploid and C) mixoploid bud flower. D) Haploid and E) mixoploid flower. F1) Haploid and F2) haploid fruit longitudinal cut. G1) Mixoploid and G2)mixoploid fruit longitudinal cut.

et al. (2015) reported that cape gooseberry genotypes with 2n = 48 chromosomes presented between 5.77 and 8.12 pg, while a genotype with 2n = 24 chromosomes had 2.33 pg. Likewise, cytometry flow and chloroplast numbers have been correlated in watermelon (Sari *et al.*, 1999) and poplar and black locust plants (Edwald *et al.*, 2009), indicating that the technique of counting chloroplasts can be used effectively to determine ploidy levels and could replace chromosome counting and flow cytometry, especially when the number of samples is high.

Our results indicate that this *in vitro* approach can produce chimeric plants with sectors that are still haploid in predominantly fertile DH plants. These fertile plants are very useful for plant breeding programs, quickly producing new cultivars after a strict progeny test. The highest rate of fertile plants was observed for the 15 mM colchicine concentration when applied for 2 h, producing mostly partially fertile plants. However, the 5-10 mM for 2 h treatments generated mostly fertile plants, indicating that this treatment is the best for fertility recovery since it provided new genetic material for conventional plant breeding programs. Similar results were reported by Smýkalová *et al.* (2006) in *Brassica* spp., who found inflorescences of chimeric plants containing both sterile and fertile flowers. Additionally, the 16U802 genotype presented more fertile plants (24%) than the other 3 genotypes, indicating that a genotype dependent factor was possibly involved.

In this study, the mixoploid plants had larger leaves and flowers and darker green leaves, but this could have been due to another attribute that was not accounted for, such as tolerance to an environmental stress that has been identified in this kind of plants (Kermani *et al.*, 2003; Shao *et al.*, 2003). Furthermore, the mixoploid flowers were bigger, probably because the cells with more ploidy grew more (Cheniclet *et al.*, 2005) and maintained a constant ratio between the nuclear and cytoplasmic volume (Novakova *et al.*, 2016). In addition, the haploid genotypes produced small fruits without seeds, as reported in haploid tomato plants (Ari *et al.*, 2016).

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Initial interference of *Cyperus rotundus* L. in pre-sprouted seedlings of sugarcane cultivars RB985476 and IACSP95-5000

Interferencia inicial de *Cyperus rotundus* L. en plántulas pre-brotadas de caña de azúcar cultivares RB985476 e IACSP95-5000

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ABSTRACT

Weeds compete with plants for water, light, nutrients and space. In sugarcane, planting pre-sprouted sugarcane seedlings (PSS) may mean a change in weed interference and management. The aim of this study was to generate information on the interference of *Cyperus rotundus* L. in PSS. Two experiments were carried out in a completely randomized design, one with cultivar RB985476, with seven densities of *C. rotundus* (0, 17, 35, 70, 140, 280 and 560 plants m^{-2}), and the second with cultivar IACSP95-5000, with four densities of *C. rotundus* (0, 70, 140 and 280 plants m^{-2}). For this weed, a 2x6 factorial design was used for the experiment with RB985476 and a 2x3 design was used for IACSP95-5000, the first factor being absence and presence of the crop, and the second factor the densities of the species. Biometric evaluations of height, diameter, number of tillers and leaves, leaf area and dry mass were carried out. The PSS had a reduction in height, number of leaves and leaf area in the main till at 60 d after planting (DAP) in RB985476. For the IACSP95-5000 cultivar, there was no reduction in the analyzed variables. The average dry mass per plant of *C. rotundus* decreased as the density of the species increased in the absence of PSS.

Key words: competition, Cyperaceae, densities, *Saccharum* spp.

RESUMEN

Las malezas compiten con las plantas cultivadas por el agua, la luz, los nutrientes y el espacio. En la caña de azúcar, la siembra con plántulas pre brotadas de caña de azúcar (PPB) puede significar un cambio en la interferencia y el manejo de las malezas. El objetivo de este trabajo fue evaluar la interferencia de *Cyperus rotundus* L. en plántulas pre-brotadas (PPB) de caña de azúcar. Se realizaron dos experimentos en invernadero, en diseño completamente al azar, uno con el cultivar RB985476, con siete densidades de *C. rotundus* (0, 17, 35, 70, 140, 280 y 560 plantas m^{-2}). El segundo con el cultivar IACSP95-5000, con cuatro densidades de *C. rotundus* (0, 70, 140 y 280 plantas m^{-2}). Para la maleza se adoptó un arreglo factorial de 2x6 para el experimento con RB985476 y 2x3 para IACSP95-5000, con el primer factor siendo la ausencia y presencia de caña de azúcar y el segundo factor las densidades de la especie. Se realizaron evaluaciones de altura, diámetro, número de perfiles y hojas, área foliar y masa seca de la parte aérea. Las PPB tuvieron reducción en altura, número de hojas y área foliar del perfil principal a los 60 días después del plantío en la RB985476. Para la variedad IACSP95-5000, no hubo reducción en las variables analizadas. La masa seca media por planta de *C. rotundus* se redujo con el aumento de la densidad de la especie en ausencia de PPB.

Palabras clave: competencia, Cyperaceae, densidades, *Saccharum* spp.

Introduction

A weed is any plant species that grows where it is not desired, interfering with cultivated plants, which can cause losses in production of around 20 to 30% (Lorenzi, 2014). Weeds interfere directly with crops through competition for water, light, nutrients and space. Additionally, they can interfere through allelopathic compounds capable of harming the development of crops or reducing the quality of products. They may also interfere indirectly since they are hosts of pests, diseases and nematodes, as well as harming culture and harvest practices (Pitelli, 1987).

According to Oliveira and Freitas (2008), 95 weed species have been identified in sugarcane (*Saccharum officinarum* L.), which are distributed in 74 genera and 30 families. The most representative in number of species are Poaceae, Asteraceae, Euphorbiaceae, Malvaceae, Papilionoideae and Amaranthaceae. Among these species, *Cyperus rotundus* L. (Purple Nutsedge) has obtained the Highest Value of Importance (HVI = 230.08) in relation to the infesting community.

The species *C. rotundus* is among the world's major weeds because of its high competition capacity and control

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difficulty (Kissmann and Groth, 2000) and its reproduction almost exclusively through tubers (Lorenzi, 2014). This species can have distinct levels of dormancy, which makes the management of this weed with herbicides difficult (Baloch *et al.*, 2015). In sugarcane, Durigan *et al.* (2006) observed a reduction of 43.3% in yield in the presence of this species.

Kuva *et al.* (2007) evaluated the weed community in sugarcane mechanically harvested at 120 d after cutting, and the importance of the *C. rotundus* species, which was found in 20 areas, was notable; in 13 of these areas, it was the most important species, presenting a Relative Importance index (RI%) above 90%. In another study, Kuva *et al.* (2008) observed the presence of *Cyperus* sp. in 11 of 29 studied plots in a seed bank, ranging from 9.09% to 80% in relation to the RI%.

In a conventional green cane system, difficulties controlling species such as *C. rotundus*, *Commelina benghalensis* L. (Bengal Dayflower), *Sida rhombifolia* L. (Arrowleaf Sida), *Digitaria nuda* (Crabgrass) and *Urochloa plantaginea* (Link) R.D. Webster (Alexandergrass) were identified. Of these species, *C. rotundus* presented an HVI of 54%, a relative frequency (RF) of 33.3%, a relative density (RD) of 76.3% and a relative dominance (RD) of 51.9%. In fallow, this species also obtained high values of HVI (51.1%), RD (66.7%) and RD (45.5%) (Soares *et al.*, 2016).

In relation to the influence of weed density on sugarcane, Durigan *et al.* (2005) observed that low (58-246 epigeal manifestations m^{-2}), average (318-773 epigeal manifestations m^{-2}) and high (675-1,198 epigeal manifestations m^{-2}) weed densities reduced sugarcane yield by 13.5, 29.3 and 45.2%, respectively, as compared to the control (free of interference).

The interference of *C. rotundus* in sugarcane is already recognized, but further studies are needed for pre-sprouted sugarcane seedlings (PSS). In this planting method, seedlings arrive at the field with the aerial part and roots already developed, which can guarantee a greater competitive advantage for sugarcane, to the detriment of weeds, since it can allow faster closing between lines, reducing the period of interference (Oliveira, 2016; Dias *et al.*, 2017; Silva *et al.*, 2018). According to Landell *et al.* (2012), PSS help maintain a cane field with greater vigor and quality, reduce mixtures of cultivars and maintain high phytosanity.

The aim of this study was to evaluate the initial interference of *C. rotundus* in pre-sprouted sugarcane seedlings (PSS) cultivars RB985476 and IACSP95-5000.

Materials and methods

Two experiments were carried out, the first from June to August, 2017, with cultivar RB985476, and the second from February to April, 2018, with cultivar IACSP95-5000. Both experiments were carried out in a greenhouse in Piracicaba, São Paulo, Brazil (22°42'31.2" S and 47°37'44.5" W, 547 m a.s.l.). Irrigation was maintained simulating the regional precipitation of the month of October, with an average of three mm per day (average of the last ten years) according to data collected by the meteorological station (22°42'30" S, and 47°38'30" W, 546 m a.s.l.).

The experimental design was completely randomized, with four replicates. A seedling was planted in a 10 L plastic pot, with a planting area of 572 cm². For cultivar RB985476, the treatments were composed of seven densities of *C. rotundus:* 0, 1, 2, 4, 8, 16 and 32 plants/pot, equivalent to 0, 17, 35, 70, 140, 280 and 560 plants m^{-2}, respectively. For the IACSP95-5000 cultivar, the treatments corresponded to four densities of *C. rotundus*: 0, 4, 8, and 16 plants/pot, equivalent to 0, 70, 140 and 280 plants m^{-2}, respectively.

For the *C. rotundus* weed, in both experiments, the treatments were composed of the densities in the absence and presence of the crop, with the treatments arranged in 2x7 (experiment with cultivar RB985476) and 2x4 factorial schemes (experiment with the cultivar IACSP95-5000), that is, two conditions of coexistence in seven and four densities, respectively. The plants were planted at a depth of three cm at the corresponding densities of each treatment. The tubers were radially allocated in relation to the sugarcane that was planted in the center of the pot.

The chemical characteristics according to the soil analysis were: pH ($CaCl_2$) = 4.9, P = 7 mg dm^{-3}, K = 1.69 $mmol_c\,dm^{-3}$, Ca = 48.49 $mmol_c\,dm^{-3}$, Mg = 14.65 $mmol_c\,dm^{-3}$, H + Al = 22 $mmol_c\,dm^{-3}$, organic matter = 42 g kg^{-1}, base saturation = 74.6 %, saturation by Al = 0.4%, and cation exchange capacity (CEC) = 86.8 $mmol_c\,dm^{-3}$.

Evaluations of cultivar RB985476 were performed at 30 and 60 d after planting (DAP) and up to 90 DAP for IACSP95-5000. Plant height was measured from the soil surface to the last fully developed leaf with a visible auricle, the diameter of the stem was measured with a digital caliper at 5 cm from the soil, and the number of leaves and tillers was also estimated.

In the last evaluation of each experiment, the leaf area was measured with a LiCor, LI 3000A (Nebraska, USA). The dry mass of the sugarcane plants and the Purple Nutsedge were estimated by cutting samples close to the soil surface, placing them in properly identified paper bags and oven drying them with forced air circulation at a temperature of 65°C to obtain the dry mass. The data were submitted to analysis of variance by the F test, and the means of the treatments were compared with Tukey's test ($P \leq 0.05$) (Pimentel-Gomes and Garcia, 2002).

Results and discussion

Experiment I: RB985476

According to the analysis of variance, no statistical differences were observed for the parameters: diameter (Tab. 1), number of tillers and dry mass of the sugarcane plants (Tab. 2).

Differences were observed only at 60 DAP for the variable height between the treatments, with 16 (17.75 cm) and 32 (26.62 cm) plants/pot (Tab. 1); however, neither differed from the control in the absence of the weed. The lowest values of leaf area were verified for the treatments with 1 and 16 plants of *C. rotundus*/pot when compared with the highest density of competition (Tab. 2). The number of sugarcane leaves was lower for the treatment with 16 plants/pot (3.50) as compared to the zero density (7.50) at 60 DAP (Tab. 2).

In relation to the total dry mass of the aerial part of the plants of *C. rotundus*, the analysis of variance showed an

TABLE 1. Height (cm) and diameter (mm) at 30 and 60 DAP in the PSS of sugarcane cultivar RB985476 in competition with *C. rotundus*.

Densities (plants/pot)	Height		Diameter	
	30	60	30	60
0	18.50	24.62 ab	5.50	8.00
1	16.00	19.50 ab	4.30	7.60
2	16.50	22.62 ab	5.00	8.30
4	17.50	22.62 ab	4.70	7.20
8	16.50	19.50 ab	4.70	5.80
16	17.00	17.75 b	4.60	7.30
32	15.75	26.62 a	5.20	7.60
Mean	16.82 ns	21.89 *	4.80 ns	7.40 ns
LSD	3.12	8.74	2.90	4.90
C.V. (%)	8.08	17.38	26.24	29.19

ns: non-significant; *followed by equal letters in a column means they do not differ according to the Tukey test at 5% probability; C.V.: coefficient of variation; LSD: less significant difference; DAP: days after planting.

TABLE 2. Number of leaves and tillers at 30 and 60 DAP, leaf area (cm^2) and dry mass (g) at 60 DAP of PSS of sugarcane cultivar RB985476 in competition with *C. rotundus*.

Densities (plants/pot)	Number of leaves		Number of tillers		Leaf area	Dry mass
	30	60	30	60		
0	5.75	7.50 a	0.00	0.00	490.075 ab	7.71
1	5.00	6.25 ab	0.25	0.50	270.912 b	5.14
2	5.25	6.00 ab	0.00	0.25	459.752 ab	6.93
4	5.50	7.00 a	0.00	0.50	470.567 ab	7.66
8	4.50	5.75 ab	0.25	0.75	287.920 ab	6.05
16	4.00	3.50 b	0.00	0.00	147.662 b	4.48
32	5.50	8.00 a	0.25	0.50	658.612 a	8.87
Mean	5.07 ns	6.28 *	0.10 ns	0.35 ns	397.928 *	6.69 ns
LSD	2.58	2.81	0.75	1.46	372.341	5.47
C.V. (%)	22.15	19.48	12.98	21.41	21.55	14.88

ns: non-significant; *means followed by the same letters in a column do not differ according to the Tukey test at 5% probability; C.V.: coefficient of variation; LSD: less significant difference; DAP: days after planting.

TABLE 3. *C. rotundus* dry mass of the aerial part (g) and dry mass per plant (g) at 60 DAP in the presence and absence of PSS of sugarcane cultivar RB985476.

Densities (plants/pot)	Dry mass of the aerial part			Dry mass per plant		
	Presence	Absence	Mean	Presence	Absence	Mean
1	1.67 cA	1.64 bA	1.65	1.67 aA	1.64 aA	1.65
2	3.01 cA	2.44 abA	2.72	1.50 aA	1.22 abA	1.36
4	5.38 bcA	4.20 abA	4.79	1.34 abA	1.05 abA	1.19
8	7.08 bA	1.03 bB	4.05	0.88 abA	0.12 bA	0.50
16	3.89 bcA	3.28 abA	3.58	0.24 bA	0.20 bA	0.22
32	14.01 aA	6.17 aB	10.09	0.43 abA	0.19 bA	0.31
Mean	5.84	3.13	4.48	1.01	0.74	0.87
$F_{factor\ 1}$		24.55 *			2.58 ns	
$F_{factor\ 2}$		19.42 *			8.36 *	
$F_{factor1\ x\ factor2}$		6.23 *			0.39 ns	
C.V. (%)		17.91			13.68	

ns: non-significant; *means followed by the same lowercase letters in a column and capital letters in a row do not differ according to the Tukey test at 5% probability; DAP: days after planting.

interaction between the factors (Tab. 3). In the interaction within the factor of absence and presence of sugarcane, a difference was observed between the densities of 8 plants/pot, with 7.08 g in the presence of sugarcane and 1.03 g in the absence, and 32 plants/pot, with 14.01 g in the presence and 6.17 g in the absence. For the densities factor, in the presence of sugarcane, the highest total dry mass value of *C. rotundus* was reached with 32 plants/pot, and the lowest total dry mass with 1 plant/pot, as was expected.

For dry mass per plant in the presence of sugarcane, the highest values were seen with the densities of 1 plant/pot (1.67 g) and 2 plants/pot (1.50 g), differing from 16 plants/pot (0.24 g). For the variable average dry mass per plant, within the factor absence of sugarcane, lower values for the densities of 8 (0.12 g), 16 (0.20 g) and 32 (0.19 g) were verified, as compared to 1 plant/pot (1.64 g). These results may suggest the beginning of intraspecific competition. There was no difference for the factor presence and absence of sugarcane within the density factor (Tab. 3).

TABLE 4. Height (cm) and diameter (mm) at 30, 60 and 90 DAP of PSS of sugarcane cultivar IACSP95-5000 in competition with *C. rotundus*.

Densities (plants/pot)	Height			Diameter		
	30	60	90	30	60	90
0	14.75	33.75	63.00	4.37	11.75	18.85
1	11.75	28.50	46.00	5.50	9.85	14.42
4	13.50	30.00	48.50	4.75	10.62	14.75
8	14.75	31.62	53.00	6.50	11.42	15.32
Mean	13.68 ns	30.96 ns	52.62 ns	5.28 ns	10.91 ns	15.83 ns
LSD	4.23	10.54	19.97	3.08	4.49	4.62
C.V. (%)	14.73	16.21	18.07	27.84	19.60	13.90

ns: non-significant; C.V.: coefficient of variation; LSD: less significant difference; DAP: days after planting.

Experiment II: IACSP95-5000

The analysis of variance was not significant for the variables: height, diameter (Tab. 4), number of leaves, number of tillers, leaf area, aerial part dry mass of the sugarcane (Tab. 5) or dry mass of the aerial part of *C. rotundus* (Tab. 6).

TABLE 5. Number of leaves and tillers at 30, 60 and 90 DAP, leaf area (cm^2) and dry mass of the aerial part (g) at 90 DAP of PSS of sugarcane cultivar IACSP95-5000 in competition with *C. rotundus*. Piracicaba, São Paulo, Brazil (2018).

Densities (plants/pot)	Number of leaves			Number of tillers			Leaf area	Dry mass
	30	60	90	30	60	90		
0	6.00	8.25	7.00	0.00	0.75	1.25	2117.55	65.78
1	6.25	6.25	6.00	1.25	1.25	1.00	1850.30	34.13
4	6.25	6.75	6.00	0.50	1.00	1.00	1761.09	32.41
8	5.75	6.50	6.25	0.25	0.25	0.25	1415.38	32.99
Mean	6.06 ns	6.93 ns	6.31 ns	0.50 ns	0.81 ns	0.87 ns	1786.08 ns	41.33 ns
LSD	2.12	2.20	1.05	2.14	2.28	2.53	985.93	41.59
C.V. (%)	16.67	15.15	7.58	28.08	27.34	29.47	26.29	19.83

ns: non-significant; C.V.: coefficient of variation; LSD: less significant difference; DAP: days after planting.

TABLE 6. Dry mass of aerial part (g) and dry mass per plant (g) of *C. rotundus* at 90 DAP in the presence and absence of PSS of sugarcane cultivar IACSP95-5000.

Densities (plants/pot)	Dry mass of aerial part			Dry mass per plant		
	Presence	Absence	Mean	Presence	Absence	Mean
4	1.28	4.12	2.70	0.32 B	1.03 aA	0.67
8	2.01	3.02	2.52	0.25 A	0.37 abA	0.31
16	3.65	4.84	4.25	0.22 A	0.30 bA	0.26
Mean	2.32	3.99	3.15	0.26	0.57	0.41
$F_{factor\ 1}$		4.091 ns			4.058 ns	
$F_{factor\ 2}$		1.752 ns			2.960 *	
$F_{factor\ 1\ x\ factor\ 2}$		0.491 ns			1.828 ns	
C.V. (%)		22.83			10.85	

ns: not significant; *means followed by the same lowercase letters in a column and capital letters in a row do not differ according to the Tukey test at 5% probability; C.V.: coefficient of variation; DAP: days after planting.

For the variable mean dry mass per plant of *C. rotundus*, the analysis showed a significant effect in the absence of sugarcane, with the highest value in the density of 4 plants/pot as compared to 16. However, differences were not found with the treatment with 8 plants/pot. A higher mean dry mass was also observed in the density of 4 plants/pot in the absence of sugarcane than in the treatment with presence of the crop (Tab. 6).

Hijano (2016) observed no differences in height, diameter, number of leaves of main stem, leaf area of main stem, dry mass of primary leaves and primary stem at 120 DAP of PSS of cultivars RB855156 and CTC14 when subjected to competition with *Rottboellia cochichinensis* (Lour.) Clayton (Itchgrass). Similar results were obtained in the present study, in which no reduction in height, diameter, or number of leaves and tillers at 30, 60 and 90 DAP of cultivar IACSP95-5000 was observed when subjected to competition with *C. rotundus*.

The height of PSS cultivar RB966928 was not reduced when they were in competition with *Panicum maximum* Jacq. (Guineagrass), at 60 and 90 d after emergence (DAE) of the weed at densities of 3.8, 7.7, 11.5 and 15.4 plants m^{-2}; however, at 45 DAE, a lower height was observed at the higher density (21 cm) as compared to the control (26.3 cm) (Paula, 2015). A height reduction also was observed in this study for cultivar RB985476 at 60 DAP at the density 16 plants/pot of *C. rotundus*, reducing the average height of the plants by 8.87 cm. In contrast to what was observed in this study, in which the IACSP95-5000 cultivar did not show a reduction in the height variable, Zera *et al.* (2016) verified a lower height of this cultivar at 30 DAP when subjected to competition with *U. plantaginea*, *S. rhombifolia*, *Digitaria horizontalis* Willd., *Amaranthus retroflexus* L. (Pigweed Redroot) and *P. maximum*, as compared to a control treatment (absence of weeds). In the present study, the lowest plant height at 60 DAP was found for cultivar RB985476 at the density 16 plants/pot (17.75 cm).

The mean number of tillers, height and dry mass of PSS cultivar RB966928 was not reduced at 30, 60 and 90 DAE when in competition with *Ipomoea hederifolia* L. (Morning Glory) at the densities 1, 2, 3 and 4 plants/pot, corresponding to 3.8, 7.7, 11.5 and 15.4 plants m^{-2}. The same was observed when the competition was established with *D. horizontalis* in the variables height and number of tillers; however, for dry mass, differences were observed between the control (absence of weed) (53.3 g) and treatment with a plant of *D. horizontalis*/pot (34.4 g) (Paula, 2015). This was not observed in the present study, in which there was no reduction in dry mass for the cultivars IACSP95-5000 and RB985476 when in competition with *C. rotundus* at 90 and 60 DAP.

For the cultivars of this study, differences in the number of tillers according to the density of *C. rotundus* were not observed. Hijano (2016) observed a reduction in the number of tillers when increasing the density of *R. cochichinensis* for the cultivars RB855156 (85 and 120 DAP) and CTC14 (55, 85 and 120 DAP). A reduction in the number of tillers was verified for cultivar RB966928 in coexistence with four plants of *U. decumbens*/pot at 60 and 90 DAE of the weed. In addition, a reduction in the dry mass of the sugarcane with 1 plant/pot was also observed; however, there was no reduction in height (Paula, 2015).

At 120 DAP, Zera *et al.* (2016) observed a reduction in the number of tillers in cultivar IACSP95-5000 with Plene Evolve, Plene PB and MPB-IACSP in competition with *P. maximum*. This was not observed in the present study, in which there was no reduction in the number of tillers for any of the studied cultivars in competition with *C. rotundus*. Paula (2015) observed a reduction in the number of tillers when PSS cultivar RB966928 was in competition with a density of 15.4 *P. maximum* plants m^{-2} at 60 DAE of the weed and a density of 7.7 plants m^{-2} at 90 DAE.

The competition of cultivar RB855536 with *I. hederifolia* during 229 DAP of sugarcane reduced the number of tillers by 34%, from 10.68 to 7.06 m^2. In addition, the authors observed a 33-d period prior to interference (PPI), yielding 71.65 t ha^{-1} when the crop remained free of weeds and 38.59 t ha^{-1} with competition for 229 DAP (Silva *et al.*, 2009).

The PPI, the critical period of preventing interference (CPPI), and the total period of preventing interference (TPPI) for sugarcane cultivar SP803280 (second cut) were 18, 18-137 and 137 DAP when subjected to competition with 12 weed species, with the highest importance for *P. maximum*, *Acanthospermum hispidum* DC. and *Alternanthera tenella* Colla (Joyweed). The yield in the absence of weeds was 126.8 t ha^{-1}, which was reduced to 84.4 t ha^{-1} when the crop competed for 270 DAP (Meirelles *et al.*, 2009).

For the dry mass of the aerial part of the PSS, no differences were found between the densities of *C. rotundus*. Zera *et al.* (2016) did not observe differences for cultivar IACSP95-5000 (Plene Evolve, Plene PB and MPB-IACSP technologies) in competition with *U. plantaginea*, *S. rhombifolia*, *D. horizontalis*, *A. retroflexus* and *P. maximum*.

Beyond sugarcane, other crops suffer from large interference caused by *C. rotundus*. Maize crops of the DKB 390 hybrid, in competition with *C. rotundus*, have presented reductions of approximately 10% for the variables dry mass, mass of 100 grains, grain mass and grain yield with increased weed densities in the presence of 10 plants m^{-2} (Silva *et al*., 2015).

In this study, the stem diameter variable for both cultivars of sugarcane was not reduced. Kuva *et al.* (2003) estimated a reduction of stem yield of the cultivar RB835089 in competition with *U. decumbens* and *P. maximum* (most representative species in the area) as the weed dry mass increased.

The results demonstrate the importance of studies to better understand weed management in the PSS system since the behavior of sugarcane was different from that observed for conventional planting with parts of the stem.

In addition, sugarcane plants arrive in the field with developed aerial parts and roots, which makes it difficult to manage weeds from the Poaceae family with herbicides. Another important factor that should be considered is that, in seedling planting areas, there is no straw, which can be favorable for positive photoblasts and smaller seeds (Silva *et al*., 2018).

Conclusions

It was concluded that *C. rotundus* interfered with the height, number of leaves and leaf area of the main tiller of cultivar RB985476 at 60 d after planting, mainly in the treatment with 16 plants/pot (280 plants m^{-2}).

There was no initial interference in the parameters evaluated for the IACSP95-5000 cultivar up to 90 d after planting.

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Pre-harvest factors that influence the quality of passion fruit: A review

Factores precosecha que influyen en la calidad de las frutas pasifloráceas. Revisión

Gerhard Fischer[1]*, Luz M. Melgarejo[2], and Joseph Cutler[3]

ABSTRACT

Colombia is the country with the greatest genetic diversity in passion fruit species, some of which are cultivated on an area of approximately 13,673 ha. Each variety must be planted at a suitable altitude under optimal conditions to obtain the best quality. Regarding plant nutrition, potassium has the greatest influence due to the effect of its application on the yield increase, ascorbic acid content and lifecycle to harvest. Adequate water increases the percentage of the marketable quality and amount of fruit juice, and the use of rootstocks does not significantly change the fruit quality. Ensuring a pollination of the flowers in cultivation is decisive for the fruit formation and its juice content. The species differ greatly in their quality, as purple passion fruit (*Passiflora edulis* f. *edulis*) is a fruit that develops the highest content of ascorbic acid, while sweet calabash (*P. maliformis*) forms the maximum amount of phenols and total antioxidant activity. The maturation and ripening of passion fruit is determined by the skin coloration, during which the Brix grades and the maturity index increase and the titratable acidity diminishes. Fruits harvested early in physiological maturity and with unripe peel color can be treated with ethylene in post-harvest, matching fruits that ripened in the plant. More research is needed in the improvement of the quality of the *Passifloraceae*. Giant granadilla (*P. cuadrangularis*) and sweet calabash have been studied less than banana passion fruit (*P. tripartita* var. *mollissima*), purple passion fruit, yellow passion fruit and sweet granadilla (*P. ligularis*). The last three species are the most exported fruits in the country.

Key words: *Passiflora edulis* f. *flavicarpa*, *P. edulis* f. *edulis*, *P. ligularis*, *P. tripartita* var. *mollissima*, *P. maliformis*, *P. cuadrangularis*.

RESUMEN

Colombia es el país de mayor diversidad genética en especies de pasifloras, algunas de las cuales se cultivan abarcando aproximadamente 13,673 ha. Cada variedad debe ser sembrada en sitio y piso térmico apto para desarrollar su calidad óptima, igualmente debe ser cultivada con las mejores prácticas para aprovechar su potencial. En la nutrición, es el potasio el que muestra mayor influencia ya que aumenta el rendimiento y el contenido de ácido ascórbico y acorta el tiempo para cosechar. Suministro suficiente de agua aumenta el porcentaje de calidad de fruto mercadeable, así como el jugo del fruto, mientras que el uso de patrones no influye significativamente en la calidad de los frutos. Garantizar una polinización de las flores en cultivo es decisivo para la formación del fruto y el jugo. Las especies difieren mucho en su calidad, siendo la gulupa (*Passiflora edulis* f. *edulis*) la que desarrolla un contenido más alto en ácido ascórbico, mientras la cholupa (*P. maliformis*) se destaca por el máximo en fenoles y actividad antioxidante total. La maduración de los frutos de las pasifloráceas está bien determinada por la coloración de la cáscara, durante la cual los grados Brix y el índice de madurez aumentan, y la acidez titulable disminuye. Los frutos cosechados tempranamente en madurez fisiológica y con cáscara de poca coloración pueden ser tratados con etileno en poscosecha igualando a los frutos madurados en planta. Más investigación es necesaria para el mejoramiento de la calidad de las pasifloráceas, siendo la badea (*P. cuadrangulares*) y la cholupa las menos estudiadas, en comparación con la curuba (*P. tripartita* var. *mollissima*), la gulupa, el maracuyá y la granadilla (*P. ligularis*), de las cuales las últimas tres están dentro de las frutas más exportadas del País.

Palabras clave: *Passiflora edulis* f. *flavicarpa*, *P. edulis* f. *edulis*, *P. ligularis*, *P. tripartita* var. *mollissima*, *P. maliformis*, *P. cuadrangularis*.

Introduction

Colombia is a country with the highest diversity rates of *Passifloraceae* species (Ocampo *et al*., 2007), nine species are commercially cultivated to supply local and international markets (Ligarreto, 2012). Six of these species have been statistically assessed, and according to Agronet (2018) in 2016, a total of 195,942.2 t were produced on 13,673.45 ha. Provinces of Antioquia and Huila produce 119,388.87 t of yellow passion fruit (7,192.30 ha); Huila, Cundinamarca and Antioquia 42,950.51 t of sweet granadilla (3,793.55 ha); Antioquia, Tolima and Cundinamarca 15,945.67 t of purple

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passion fruit (1,001.55 ha); Boyaca, Norte de Santander and Tolima 13,741.8 t of banana passion fruit (1,319.1 ha); Huila, Santander and Choco 2,352.55 t of giant granadilla (156.05 ha); and finally, Huila produces 1,562.8 t of sweet calabash (211 ha).

The most exported passiflora from Colombia is purple passion fruit, which generated 25.8 million USD for the country in 2017 and is exported mainly to The Netherlands, Germany and the United Kingdom (La Nota Económica, 2018).

The physiological behavior, quality and perishability of the fruits depends on the characteristics of each fruit, environmental conditions and crop management, as well as postharvest care (Herrera, 2012). The word "quality" comes from the Latin "qualitas" which means attribute, property or basic nature of a product; however, nowadays it can be defined as the "level of excellence or superiority" (Kader, 1986), in terms of appearance, taste, nutritional value and safety, among others (López, 2004). Quality is a combination of the attributes, properties or characteristics that each product value confers in terms of its use (*e.g.* raw, cooked or processed) (Kader, 2002). The set of all the qualitative factors indicates the rate of deterioration, and the lack of control of these factors leads to post-harvest losses on a larger scale (Barmann *et al*., 2015). Kader (2002) estimates, in general, that a third of all fruits is lost before reaching the consumer.

Due to the fact that during the post-harvest of fruits, less importance has been given to the investigation of the influence of some pre-harvest factors, such as climate and crop management on physiology, quality and longevity (Barman *et al*., 2015), the objective of this review is to deal with these issues in the case of commercially cultivated passion fruits.

Development of the fruit quality during cultivation

Of the factors that influence the quality of the product in cultivation (pre-harvest), the most determining factors are the genetics of the variety, the environmental conditions, the further interaction between genotype and environment, and the crop management strategies (Herrera, 2012).

Environmental factors and quality

The environmental conditions of the site (climate and soil) are crucial for the process of crop quality formation, and are the basis for the development of commercial fruit growing in a country or region (Fischer and Miranda, 2012; Fischer and Orduz-Rodríguez, 2012). The environment influences processes such as photosynthesis, transpiration, respiration, translocation of photoassimilates and the metabolism of the plant, which are decisive for the internal and external quality of the fruit and its longevity in postharvest (Ladaniya, 2008).

The effect of the physical, chemical and biotic environment on the physiological mechanisms of the plant is known as plant ecophysiology (Larcher, 2003; Lambers *et al*., 2008). Different cultivated passion fruit species are found in Brazil, Colombia and, in general, in tropical America and Central America (Miranda, 2012). The ecophysiological demands and climatic conditions of the different types of passion fruit are diverse, as shown in Table 1.

In Colombia, the altitude defines factors such as temperature. With an increase of 100 m the temperature decreases on average 0.6°C, while the UV radiation increases, and the atmospheric pressure and relative humidity decrease (Fischer and Orduz-Rodríguez, 2012). As shown in Table 1, different *Passifloraceae* can be grown commercially between 0 and 3,000 m a.s.l.

TABLE 1. Climatic factors of growth and production of passion fruits in Colombia.

Climatic factor	Yellow passion fruit (*P. edulis* f. *flavicarpa*)	Purple passion fruit (*P. edulis* f. *edulis*)	Sweet granadilla (*P. ligularis*)	Banana passion fruit (*P. tripartita* var. *mollissima*	Sweet calabash (*P. maliformis*)	Giant granadilla (*P. cuadrangularis*)
Temperature (°C)	24-28	15-18	16-20	13-16	26-32	20-24
Altitude (m a.s.l.)	0-1,300	1,700-2,200	2,000-2,500	1,800-3,000	600-1,000	0-1,000
Precipitation (mm)	800-1,500	1,300-1,800	2,000-2,500	1,000-1,500	1,200-1,450	1,000-1,800
Rel. humidity (%)	70	80-94	70-75	70-80	60-70	80
Light/solar radiation	<5 h/day direct sunshine	9-13 h light/day		7-8 h/day direct sunshine	8-11 h light/day	
Authors	Cleves *et al*., 2012	Ocampo and Wyckhuis, 2012	Miranda, 2012	Campos and Quintero, 2012; Ángulo and Fischer, 1999	Ocampo *et al*., 2015	Carrión and Pontón, 2002

Due to the increase in UV light with elevation, juicy fruits, as in the case of *Passifloraceae*, are more prone to sunburn (Fischer *et al*., 2016) and, in addition, a high and prolonged solar radiation increases both the temperature of the irradiated cells that can cause the splitting of the epidermis and a subsequent entry of pathogens to these unprotected tissues (Fischer, 2000). For this reason, the plants need leaves to cover fruits. However, it is important that part of the UV light reaches the fruits during ripening to stimulate the production of antioxidants that improve their nutritional value (Fischer *et al*., 2016).

At higher altitudes, due to the colder environment, production starts later and fruit development lasts longer than at lower sites, as Mayorga (2017) found in banana passion fruit. Outside their altitudinal range, crops can have a higher incidence of pests and diseases, and pollination processes are less effective (Fischer *et al*., 2009; Ocampo *et al*., 2015). In banana passion fruit at higher altitudes, the epidermis of the fruit is thicker and more resistant to anthracnose (Campos and Quintero, 2012). In sweet granadilla, at altitudes lower than 1,700 m a.s.l., fruit size is reduced producing 50% of second grade quality fruits (Castro, 2001).

After assessing *Passifloraceae* fruits in a high altitudinal zone (2,498 m, average 14.9°C), Mayorga (2017) found that in Pasca (Cundinamarca, Colombia) the banana passion fruits had a higher weight, size, citric and ascorbic acid contents; in contrast, the total soluble solids content (TSS) was lower in the high zone compared to fruits produced in lower areas (2,006 m a.s.l. and 17.9°C). It is possible that the organic acids in the higher area were not metabolized at the same rate due to the colder environment (Tellez *et al*., 2007) and the higher TSS at the lower elevation (2,006 m), leading to increase the transformation rate of starch in sugar by hydrolysis process. This was stated assuming that the TSS is basically a sugar dilution (80.95%) that does not depend directly on leaf photosynthesis rather than on the climatic conditions (Flórez *et al*., 2012), considering that in this research the photosynthetic rate increased proportionally to the elevation rate.

For banana passion fruit located in foggy areas, the espalier and the conduction of the canopy were exposed with an angle of 45° and an orientation towards the east, increasing the capture of light by the leaves and the production of fruits. These features produce 90 fruits per m^2 of canopy in low radiation sites and up to 100-150 fruits per m^2 in those of high radiation with productions as high as 40 t ha^{-1} $year^{-1}$, with 2.5 cycles annually for the cv. Momix (O.C. Quintero, personal communication, 2004).

Genetic material and quality

The highest genetic diversity of the passion fruit is found in Colombia, where Ocampo *et al*. (2007) observed 167 species, from which 165 were native, followed by Brazil with 127 and Ecuador with 90. According to Ligarreto (2012), in the National System of Germplasm Banks administered by Corpoica (Colombia), there are 170 accessions of passion fruits, with 52 molecularly characterized, including details about their biochemical and agro-industrial properties. The quality enhancement is the most important objective of genetic breeding, improving the flavor and acidity of the fruit for the industrialized beverages manufacture, increasing the content of TSS and the size of the fruit (Ligarreto, 2012). In the case of yellow passion fruit, the same author mentions that oval shaped fruits are preferred because they contain up to 10% more juice than the round ones.

Ocampo *et al*. (2015) collected and characterized sweet granadilla fruits in 35 municipalities of 11 provinces of the Andean region in Colombia to establish the degree of genetic variability as an important input for genetic improvement. Eleven physicochemical variables were characterized and seven elite accessions were identified through the quality parameters: fruit weight (>34 g), °Brix (>14.4) and pulp + seed (>52%).

Regarding the influence of the genetic material on the quality and production of the *Passifloraceae*, there are great differences between the species and the variety. In yellow passion fruit in Brazil, the kinetics of chlorophyll fluorescence allowed an accurate evaluation of the functional states of the photosynthetic apparatus in the different cultivars, which indicated that 'FB 300', 'BRS Sol do Cerrado' and 'BRS Ouro Vermelho' are photosynthetically more efficient and have better fruit quality (Gama *et al*., 2013).

Ramaiya *et al*. (2013) compared antioxidant contents (ascorbic acid, total phenols and total antioxidant activity) in four passion fruit species and found the highest content of ascorbic acid in purple passion fruit (0.32 g kg^{-1} FW) and the lowest in sweet calabash (0.15 g kg^{-1} FW). Regarding the total phenols, the highest content was found in sweet calabash (277.00 mg GAE/L FW) and the lowest in giant granadilla (272.96 mg GAE/L FW), while the highest total antioxidant activity was found in sweet calabash (1,685.00 μmol Trolox/L FW) and the lowest in purple passion fruit (547.70 μmol Trolox/L FW); yellow passion fruit obtained intermediate values of these antioxidants (Tab. 2).

TABLE 2. Some antioxidants, predominant organic acids and sugars contained in the pulp of passion fruits.

Fruit species	Antioxidants	Organic acids	Sugars
Yellow passion fruit	[1]Ascorbid acid 0.24 g kg^{-1} FW [1]Total phenols 361.73 mg GAE L^{-1} FW [1]TAA 524.00 μmol Trolox L^{-1} FW	[3]Citric acid 55.0 meq 100 g^{-1} [3]Malic acid 10.5 meq 100 g^{-1} [3]Lactic acid 0.5 meq 100 g^{-1} [3]Malonic acid 0.1 meq 100 g^{-1}	[1]Sucrose 28.6 g kg^{-1} FW [1]Glucose 14.1 g kg^{-1} FW [1]Fructose 14.6 g kg^{-1} FW
Purple passion fruit	[1]Ascorbic acid 0.32 g kg^{-1} FW [1]Total phenols 362.00 mg GAE L^{-1} FW [1]TAA 547.70 μmol μmol Trolox L^{-1} FW	[3]Citric acid 13.1 meq 100 g^{-1} [3]Malic acid 3.8 meq 100 g^{-1}, [3]Lactic acid 7.4 meq 100 g^{-1} [3]Malonic acid 4.9 meq 100 g^{-1}	[1]Sucrose 45.5 g kg^{-1} FW [1]Glucose 30.1 g kg^{-1} FW [1]Fructose 31.3 g kg^{-1} FW
Sweet granadilla	[2]Ascorbic acid 0.14 g kg^{-1} FW	[2]Citric acid 7.12 mg g^{-1} FW [2]Malic acid 2.86 mg g^{-1} FW [2]Oxalic acid 0.019 mg g^{-1} FW	[2]Sucrose 20.67 g kg^{-1} FW [2]Glucose 35.6 g kg^{-1} FW [2]Fructose 35.6 g kg^{-1} FW
Giant granadilla	[1]Ascorbic acid 0.22 g kg^{-1} FW [1]Total phenols 272.96 mg GAE L^{-1} FW [1]TAA 1,352.30 μmol Trolox L^{-1} FW	[4]Citric acid 15.2 mg g^{-1} FW	[1]Sucrose 28.1 g kg^{-1} FW [1]Glucose 43.7 g kg^{-1} FW [1]Fructose 39.0 g kg^{-1} FW
Sweet calabash	[1]Ascorbic acid 0.15 g g kg^{-1} FW [1]Total phenols 277.0 mg GAE L^{-1} FW [1]TAA 1,685.00 μmol μmol Trolox L^{-1} FW	[5]Acidity 3.0 %	[1]Sucrose 17.0 g kg^{-1} FW [1]Glucose 23.4 g kg^{-1} FW [1]Fructose 22.1 g kg^{-1} FW

[1]Ramaiya *et al*. (2013); [2]Espinosa *et al*. (2018); [3]Chan *et al*. (1972); [4]Sánchez *et al*. (2014); [5]Ocampo *et al*. (2015).

TAA - Total antioxidant activity

In a compilation by Casierra-Posada and Jarma-Orozco (2016) on the nutritive values of different passion fruits vary depending on the species. For example, according to the reports of Chan *et al*. (1972), the highest content of organic acids in yellow passion fruit are: citric acid (55.0 meq/100 g fruit juice), malic acid (10.5 meq), lactic acid (0.5 meq) and malonic acid (0.1 meq). In purple passion fruit, citric acid also presented the highest content, but only with 13.1 meq followed by lactic (7.4 meq), malonic (4.9 meq) and malic (3.8 meq) acids (Tab. 2).

Moreover, the different commercial passion fruit species differ in the content of sugars in the fruit juice. Ramaiya *et al*. (2013) analyzed the highest sucrose content in purple passion fruit (45.5 g kg^{-1} FW) and the lowest in sweet calabash (17.0 g), while the glucose content was the highest in giant granadilla (43.7 g) and the lowest in yellow passion fruit (14.1 g). Likewise, the lowest level of monosaccharide fructose was found in yellow passion fruit (14.6 g), and the highest one was reported in giant granadilla (39.0 g) (Tab. 2).

Some cultural practices and quality

Nutrition

Good crop management increases the potential of the variety. Plant nutrition is one of the key factors for the development of the typical fruit quality (Fischer and Álvarez, 2008), and due to its effect on the growth it highly influences the production and quality of the fruit (Aular *et al*., 2014). Important practices can be lead to promote the development and quality of the fruit in purple passion fruit crop. For example, around 70 to 80 g of fertilizer 10-20-20 (NPK) should be applied seven times between 200 and 295 d after sowing (Jiménez *et al*., 2012).

The absorption of nutrients increases at the beginning of the reproductive phase, for example in yellow passion fruit, the fertilizer demand increases at 250 to 280 d after transplanting, when the plants begin to grow exponentially. In this phenological stage, the absorption of nutrients such as nitrogen (N), potassium (K) and calcium (Ca) and the micronutrients rises, especially manganese (Mn) and iron (Fe) (Borges and Lima, 2007). In addition, the same authors reported that temperatures below 18°C decrease passion fruit growth which, consequently, reduces nutrient absorption and fruit production.

In order to understand the influence of N on yellow passion fruit, Borges *et al*. (2006) applied two sources, urea and calcium nitrate, in five doses (0 to 800 kg ha^{-1}) and found the maximum fruit yield (34.3 t ha^{-1}) with the application of 457 kg ha^{-1} of N in the form of urea, without influencing the characteristics of the fruit and the quality of the juice.

Freitas *et al*. (2006) studied the deficiencies of macronutrients and boron (B) in yellow passion fruit and found that the lack of Mg, N, phosphorus (P) and sulfur (S) in the nutrient solution caused the lowest number of fruits per plant (0, 2, 3 and 4, respectively), compared to the control (10 fruits). The authors also found that TSS were lower in fruits deficient in N, P and K, and the content of ascorbic acid decreased with the lack of N, K and S in the nutrient solution.

With different levels of K in the nutrient solution, Araújo *et al*. (2006) found that 6 mmol L^{-1} of K produced fruits of greater weight and yield/plant, while the thickness and relative water content of the pericarp as well as the concentration of vitamin C increased with the dose of K (from 0 to 8 mmol L^{-1}). Also, the increase in K concentration to 8 mmol L^{-1} shortened the time to fruit maturity by around 25 d (Araújo *et al*., 2005).

Passion fruits are notable for their high content of mineral nutrients. Martin and Nakasone (1970) reported higher contents of Ca and P (measured in 100 g of fresh weight) in purple passion fruit (14 g and 41 mg, respectively), while sweet granadilla excelled in Fe (0.8 mg), but yellow passion fruit and banana passion fruit presented low Ca contents (4 g).

Water and irrigation

In yellow passion fruit cultivated in the semi-arid region of Paraíba (Brazil) and irrigated with 133% of the crop evapotranspiration (Eto), gas exchange rates were higher in the 'BRS Sol do Cerrado' genotype, in addition to fruit production with a higher market classification, compared to 'BRS Gigante Amarelo' which was more prone to develop in conditions of low water availability due to its lower stomatal conductance (Melo *et al*., 2014). In the same semi-arid region, Suassuna *et al*. (2011) found in the yellow passion fruit hybrid IAC 273/277 that the irrigation level at 120% of ETo promoted a greater proportion of fruits with fresh weights higher than 150 g, an increase in juice yield and a higher percentage of skin compared to lower and higher levels of irrigation.

A symptom of severe deterioration is the cracking of the passion fruits due to possible effects such as higher water influx to organs, drastic change between night and daytime temperature and/or lack of B, Ca, K or Mg (Rivera *et al*., 2002; Fischer *et al*., 2009; Parra-Coronado and Miranda, 2016), which is observed especially in sweet granadilla and banana passion fruit.

Pruning

Regarding pruning, growing yellow passion fruits with a different number of reproductive branches (40, 30, 24, 20 and 14 branches/plant) evidenced that a smaller number of tertiary branches decreased production (number of fruits/plant) and productivity (kg ha^{-1} of fruits) and juice yield, but increased the average fruit weight without modifying the internal characteristics of organs (Hafle *et al*., 2009). According to Weber *et al*. (2017), fruit weight accumulation includes competition for compounds produced by photosynthesis, and a lower fruit number increases the available assimilates for fruit filling.

Grafting

In different combinations of yellow passion fruits in Viçosa-MG (Brazil) grafted on two wild species (*P. gibertti* and *P. mucronata*), Salazar *et al*. (2016) observed significant correlations in fruits for ascorbic acid, brightness values, chroma and angle Hue. However, the content of β-carotene in the fruit did not show significant differences, which indicates that the two rootstocks did not influence this compound; nevertheless, there was a decrease in the ascorbic acid content, compared to non-grafted plants. The results indicate the potential use of wild rootstocks due to their positive effects on grafted plants, while maintaining the commercial quality of the fruits.

Pollination

Akamine and Girolami (1959) reported that the fruit set, the number of seeds, the weight of the fruit and the fruit yield depend on the amount of pollen deposited on the stigma. Seed development is directly correlated to the juice content (Knight and Winters, 1962). The *Passifloraceae* species are commonly pollinated by bumblebees, especially by *Xylocopa* sp. (Miranda, 2012) and also by *Centris* sp., *Epicharis* sp., *Eulaema* sp., *Bombus* sp. and *Ptiloglossa* sp. (Schotsmans and Fischer, 2011). In the allogamous yellow passion fruit, artificial pollination is very common, achieving a fruit set of 80% (Cleves *et al*., 2012). Rodriguez-Amaya (2003) reports that manual pollination will produce larger and more succulent fruits.

Phytosanitary status

Bacteria affecting quality are: *Xanthomonas axonopodis* pv. *passiflorae* causing dark brownish green spot lesions (Castilho and Granada, 1995) and *Pseudomonas syringae* pv. *passiflorae* causing small green spots that develop into golden brown sticky necrotic lesions (Fischer and Rezende, 2008). The following fungi are causal agents affecting quality: *Fusarium oxyporum* f. sp. *passiflorae* causes a pale beige color in fruits (Fischer and Rezende, 2008), *Phytophtera* sp. causes gray spots (Varón de Agudelo, 1993), *Colletotrichum gloeosporioides* induces lesions on fruit skin that become corky and brown, *Alternaria passiflorae* causes browning, and *Septoria* sp. causes blotch. Of the viruses found in passion fruit, Cowpea-aphid borne mosaic virus (CaBMV) causes fruit deformation, East Asian Passiflora virus (EAPV) induces fading in fruit color, Passion fruit vein clearing virus (PVCV) reduces fruit size (Kitajima et al. 1986), Passion fruit green spot virus (PGSV) causes green spots on yellow passion fruit (Kitajima et al., 2003), and Passion fruit mottle virus (PaMV) induces fruit skin mottling (Fischer and Rezende, 2008).

Fruit ripening and quality

Ripening can be defined as the integration of external and internal changes, including the taste and texture that a fruit provides when it completes its growth (Agustí, 2004). The cultivated passion fruits are climacteric (Hernández and Fischer, 2009); it means that berries growth and development take place while attached to the plant or after the harvesting process, depending on the state of fruit development.

In two sweet granadilla plantations in the municipalities of Santa María and La Argentina in the province of Huila (Colombia), Espinosa *et al.* (2015) grouped the fruits into four stages of maturity, recording the change in color, the increase in TSS (Brix degrees) and maturity index (TSS/TTA), and the decrease in total titratable acidity (TTA) (Tab. 3). Since the color change of the epidermis during the maturation of the passion fruit is the most notable characteristic (Schotsmans and Fischer, 2011), the change of the passion fruit from green to yellow is due to the degradation of chlorophyll and the production of new pigments, mainly carotenoids, which generate the yellow to red coloration of the tissues (Valero and Serrano, 2010).

Similar results were obtained by Pinzón *et al.* (2007) for purple passion fruit, only differing in the purple skin color and the maturity index (MI). The purple passion fruit retains a higher content of TTA at the consumption ripeness, recording a MI of 4.14 (with a TTA of 3.92), regardless of the higher concentration of TSS (16.21), compared to 13.1 of the sweet granadilla (Tab. 2). After analyzing the stages immature (I), color change (breaker) (II) and mature (III) for purple passion fruits, Jiménez *et al.* (2011) found an increase of the TSS from 13.5 to 17.4, a decrease in the TTA from 4.68 to 2.51 and an increase in the concentration of anthocyanin, between stage II and III, from 0.45 to 1.73 g cy-3glu equiv./kg fruit.

The maturity index (TSS/TTA), which is the balance between sugars and acids giving the characteristic flavor to the fruit (Flórez *et al.*, 2012), is a parameter stated to identify the maturity of passion fruit. For example, in the case of purple passion fruit, the consumption quality improves mainly due to the decrease in acidity (Shiomi *et al.*, 1996a). In several sensory tests, it has been found that acidity masks the sensation of sweetness and as the acidity decreases due to the reduction of TTA during post-harvest, the sweetness is evident (Schotsmans and Fischer, 2011).

In relation to ascorbic acid, Patel *et al.* (2014) recorded the maturity of the purple and yellow passion fruits to contain the highest content (48.75 mg 100 mL^{-1}) of this antioxidant, produced in purple passion fruit (var. Megha Purple) at the end of maturation (90 d), while in yellow passion fruit (var. Kerala Yellow) the highest ascorbic acid concentration

TABLE 3. Development of sweet granadilla fruit quality grouped into four stages of maturity.

Maturity stage		Description	Color scale	TSS (°Brix)	TTA (%)	Maturity index (TSS/TTA)
1		Fruit with 100% growth, 100% green, 49-105 daa	L = 38.49 C = 15.08 h = 102.35	10.2	1.4	7.6
2		Fruit with 100% growth, 60% green - 40% yellow, 109-117 daa	L = 53.65 C = 40.41 h = 79.21	12.8	1.1	12.2
3		Fruit with 100% growth, 40% green - 60% yellow, 121-129 daa	L = 61.96 C = 57.86 h = 67.09	12.5	0.7	17.4
4		Fruit with 100% growth, 100% yellow, 133-141 daa	L = 61.77 C = 57.04 h = 64.53	13.1	0.7	18.5

daa - days after anthesis. Photos: D.D. Espinosa. Table and photos with permission from Espinosa *et al.* (2015) and Universidad Nacional de Colombia, Bogota.

(28.4 mg 100 mL^{-1}) is produced until 83 d and then decreases. The reduction of ascorbic acid in the final stage of fruit ripening was attributed by these authors to the oxidation of L-ascorbic acid to dehydro-ascorbic acid during the metabolic process.

Pongener *et al*. (2014) reported for purple passion fruit that the quality attributes of these fruits were better when they were harvested after the breaker state in which the fruits had developed 50% of their purple coloration (Tab. 4). The ethylene evolution rate increased almost 8.15 times the value with maximum levels of 505.35 µL kg^{-1} h^{-1} of C_2H_4 in fruits harvested in the color change (breaker) state. The changes in the color values L*, a* and b* indicated an optimal color development only in harvested fruits after 50% of its coloring. These authors concluded that the purple passion fruit should be harvested only after 50% of coloring on its fruit surface, for an optimal storage, appropriate ripening and development of flavor and quality.

In contrast, Shiomi *et al*. (1996b), in an experimental orchard of purple passion fruit in Kenya, observed that harvested fruits at 70 d after flowering (daf; "ripe" green stage), showed characteristics almost similar to the more developed fruits (80 and 90 daf). If the color can develop normally after the application of exogenous ethylene in post-harvest, it should be possible to harvest the purple passion fruit in a stage of "ripe" green or in the change from green to purple (breaker). This is much earlier than the usual maturity of the harvest and would give the product a good export quality, with a longer shelf life when marketed and transported abroad. Shiomi *et al.* (1996a) reported that the application of ethylene in post-harvest of the purple passion fruit should be at least 1 d after harvesting, given that the ethylene biosynthesis regulated mainly by the activity of the ACC synthase could change its sensitivity after the harvest when the fruit is ripening.

Flórez *et al*. (2012) harvested purple passion fruits in different ripening stages, from physiological (100% green) to mature (100% purple), registering in all of these a peak of ethylene production on day 18 of post-harvest. The fruits harvested in the states 70% (30% green, 70% purple) and 100% purple presented the highest ethylene production rate. In addition, these authors found that the climacteric peak (of respiration) preceded the ethylene production, which confirms the hypothesis that this hormone is not responsible for the respiratory increase in the purple passion fruits, which, in the 100% state, obtained the maximum CO_2 production.

Vianna-Silva *et al*. (2010) found a different behavior in yellow passion fruit, as fruits ripen differently depending on whether they are attached to the plant or have already been removed from it. Fruits delayed their coloration and decreased the thickness of the epidermis, compared with fruits of the same age already stored. The same authors stated that the optimum harvest time is 63 d after anthesis (daa), while when harvested at 54 daa fruits had 21% less juice yield.

Conclusions

In order to produce high quality passion fruit it is important to choose the suitable species and variety adapted to specific altitudinal and climatic conditions.

There are major differences between the species regarding quality, for example the purple passion fruit develops a very high content of ascorbic acid, while the sweet calabash contains the maximum amount of phenols and total antioxidant activity.

Regarding the crop management, an accurate plant nutrition strategy is decisive in the quality of yellow passion fruit, especially K supplement, which is the element with the highest effect on yield, content of ascorbic acid and the reduction of the fruit development time. Irrigation increases the percentage of the marketable qualities and the juice of the fruit, while the use of rootstocks does not

TABLE 4. Recommended harvest indices for different passion fruit crops.

Species	Maturity index[1]	Authors
Purple passion fruit	Skin with 50% purple color - 50% green	Pinzón *et al*., 2007; Pongener *et al*., 2014
Sweet granadilla	Skin with 40% green color - 60% yellow	Espinosa *et al*., 2015
Yellow passion fruit	Skin with 60% green color - 40% yellow	Hernández and Fischer, 2009
Banana passion fruit	Skin color $\leq$ 30% yellow	Campos and Quintero, 2012
Giant granadilla	Light yellowish or redness of the apical fruit part - opaque green fruit	Reina *et al*., 1996
Sweet calabash	Green or yellowish green fruit, before falling to the ground, with dry bracts	Ocampo *et al*., 2015

[1]The indices may vary according to the destination of the fruits.

significantly change the fruit quality. Guaranteeing a pollination of the flowers is decisive for the production of the fruit and its juice.

Fruit ripening is well determined by the change of color, an increment in sugar content (Brix degrees) and maturity index along to a decrease in the titratable acidity rate. Fruits harvested early in the physiological maturity and with a lower color expression in the peel can be treated with ethylene at post-harvest.

More research is needed on the improvement of the quality of the *Passifloraceae* in Colombia, as the purple passion fruit, yellow passion fruit and sweet granadilla are among the five most exported fruits in the country, and the giant granadilla and sweet calabash are the least studied so far.

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Physicochemical characteristics of strawberry (*Fragaria x ananassa* Duch.) fruits from four production zones in Cundinamarca, Colombia

Características fisicoquímicas de frutos de fresa (*Fragaria x ananassa* Duch.) provenientes de cuatro zonas productoras de Cundinamarca, Colombia

Manuel Vergara[1]*, Jessica Vargas[1], and John Acuña[1]

ABSTRACT

Albion is a strawberry (*Fragaria* x *ananassa* Duch.) plant variety that belongs to the Rosaceae family, which is notable in Colombia because of its vitamin content and organoleptic characteristics. Fruits of this variety from four municipalities (Mosquera, Guasca, Sibate, Facatativa) in the province of Cundinamarca were evaluated. Significant differences in physical and chemical characteristics were detected between fruits of the four areas. For the physical parameters, fruits from Mosquera were notable because of their large size and biomass content. The specific chemical qualities of the fruits from Guasca make them important for the industry and processing. The fruits from Sibate had an acceptable content of citric acid and, at the same time, a high content of sugars, qualities that result in high acceptance for products in a fresh market. Finally, the fruits from Facatativa were characterized by intense colors and attractiveness to final consumer. Because of the varying conditions of each municipality, the fruits presented different strengths, so the implementation of new harvesting technologies is recommended so that all producers in the province can achieve the same high-quality characteristics in order to obtain worldwide recognition.

Key words: quality, citric acid, sugar content, industry, international marketing.

RESUMEN

Albión es una variedad de fresa (*Fragaria* x *ananassa* Duch.), planta de la familia Rosaceae de gran relevancia en Colombia por su contenido vitamínico y sus características organolépticas. En el presente trabajo se evaluaron los frutos provenientes de cuatro municipios productores del departamento de Cundinamarca (Mosquera, Guasca, Sibaté, Facatativá). Se detectaron diferencias significativas en las características físicas y químicas entre los frutos de las cuatro áreas de cultivo. En los parámetros físicos se destacaron los frutos provenientes del municipio de Mosquera por su gran tamaño y contenido de biomasa. Las cualidades químicas específicas de los frutos del municipio de Guasca los hacen importantes para la industria y el procesamiento. Los frutos de Sibaté se destacan por un contenido más aceptable de ácido cítrico y al mismo tiempo un alto contenido de azúcares, las cuales son cualidades que los perfilan como productos de alta aceptación para un mercado en fresco. Finalmente, los frutos provenientes de Facatativá se caracterizan por tener colores más intensos y más atractivos para el consumidor final. Debido a que los frutos cultivados en cada municipio tienen diferentes fortalezas, se recomienda trabajar en la implementación de nuevas tecnologías de cosecha, para que todos los productores del departamento logren obtener las mismas cualidades de alta calidad, y así obtener reconocimiento a nivel mundial.

Palabras clave: calidad, ácido cítrico, contenido de azúcar, industria, mercado internacional.

Introduction

In 2012, 4.6 million tons (t) of strawberries were produced worldwide, with the United States being the main producer with 1,366,850 t, followed by Mexico with 360,426 t, Turkey with 353,173 t, Spain with 289,900 t and Egypt with 242,297 t (Wu *et al.,* 2015). The principal importing countries for 2013 were the United Kingdom (470,770 t), Canada (123,463 t), the United States (110,457 t), France (90,587 t) and the Netherlands (28,937 t) (Camara de Comercio de Bogota CCB, 2015). In Colombia in 2013, 42,453 t of strawberries were produced, with Cundinamarca being the province with the highest production with 22,562 t, followed by Antioquia with 12,545 t, Norte de Santander with 3,360 t, Cauca with 2,808 t and Boyaca with 542.2 t (CCB, 2015), to date these amounts have not been significantly improved.

This fruit stands out because of its content of vitamin C, tannins, flavonoids, anthocyanins, catechin, quercetin and kaempferol, organic acids (citric, malic, oxalic, salicylic

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and ellagic) and minerals (K, P, Ca, Na and Fe), as well as pigments and essential oils (Özcan and Haciseferogullar, 2007; Pinto *et al.*, 2008). It has a unique taste that makes it desirable, a pleasant aroma and an immense source of sugars (Sharma and Sharma, 2004). However, the strawberry is a highly perishable product because of its high respiration rate (Manning, 1993). Its postharvest life is very short and very susceptible to microorganisms and physical damage during handling, storage and commercialization (Sistrunk and Morris, 1985). The market and distribution over long distances is restricted because of its perishability and senescence, as well as the appearance of postharvest diseases that can cause significant economic losses. This short shelf-life is related to the reduction of characteristics such as aromatic properties, flavor and brightness, which cause early deterioration (Cuncha, 2011).

Many studies have been carried out evaluating the effect of different factors on sensory and nutritional treatments of fruits and their products (Shin *et al.*, 2012). Information on the physical, mechanical and chemical properties of the strawberry are of great importance in the design of harvesting equipment and postharvest technology, such as transportation, storage, cleaning, sorting, packing and processing (Martínez-Soto *et al.*, 2008).

In order to know the properties of harvested fruits from different strawberry producing municipalities in the province of Cundinamarca, Colombia, the main goal of this study was to provide useful information in order to establish quality standards for national and international markets.

Materials and methods

Location

This study was conducted in four representative producer areas of the province of Cundinamarca, shown in Table 1. These zones were strategically chosen because of their agroclimatic potential, which promotes better plant growth, fruit development and a larger strawberry crop tradition.

TABLE 1. Location of each production area.

Municipality	Elevation (m a.s.l.)	Geographic coordinates
Mosquera	2,630	4º40'34.88'' N and 74º12'48.90'' W
Guasca	2,655	4º51'40.94'' N and 73º52'41.28'' W
Sibate	2,576	4º20'50.75'' N and 74º15'50.76'' W
Facatativa	2,582	4º47'26.15'' N and 74º20'57.56'' W

Environmental data

Recorded data for the mean day temperature and relative air humidity of the producer municipalities were obtained from the database of the "Instituto de Hidrologia, Meteorologia y Estudios Ambientales (IDEAM)" from the Ministry of Environment and Sustainable Development of Colombia for the plant development period of 12 months (January 2015 to December 2015). Additionally, the relative air humidity and average day temperature in each crop area where the fruits were collected for this study were registered with data loggers (RHT10, Extech Instruments® Waltham, MA) (Fig. 1 and 2).

To carry out this study, a representative sample of fruits of the Albion variety was collected and classified in three quality states (1, 2 and 3) and three maturity stages (4, 5 and 6) according to the Colombian Technical Standard (NTC 4103). From each sample, fruits of each state were taken for variable evaluation. The tests were performed one day after harvest in the postharvest laboratory of the Agricultural Engineering Department of the Universidad Nacional de Colombia, Bogota campus, following the guide for postharvest analysis of perishables (Herrera, 2010), using 5 samples for data collection for each of the variables explained below:

Dry mass

In order to obtain the dry weight of the fruits, samples were taken, differentiating them by quality grades and maturity stages. Then the fruits were dried in an oven at 90ºC, recording weight measurements every 24 h, taking as the final dry weight the stabilized weight used to obtain a percentage with the following formula:

$$Dry\ matter\ (\%) = \frac{Initial\ weight}{Final\ weight} \times 100 \quad (1)$$

Consistency

To determine the consistency, a CT3 texturometer (Brookfield Engineering, USA) was used with an accessory punch with a 0.2 cm diameter, using the final load as the reference.

pH and total titratable acidity (TTA)

The TTA was determined with a TitroLine® WA20 potentiometer (SI Analytics, Weilheim, Germany). To obtain a 1:1 solution, 10 g of pure fruit extract and 10 g of distilled water were prepared. A computer showed the pH and the volume of reagent required for titration in millimeters (mm), and the percentage of acidity (citric acid for strawberry fruits) was calculated with the following formula:

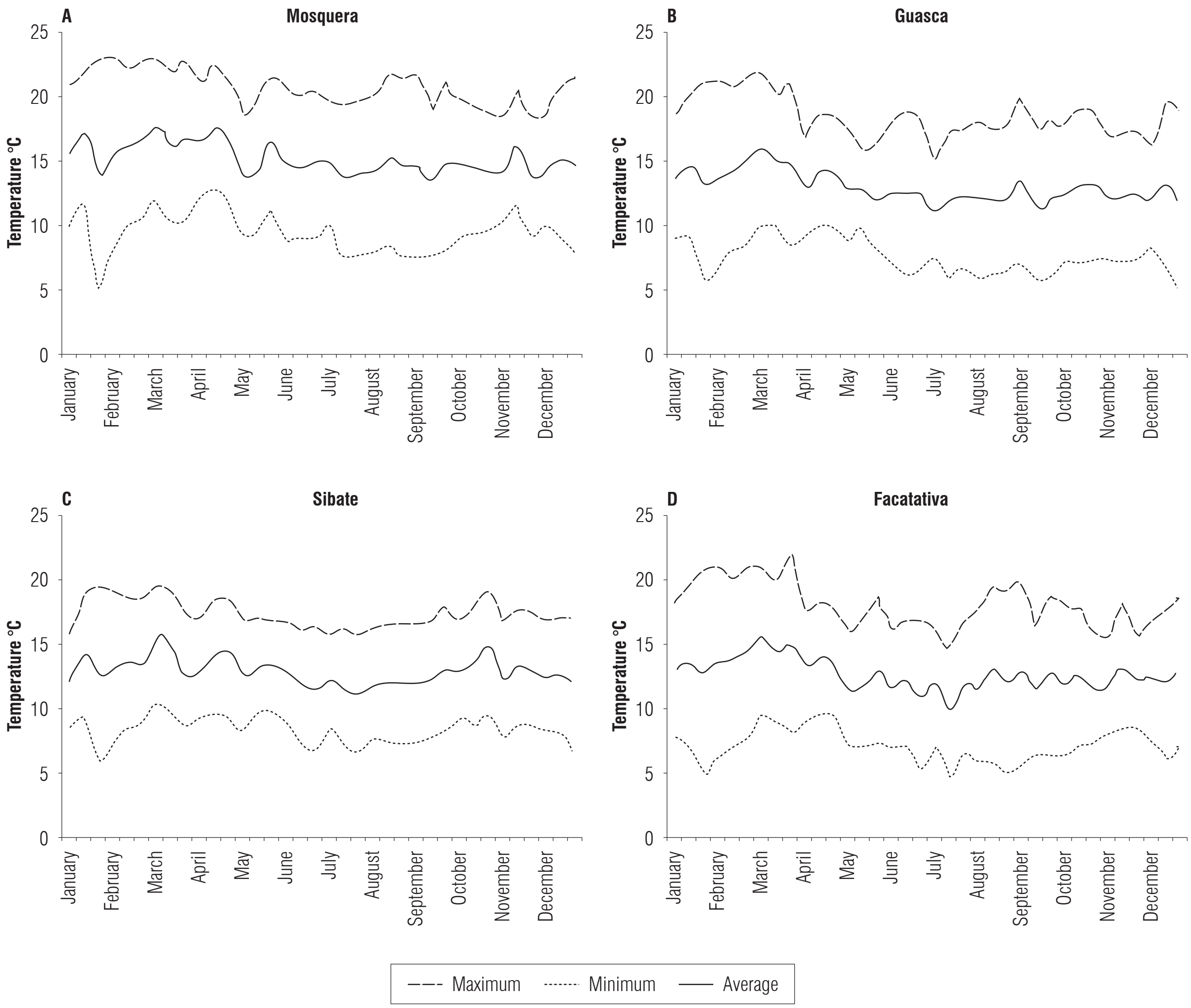

FIGURE 1. Temperature in the growing areas of each municipality for one year, obtained with the IDEAM database.

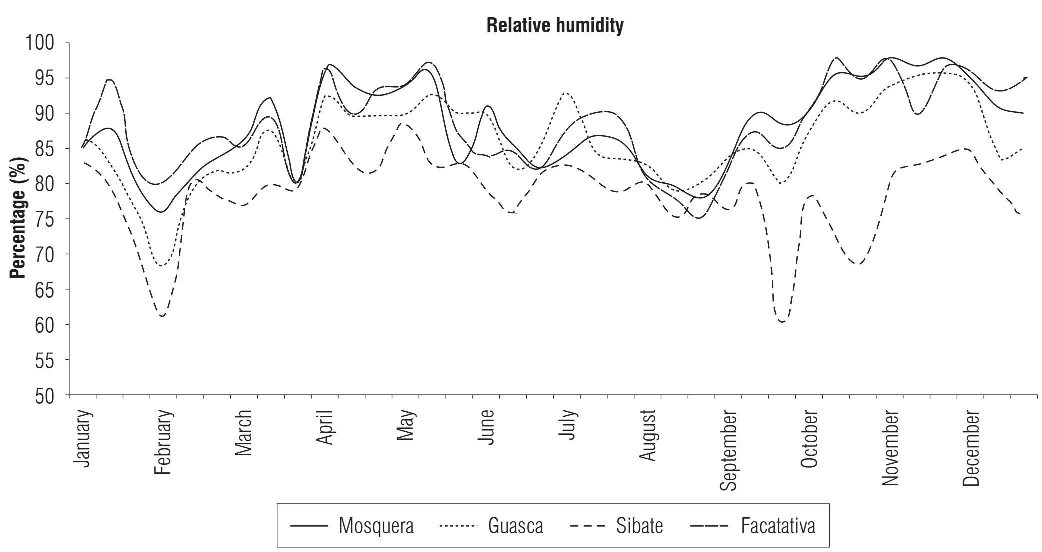

FIGURE 2. Average relative air humidity in the growing areas of each municipality for one year, obtained with the IDEAM database.

$$Acidity\ (\%) = \frac{V \times N \times C}{W} \ x\ 100 \quad (2)$$

where:

V: NaOH volume (mL) used

N: NaOH normality (0.1 mol/L)

C: Citric acid constant (0.064 g meq^{-1})

W: Sample weight (g)

Total soluble solids (TSS)

For the TSS determination, a J357 refractometer (Rudolph Research Analytical, Hackettstown, NJ, USA) was used with one pure strawberry juice extract as the sample, and results were expressed in percentage units. Additionally, the reading was corrected for acidity using the formula obtained by Herrera (2010):

$$Corrected\ TSS = TSS(\%) + (acid(\%) \times (acid(\%) \times 0.0118) \quad (3)$$

Color

For the color measurement, the Hunter Lab system and a Chroma meter CR 400 (Konica Minolta Sensing Europe, Bremen, Germany) were used. After calibration, the readings were taken at three different sites of each fruit.

Statistical analysis

All statistical analyses were performed with *R* software (v 2.15.2) (R Development Core Team 2012). The data were analyzed using a mixed lineal effect model, with Tukey test for variance and homogeneity with a reliability level of $\alpha = 0.05$ to identify significant differences between the evaluated variables.

Results and discussion

Three maturity stages of the Albion variety for the 4 producer municipalities were evaluated. Production in Guasca is focused on obtaining stage 6 fruit maturity fruits for industrial use. Production in Sibate and Facatativa does not reach stage 6 of maturity in order to avoid losses from deformation or mistreatment of fruits during the harvest, packing and transportation process. Finally, in the municipality of Mosquera, the production process is carried out in order to make comparisons in terms of applied technology versus traditional strawberry treatments.

Length and width

As the quality decreased, so did the size. The strawberry fruits from these municipalities showed significant differences for both length (Fig. 3) and width (Fig. 4). Mosquera presented a length average of 5.02 cm, Guasca had 4.99 cm and Facatativa showed 4.57 cm. The most contrasting difference occurred between the municipalities of Mosquera and Sibate. All measurements were higher than the NTC 4103 standard, in which strawberries of the Chandler variety were used; the largest measured width (A) was 3.4 cm.

A study carried out by Casierra-Posada *et al.* (2011), in which the development of strawberry plants with different color coverings was analyzed, showed that the highest length average with red coverage results in 3.16 cm in final products. On the other hand, Martinez-Soto (2008) evaluated the physicochemical properties of six different varieties of strawberry, in which the average length of the Albion variety was 3.56 cm; these results were similar to those established by the NTC 4103 standard. Finally, a study conducted in India by Pandey *et al.* (2015), in which different growth environments were evaluated, showed that the 'Winter dawn' variety planted in open fields presented an average length of 4.77 cm, which is closest to that obtained in the four evaluated municipalities; however, the municipality of Mosquera, for quality 1 and stage 6 of maturity, presented an average length of 5.87 cm, a size of commercial interest.

The width of the fruits had a behavior that was very similar to that of the length, but in this case, the differences had less contrast between the municipalities. As the quality decreased, so did the size, resulting in an average of 3.29 cm for Mosquera, of 3.51 cm for Guasca, of 3.07 cm for Sibate and of 3.27 cm for Facatativa. The greatest difference was obtained between the municipalities of Guasca with the highest average and Sibate with the lowest average.

Dry matter of fruits

Dry matter is a representation of chemical compounds of plant tissue such as carbohydrates, nitrogen and lipid compounds, minerals, vitamins, fiber and organic acids (Ayala *et al*., 2013). So, it was important to determine their percentage accumulation within the total weight of each strawberry fruit.

The results obtained in this study showed statistically significant differences (Fig. 5); the fruits of the municipality of Mosquera had higher percentages of dry mass than the other production areas, with a total accumulated average of 11.88%, in comparison to 8.47%, 9.58%, and 7.11% accumulated for the municipalities of Guasca, Sibate and Facatativa, respectively. Studies by Ávila and Soler (2001) showed that the water content in fruits was 85.2%, which left a cumulative dry matter of 14.8%. Thus, the results obtained in this study agree with normal fruit development.

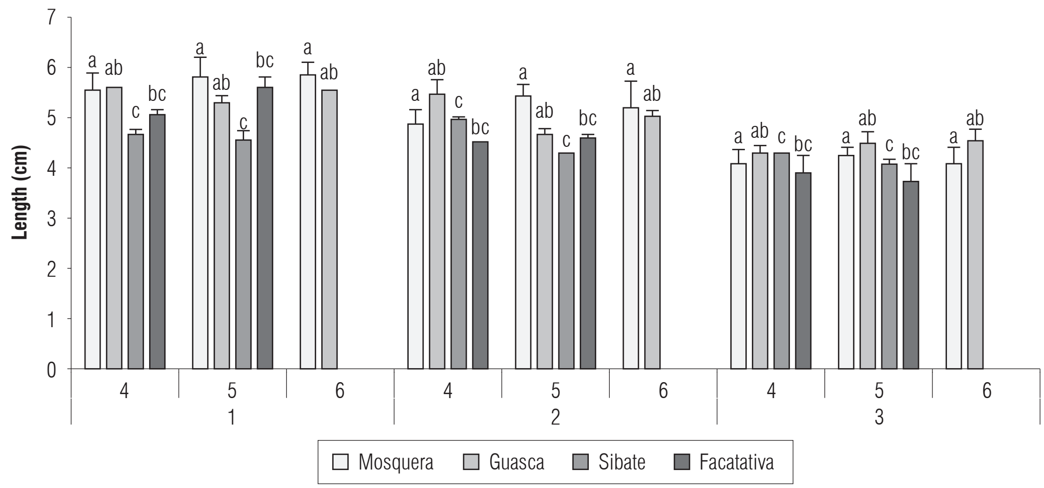

FIGURE 3. Average length of Albion fruits selected by degree of quality and maturity stage. Error bars indicate standard deviation. Means with different letters indicate significant differences according to the Tukey test ($P\leq0.05$).

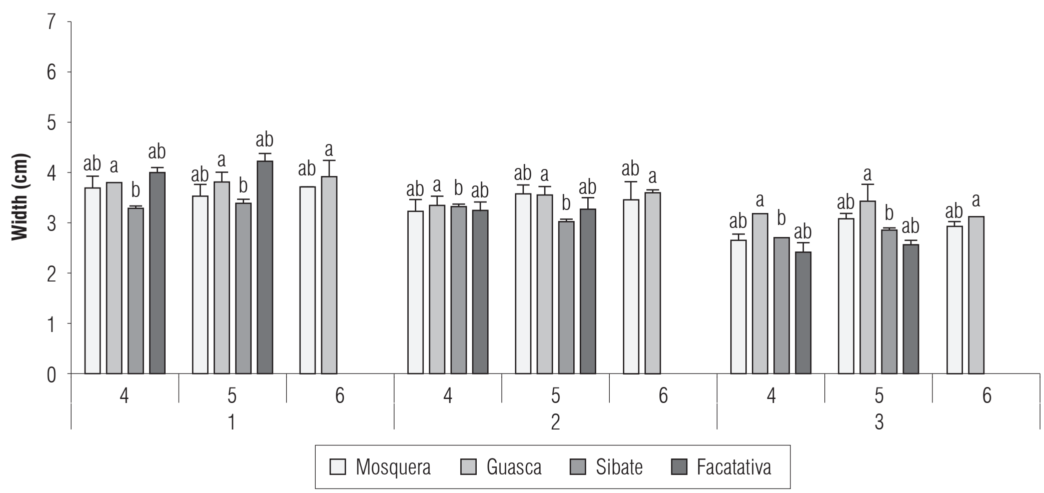

FIGURE 4. Average width of Albion fruits selected by degree of quality and maturity stage. Error bars indicate standard deviation. Means with different letters indicate significant differences according to the Tukey test ($P\leq0.05$).

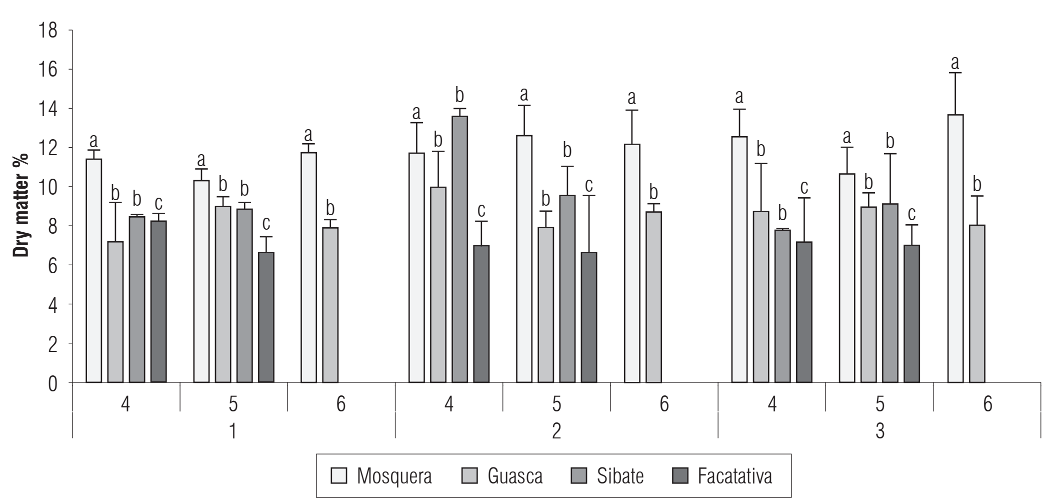

FIGURE 5. Percentage of dry matter of Albion fruits selected by degree of quality and stage of maturity. Error bars indicate standard deviation. Means with different letters indicate significant differences according to the Tukey test ($P\leq0.05$).

Hence, the greater accumulation of differentiated dry matter by the fruits from the municipality of Mosquera may have resulted from the crop system established inside greenhouse conditions. As shown in Figure 1, it had the highest temperature among the municipalities, which improved the development of plants and harvestable organs by accelerating plant metabolism (Opstad *et al.*, 2011). Likewise, plastic with a UV filter can generate a more assimilable wavelength and the arrival of more uniform and stable light to the foliage of plants. Estrada (2011) commented in his study on the positive correlation that is generated in the photosynthetic apparatus on the production of fresh and dry matter in strawberry crops.

The weight variation was not determined directly with the biomass, but with the amount of water contained in the cells. Water is the most abundant component in fruits and vegetables, comprising about 90% of the mass. Water content values vary according to the structure of products (Vicente *et al.*, 2009), which is why fruits and vegetables are highly perishable, since they can easily generate the development of microorganisms.

Consistency

The consistency assessment in this study had an interesting behavior that demonstrated two important aspects: first, a greater resistance to deformation in third quality fruits, in which smaller sizes showed a more compact and rigid cellular order in the fruits, and second, a lower deformation resistance as the degree of maturity advanced. Figure 6 shows how, at the municipality level, Guasca and Sibate presented 10.32 and 10.34 mm, respectively, whereas Mosquera and Facatativa showed averages of 8.96 and 7.92 mm, respectively. Fruit texture changes as a result of hydrolysis of starch and pectin, reduction of fiber content and degradation processes in cell walls, so that fruits become soft and more susceptible during post-harvest management (Arias and Toledo, 2000).

As the physiological and organoleptic maturity of fruits proceeds, they lose resistance to penetration because of the degradation of polymeric carbohydrates, especially peptic substances and hemicelluloses. This is why cellular walls and the cohesive forces that keep cells united weaken, resulting in disintegration of the cellular structure and the internal movement of water that is related to the processes of osmosis (Agón *et al.*, 2006).

The strawberry also suffers problems of softening during maturation and senescence processes. Softening is related to biochemical alterations by enzymes such as polygalacturonases and pectin methyl esterases, which degrade the peptic structure of the cell wall of the middle lamina as a result of the decrease of levels of extracellular calcium seen in mature fruits (Ferguson *et al.*, 1995). The firmness of fruits, an important attribute of quality, is affected by these enzymes, which cause the softening of fruit tissues, making them susceptible to fungal contamination (Hernández-Muñoz *et al.*, 2008).

pH, TTA and TSS

The values of pH, TTA and TSS (Tab. 2) showed significant differences in each municipality. The pH had a different value in Sibate, with an average of 3.37; the other municipalities did not present significant differences, with average results of 3.53, 3.51 and 3.47 for Mosquera, Guasca and

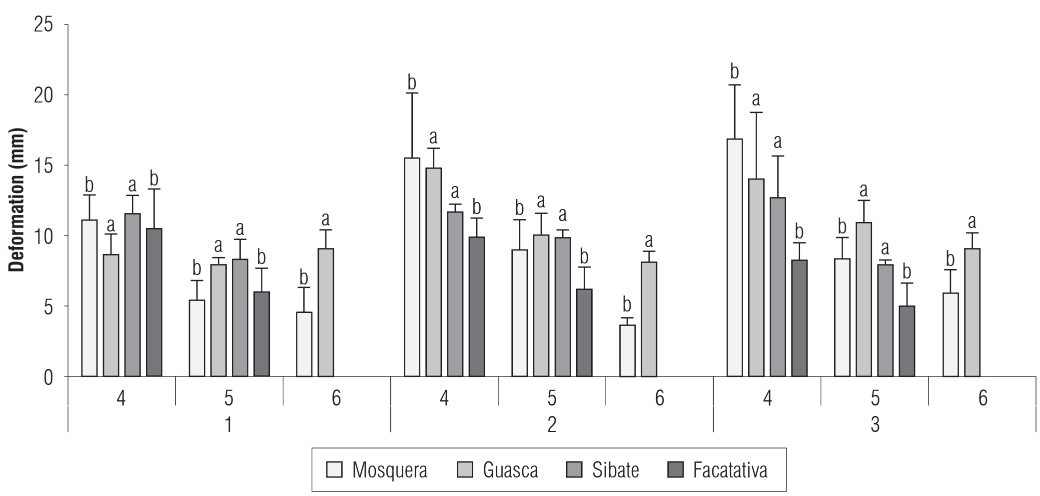

FIGURE 6. Resultant deformation on Albion fruits selected by degree of quality and stage of maturity. Error bars indicate standard deviation. Means with different letters indicate significant differences according to the Tukey test ($P \leq 0.05$).

Facatativa, respectively. The slight increase in pH in the three municipalities resulted from a reduction of the acidity generated by the use of organic acids as an energy source to support the normal process of fruit ripening (Chitarra and Chitarra, 2005).

The municipality of Sibate presented the highest average TTA value (expressed as a percentage of citric acid), with a total of 0.55% differing significantly from the other municipalities; Mosquera showed a value of 0.49%, Facatativa had a similar value with 0.51% and Guasca showed the lowest average value with 0.55%. The highest TTA single value was detected in Facatativa (quality 1, maturity stage 4) with 0.66%. This value is very different from 0.83%, which is the minimum standardized value in maturity stage 4 of NTC 4103.

However, Kader (1999) recommended a maximum concentration of citric acid of 0.8% in order to achieve an acceptable flavor since less acidic fruits are preferred for fresh consumption, while those with a higher concentration of citric acid can be used for processed products of higher quality (Quian *et al.*, 2005). On the other hand, Cordenunsi *et al.* (2002) found that citric acid contributes to 92% of strawberry acidity, which is why it is established in terms of percentage. Pinto *et al.* (2008) stated that the acids contained in fruits are consumed in the respiratory process over time, giving place to new compounds, which explains their decrease during the ripening process.

As for the TSS, the municipalities of Guasca and Sibate were significantly different, with averages of 8.19 and 8.38, respectively, while Mosquera and Facatativa presented 7.66 and 7.77, respectively. Compared to NTC 4103, these values are appropriately adjusted to those proposed by the standard.

The TSS measurement is associated with the dissolved sugars in the cellular juice (Osterloh *et al.*, 1996). These authors stated that the amount of sugars in a fruit is determined by the variety. A recent study with seven different varieties supports this statement, where the authors clearly showed significant differences between seven different strawberry varieties, cultivated in very similar regions of the country (López-Valencia *et al.*, 2018).

Previous studies have shown that °Brix increases if starch is completely hydrolyzed and then decreases as a result of respiration, which increases as maturation progress does (Salamat, 2013). According to Kays (2004), sucrose is translocated from leaves to fruits and, during the development of fruits, sugars are transported to growing fruits, where they are turned into starch. Accordingly, total soluble solids increase in the maturation process, so that expected TSS values would tend to increase °Brix (Manning, 1993).

TABLE 2. Results of pH, TTA and TSS in Albion fruits evaluated by degree of quality and stage of maturity.

Municipality	Quality	Maturity	pH	TTA	TSS
Mosquera	1	4	3.23 b	0.60 b	7.20 b
		5	3.44 b	0.55 b	6.87 b
		6	3.52 b	0.35 b	11.30 b
	2	4	3.42 b	0.61 b	5.60 b
		5	3.36 b	0.54 b	7.90 b
		6	3.92 b	0.26 b	7.73 b
	3	4	3.25 b	0.62 b	4.23 b
		5	3.95 b	0.63 b	8.17 b
		6	3.72 b	0.22 b	9.97 b
Guasca	1	4	3.48 b	0.24 c	6.73 a
		5	3.51 b	0.28 c	8.93 a
		6	3.73 b	0.24 c	9.03 a
	2	4	3.28 b	0.34 c	7.23 a
		5	3.41 b	0.30 c	8.27 a
		6	3.60 b	0.30 c	8.73 a
	3	4	3.49 b	0.32 c	8.00 a
		5	3.54 b	0.30 c	8.57 a
		6	3.54 b	0.31 c	8.17 a
Sibate	1	4	3.47 a	0.55 a	8.02 a
		5	3.37 a	0.53 a	9.02 a
		6	0	0	0
	2	4	3.32 a	0.55 a	8.30 a
		5	3.25 a	0.51 a	8.25 a
		6	0	0	0
	3	4	3.14 a	0.60 a	8.10 a
		5	3.72 a	0.52 a	8.58 a
		6	0	0	0
Facatativa	1	4	3.60 b	0.66 b	7.30 b
		5	3.26 b	0.45 b	9.67 b
		6	0	0	0
	2	4	3.36 b	0.53 b	6.83 b
		5	3.63 b	0.43 b	7.73 b
		6	0	0	0
	3	4	3.73 b	0.46 b	7.30 b
		5	3.23 b	0.50 b	7.80 b
		6	0	0	0

Means with different letters indicate significant difference according to the Tukey test ($P \leq 0.05$).

Color

In order to evaluate this parameter, quality 1 fruits were analyzed because of the high similarity in the color of the three different quality stages. Figure 7 shows the behavior of the L, a and b parameters based on the Hunter Lab scale for the four evaluated municipalities. Component L represents the variation between black and white, where black is = 100 and white is = 0. Parameter a does not have a defined numerical limit, and its positive value indicates a tendency towards the red tone, while its negative value indicates a tendency towards the green one. Parameter b also has no defined numerical limit; its positive value indicates a tendency towards the yellow tone, while its negative value indicates a tendency towards the blue tone (Hunterlab, 2012).

A clear decrease of the L parameter was observed as the maturity stage progressed, indicating the tendency of the fruits to become darker, just as the value b was abruptly reduced, showing a trend towards the blue tone. In the graphs, no negative values were observed, eliminating the green color in the evaluated fruits because of their advanced maturity stages. However, this indicates a positive decrease, turning the fruits towards the red tone, as expected. This reduction was explained by the change in the purple red hue of the fruits during maturation because of the appearance of pigments such as anthocyanins and carotenoids (García, 2012).

The color change tendency in the harvested fruits was the same; however, significant differences in color intensity were evidenced. The Guasca and Sibate fruits were the most attractive because they had more striking and intense red colors. Terry *et al.* (2007) demonstrated that an absence of irrigation results in strawberries with a higher concentration of anthocyanin and antioxidant capacity. On the other hand, the fruits from Mosquera and Facatativa presented a lower color intensity, showing lighter and less attractive reds. However, compared to the color scale suggested by NTC 4103 standards, the four municipalities easily meet the minimum requirements for gaining access to a high-quality commercial field.

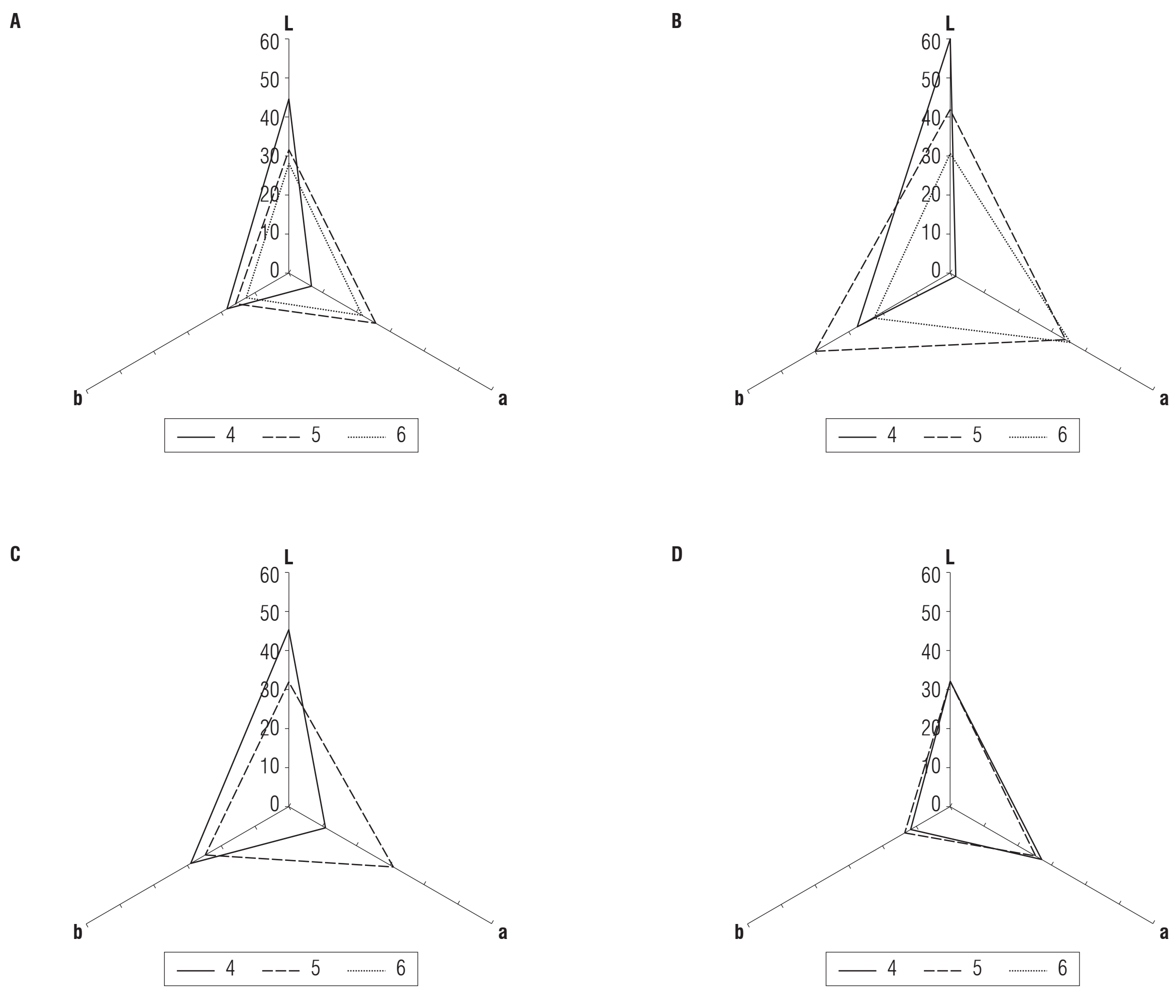

FIGURE 7. Hunter Lab graphs obtained from the Albion fruits selected for first quality and by stage of maturity (4: black line, 5: segmented line and 6: grey line) of each municipality: A) Mosquera, B) Guasca, C) Sibate and D) Facatativa.

Conclusions

The sizes, volumes and densities of the fruits measured in the present study were even higher than those proposed by the current standard for gaining access to a high quality fresh trade; however, this may present some drawbacks because of the care that must be taken at the time of packing or storing fruits because of the standard measurements. Large sizes can be very attractive for national and international marketing. These characteristics should be included in the generation of a new standard for strawberry quality in Colombia.

As for the organoleptic characteristics, the fruits with a sugar content were suitable for fresh consumption. However, a market based on industrial transformation requires higher citric acid percentage values. According to this research, this is the only parameter in which more work is needed to improve the minimum levels required by the Colombian technical standard (NTC 4103).

Although the fruits from Mosquera presented favorable results compared to the other producer areas, work is under way to improve the infrastructure and technology applied in order to obtain representative crops with high quality standards that can be used as a demonstration and reference example for the producers of Cundinamarca and, of course, the whole country.

Acknowledgments

The authors thank the Corredor Tecnologico Agroindustrial II - Fresa y Mora project for allowing this study to exchange experiences with strawberry producers and farmers in each crop area. The authors also thank the students and professionals of the Universidad Nacional de Colombia who supported the practical work and collection of laboratory data.

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Spectral and thermal response of *Heliconia psittacorum* species to induced water stress

Respuesta espectral y térmica de la especie *Heliconia psittacorum* ante estrés hídrico

David Revelo-Luna[1*], Aldemar Reyes-Trujillo[2], and Miguel Peña-Varón[3]

ABSTRACT

An important limitation in agricultural production is stress resulting from water deficit. Flower production and post-harvest life both decrease in *Heliconia psittacorum* affected by water stress. Remote sensing provides tools for estimating the water status of plant species using spectral information in the visible and infrared range. This paper presents a study of reflectance in the 350-800 nm range and the response in the thermal infrared of leaf tissue under different irrigation regimes. For the measurement of reflectance, an OceanOptics® Micro-Spectrometer was used, while for the thermal infrared measurements, a FLIRE40® camera was used. Three irrigation regimes were established: T1: 100% field capacity (FC), T2: 50% FC, and T3: 10% FC. Significant differences were found between treatment T1 and treatments T2-T3 in the water stress index (CWSI) and stomatal conductance index (GI). The reflectance around 800 nm decreased for T2 and T3. Significant differences were obtained between T1 and T2-T3 in the maximum of the first derivative of the reflectance between 700 and 750 nm. It was found that, in the range 350-800 nm, the thermal indices were better indicators of the water status of the *Heliconia* species than the spectral indices.

Key words: thermal indices, spectral reflectance, water deficit, vegetation indices.

RESUMEN

Un limitante importante en la producción agrícola es el estrés por déficit hídrico. La producción de flores y la vida de poscosecha disminuyen en *Heliconias psittacorum* afectadas por estrés hídrico. El sensado remoto proporciona herramientas para la estimación del estado hídrico de especies vegetales usando información espectral en el rango visible e infrarrojo. En este trabajo, se presenta el estudio de la reflectancia en el rango 350-800 nm, y la respuesta en el infrarrojo térmico del tejido foliar en diferentes tratamientos de riego. Para la medida de reflectancia se usó un Micro-Spectrometer OceanOptics® y para las medidas en el infrarrojo térmico se usó la cámara FLIRE40®. Se establecieron tres regímenes de riego: T1: Capacidad de Campo (CC) 100%, T2: CC 50%, y T3: CC 10%. Se encontraron diferencias significativas entre el tratamiento T1 y los tratamientos T2-T3 para el índice de estrés hídrico (CWSI) y el índice de conductancia estomática (GI). La reflectancia alrededor de 800 nm se disminuyó para T2 y T3. Se obtuvieron diferencias significativas entre T1 y T2-T3 en el máximo de la primera derivada de la reflectancia entre 700 y 750 nm. Se encontró que los índices térmicos son mejores indicadores del estado hídrico de la especie *Heliconia* que los índices espectrales en el rango 350-800 nm.

Palabras clave: índices térmicos, reflectancia espectral, déficit hídrico, índices de vegetación.

Introduction

Heliconia sp. is an herbaceous species of the family *Heliconiaceae* that is distributed mainly in Brazil, Colombia, French Guiana, Guyana, Surinam, Trinidad and Venezuela. The genus *Heliconia* L. grows in neo-tropical habitats in warm protected areas in temperate climates. This genus has an important commercial application because of its ornamental uses, with 93 species preliminarily recognized in Colombia. *Heliconia psittacorum* grows between 0.5 m and 1.5 m tall, preferably under both full sun and partial shade, with moist and well-drained soil that is rich in organic matter. This tropical species is particularly important because of its use in phytoremediation studies in environments affected by a variety of pollutants, such as Chemical oxygen demand (COD), nitrogen, Hg^{+2}, Cd^{+2}, Cr^{+6}, and Pb^{+2} (Madera *et al.*, 2014; Wood, 2015).

The water status of ornamental plants influences their flower production. The water requirement in an irrigation level of the species *Heliconia psittacorum* (*He*) varies around 10 cm d^{-1}, depending on the vegetative stage of the crop, the soil type, and the climatic conditions (Sosa, 2013). Water deficiency in plants can cause water stress, resulting in growth inhibition, decreased production, and death (Lisar *et al.*, 2012; Etesami and Jeong, 2018). A plant under water

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stress reduces its photosynthetic activities by decreasing the leaf area and the exchange of gases and by rejecting light energy that is useful for the photosynthetic process; other effects include the inhibition of growth cells, decrease in stomatal opening, mainly caused by an increase in the concentration of gaseous carbon in the leaves, and decrease in the water potential (Akıncı and Lösel, 2012; Kögler and Söffker, 2017). Water status is estimated using traditional methods, such as direct measurement, water potential and relative water content; however, it is also estimated indirectly with variables, such as stomatal conductance, photosynthetic activity, chlorophyll fluorescence, and gas exchange (CO_2 assimilation and transpiration). The measurement of water potential and relative water content, in general, are destructive and require a lot of time (Farifteh *et al*., 2013). There is, therefore, an evident need to develop non-invasive and non-destructive techniques that monitor, in real time, the water status of plants and crops.

Remote sensing techniques provide tools to monitor water status, using information on the spectral response of the plants. The first advances in this subject were made in the 1980s with studies on low spatial and spectral resolution, using the vegetation response in bands of the visible spectrum (VIS) and the near infrared (NIR) (Wójtowicz *et al*., 2016). In recent years, remote sensing techniques have made significant progress because of the development of hyper-spectral sensors, which make it possible to carry out detailed studies of pigment content (Uiboupin *et al*., 2012; Fan *et al*., 2014; Li *et al*., 2015), leaf tissue structure and water content in plants (Shimada *et al*., 2012; Farifteh *et al*., 2013; Genc *et al*., 2013; Ge *et al*., 2016).

In several studies, the thermal response of foliar tissue of plants and crops has been associated with indirect effects caused by water deficit. The development of low cost thermographic cameras has allowed an important advance in the study of the water status of plants, providing spatial resolution. Because of the alteration in the transpiration and the opening of stomata in leaf tissue, thermal variations are produced that can be measured in images using thermal indices, such as the stomatal conductance index (GI) or the crop water stress index (CWSI). Several studies have found high correlation between thermal indices and measurements of physiological parameters, such as stomatal conductance, water potential and gas exchange (Fuentes *et al*., 2012; Gómez-Bellot *et al*., 2015; Yuan *et al*., 2015; Lima *et al*., 2016; Mangus *et al*., 2016). The main objective of this study was to determine variations in the spectral response in the VIS, NIR and Long-wave infrared (LWIR) bands of the *He* species in response to water stress induced by different irrigation levels.

Materials and methods

Experimental design

This experiment was carried out in a greenhouse located in the Experimental Station of the Agricultural Water and Soils Laboratory of the Universidad del Valle, Cali, Colombia, where the variables ambient temperature and relative humidity were monitored (average temperature of 24.5°C, 79% relative humidity, and 30% poly shade). Young individuals of the plant species *Heliconia psittacorum* (*He*), not more than 0.15 m tall, were taken from a sub-surface wetland of horizontal flow from the wastewater treatment plant of Ginebra-Valle, Colombia. The plants were acclimated for two months under saturated substrate conditions. The experimental design was completely randomized with one factor (irrigation). Three levels of the irrigation factor were established (100% Field Capacity (FC), 50% FC and 10% FC). The FC value of the substrate was estimated using the sand box method (Jaramillo, 2002). Three treatments were performed with 4 replicates and 12 experimental units (EU). Each EU was an individual of the *He* species planted in 6 L pots with the substrate, which provided nutritional support, composed of soil with organic fertilizer, to which macro and micronutrients were added according to the requirements of nitrogen, phosphorus and potassium reported by Sosa (2013). Tests of analysis of variances (ANOVA) and Honestly Significant Difference (HSD) of Tukey ($P \leq 0.05$) were used prior to the normalization and verification of homoscedasticity to determine if there were significant differences between the treatments. The experiments began on November 20, 2016 and ended on January 27, 2017. Statistical tests were performed to evaluate significant differences between treatments on the days shown in Table 1.

TABLE 1. Start and end dates of experiments and days chosen to carry out hypothesis tests.

Sampling day	Date
Start of experiment	20-11-2016
5	02-12-2016
9	12-12-2016
14	27-12-2016
16	10-01-2017
18	17-01-2017
19*	20-01-2017
20 (End of experiment)	27-01-2017

* Substrate moisture was restored to FC for T2.

Irrigation control

The substrate moisture of each EU was measured using time domain refractometry (TDR) equipment (MPM160 ICT-International, Armidale-Autralia) three times per week at a distance equal to the effective root depth (0.07 m). Each measurement was taken three times in random points, and the average value was recorded. According to the volumetric moisture value of each EU, the moisture of the substrate was adjusted according to each treatment. The FC value of the substrate was estimated using the sand box method (Jaramillo, 2002). All the EUs started at FC; treatments T2 and T3 were drained until reaching the respective depletion level for each group, while T1 was maintained at FC throughout the experiment.

Measurement of spectral reflectance

The leaf tissue reflectance (%) of each EU was measured using an STS-VIS Mini-Spectrometer (Ocean Optics®, Florida, USA). The equipment has a measurement range between 350 and 800 nm, with an integration time of 100 ms and a spectral resolution of 1 nm. The measurements were taken at a distance of 0.05 m from the leaf tissue, between 10:00 am and 12:00 midday. One measurement was taken per leaf in the central part and to one side of the midrib. The calibration was done using the reflectance blank prior to each measurement. A medium low pass filter was used to eliminate high frequency and noise components before the calculation of the spectral indices. The Normalized Difference Vegetation Index (NDVI) and the Variation of NDVI in the range 705-750 nm (NDVI705) spectral indices were estimated using Equations 1 and 2, respectively. The maximum value of the first derivative of the reflectance curve (*D*max) between 700 and 750 nm (Eq. 3) was also calculated:

$$NDVI = \frac{R_{650} - R_{800}}{R_{650} + R_{800}} \quad (1)$$

$$NDVI705 = \frac{R_{750} - R_{705}}{R_{750} + R_{705}} \quad (2)$$

$$D\text{max} = max\left(\frac{d_R}{d\lambda}\right) \quad (3)$$

where R_{650}, R_{705}, R_{750} and R_{800} are the percentages of light reflected with respect to the calibration blank at wavelengths 650, 705, 750 and 800 nm, respectively (%), and $\frac{d_R}{d\lambda}$ is the first derivative of the reflectance with respect to the wavelength (% nm^{-1}).

Measurement of thermal indices

The CWSI and GI values were calculated according to the model described by Leinonen and Jones (2004) (Eq. 4 and 5).

$$CWSI = \frac{T_{leaf} - T_{wet}}{T_{dry} - T_{wet}} \quad (4)$$

$$GI = \frac{T_{dry} - T_{leaf}}{T_{leaf} + T_{wet}} \quad (5)$$

where T_{leaf} is the temperature of the leaf (°C), T_{wet} is the reference temperature of wet tissue (°C) and T_{dry} is the reference temperature of dry tissue (°C). The temperature measurement was taken with thermographs of each EU, using the FLIR E40® camera (Oregon, USA), with a thermal sensitivity of 0.07°C at 25°C, 160x120 pixels resolution, and a spectral range from 7.5 to 13 µm. The images were taken at a height of 1.5 m directly above each EU. Two reference temperatures were used: T_{dry} (dry tissue) and T_{wet} (wet tissue). T_{dry} was obtained by taking 5°C above air temperature (Rud *et al*., 2014; Mangus *et al*., 2016). The value of the reference temperature T_{wet} was measured using the most frequent value of the histogram of the thermography in wet leaf tissue. The thermal information of the images (*Raw-Data*) was exported in the .csv format using the FlirTools© (Oregon, USA). The data were then processed for the calculation of the thermal indices in free software developed by the main author in C++ using image processing libraries from OpenCV2.0.

Results and discussion

Soil moisture

All EU started with the volumetric moisture of the substrate at FC (76% volume content). The substrate moisture was monitored and adjusted three times per week until it reached the values of each treatment T1, T2 and T3. Figure 1 shows the variation of the average substrate moisture (volume-basis) throughout the experiment. On d 19, the moisture of the T2 treatment was restored to 100% FC in order to determine if the changes in the thermal and spectral response of the EUs corresponded to the variation in the irrigation.

Spectral reflectance and spectral indices

The leaf tissue reflectance of the EUs presented the typical response of plant tissue, in which the percentage reflected in the NIR band (around 800 nm) was higher than the

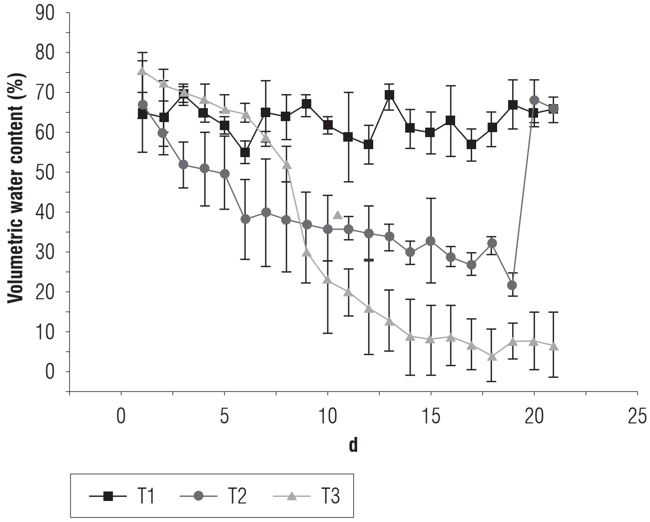

FIGURE 1. Variation of substrate moisture (volume-basis) for 3 treatments: T1 (100% FC), T2 (50% FC) and T3 (10% FC).

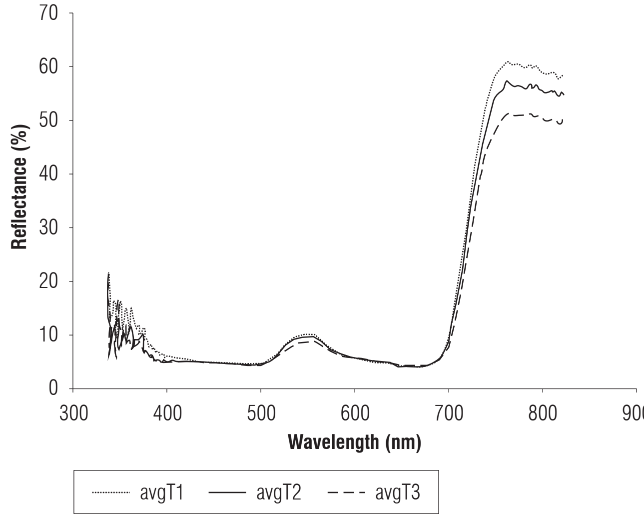

FIGURE 2. Average reflectance for treatments T1 (100% FC), T2 (50% FC) and T3 (10% FC) at d 14 of the experiment.

percentage reflected in the visible band (400-700 nm). However, it was found that the NIR reflectance for the T2 and T3 treatments was lower than for the T1 treatment. Changes in morphology of the leaf and the structure of the mesophyll have a strong influence on the spectral properties of leaves in this range (Wójtowicz *et al.*, 2016). Decrease in reflectance in the near infrared could occur because of the structural modification of mesophyll, which is possibly related to a strong water deficit (Semenova *et al.*, 2014). Figure 2 shows the average of the reflectance curves for each treatment at d 14 of the experiment. These results are in agreement with those obtained in other species subjected to a water deficit. In a study with *Zea mays* L., Genc *et al.* (2013) found similar results for four treatments (100% FC, 66% FC, 33% FC and 0% FC). In the species *Spinacia oleracea* under water stress, Corti *et al.* (2017) reported the same effect of a decrease on NIR reflectance measured on green leaves.

No significant differences were found (P>0.05) between any of the treatments for the NDVI and NDVI705 vegetation indices (Tab. 2); Shimada *et al.* (2012) reported that there were no significant differences between the NDVI index and the water potential of the species *Hibiscus rosa-sinensis* subjected to water stress. Elvanidi *et al.* (2017) conducted an experiment to study the water status of *Solanum lycopersicum* in an environment with a controlled microclimate. They found no significant differences in treatments using the NDVI index. However, the normalized difference indices showed greater sensitivity in changes generated by the structure at the canopy level (Duan *et al.*, 2017), especially when the leaf area index was low and in the face of lengthy follow-up times (Gamon *et al.*, 2013; Morgounov *et al.*, 2014).

The vibrational modes of water molecules have associated wavelengths of 1,405 nm (2v1) and 1,896 nm (v1+v2); some weak vibrations are associated with the bands 1,150 nm (v1+3v2) and 960 nm (v1+4v2), the latter being the weakest (Yang *et al.*, 2011; Steidle *et al.*, 2017). These wavelengths are all outside the range of the spectrometer used in this study. As such, it was not possible to directly associate the spectral indices in this range with the water status of the plants. However, one effect water stress produces is the inhibition of the synthesis of chlorophyll (Lisar *et al.*, 2012; Etesami and Jeong, 2018), a pigment that does have optical activity in the visible spectrum.

Red edge variations

The treatments with a water deficit showed a decrease in the slope between 700 and 750 nm. As a consequence, the maximum value of the first derivative of the reflectance curve (Eq. 3) decreased for T2 and T3. Figure 3 shows the average behavior for d 14 of the measurements in the three treatments.

Significant differences were found between the T1 treatment and the T2 and T3 treatments from d 9 of the measurements for values with a significance of P≤0.05 (Tab. 2). The inhibition in chlorophyll synthesis in plants subjected to water stress can explain the statistically significant difference in the spectral response in the 700-750 nm range between the T1 treatment and the T2 and T3 treatments. Authors such as Mielke *et al.* (2012), Dian *et al.* (2016), and

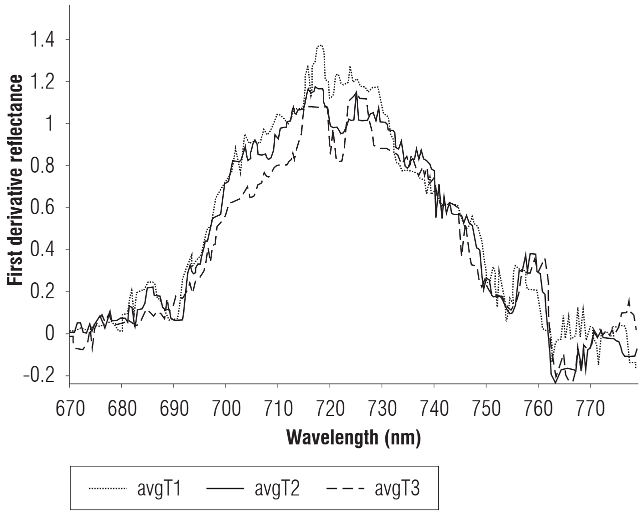

FIGURE 3. First derivative curve of reflectance treatments T1 (100% FC), T2 (50% FC) and T3 (10% FC).

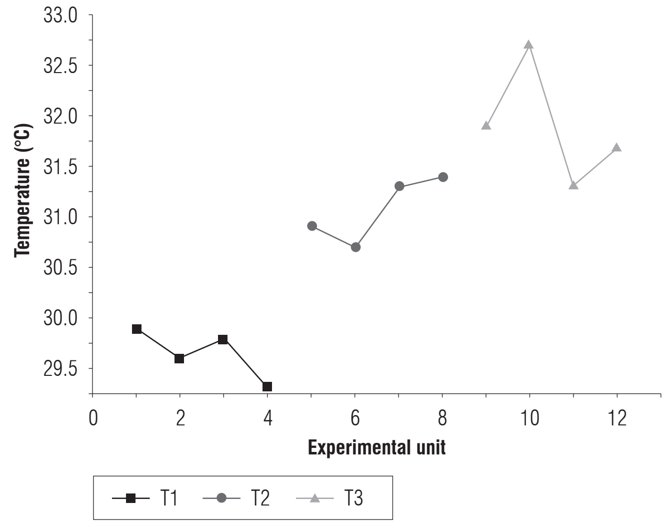

FIGURE 4. Leaf temperature of the EUs in treatments T1 (100% FC), T2 (50% FC) and T3 (10% FC).

Liu *et al.* (2016) have considered spectral indices in the red edge band as good indicators of the leaf chlorophyll content of different plant species. Yang *et al.* (2015) established a correlation model for chlorophyll content that considers the maximum of the first derivative of the reflectance in the range 670-780 nm as one of its variables.

Leaf temperature and thermal indices

The temperature of the leaf tissue was found to increase for treatments with a water deficit (T2 and T3). Figure 4 shows the leaf tissue temperature of the EUs in the three treatments on d 14 of the measurements. The data from 1 to 4 correspond to the EUs of T1, data 5 to 8 to T2, and data 9 to 12 to T3.

The mean temperature difference between treatments T1 and T3 was 2.2°C, for which there were significant differences. A similar result was presented by Farifteh *et al.* (2013), who found that the difference between the control group and the treatment with induced water stress was 2.7°C after 22 d, a time period in which significant differences were found between the treatments. The increase in leaf temperature of the treatments with a water deficit may correspond to stomatal closure, a decrease in gas exchange and a reduction in transpiration, which occur in response to low water availability (Lisar *et al.*, 2012; Etesami and Jeong, 2018). All these phenomena, in principle, contribute to a lower water circulation and contribute to a process of raising the temperature of the tissue since the rate of heat transmission to the environment is reduced. Authors such as Lima *et al.* (2016) correlated measurements of stomatal conductance (*gs*) and transpiration (*E*) with increased leaf temperature. The above is valid if it is assumed that leaf temperature is not strongly influenced by variations in wind speed, resistance to heat flow and vapor pressure deficit. In this way, the stomatal resistance model described by Jones (1999) has an inverse relationship with leaf temperature, which would explain the relationship between leaf temperature and a possible stomatal closure resulting from water stress.

The T_{wet} reference was calculated using the most frequent value of the histogram in the region of interest (ROI). Figure 5 presents the ROI for measurement of the T_{wet} and its histogram. The circle shows the most frequent value of the histogram of the ROI temperature field. Similarly, the leaf temperature of the EUs was estimated with the temperature image.

The CWSI and GI showed significant differences between the T1 treatment and the T2 and T3 treatments. After restoring irrigation to FC for T2, no significant differences were obtained between T1 and T2, which shows that the thermal response of the leaf tissue of the EUs was influenced by the irrigation levels (Tab. 2). Figure 6 presents the estimated thermal indices for the thermographs taken for the three treatments on the 14th d of experiment.

The CWSI and GI made it possible to obtain greater statistical differentiation between the T1 treatment and the T2-T3 treatments than the NDVI, NDVI705 and *D*max spectral indices studied in the 350-800 nm range (Fig. 7).

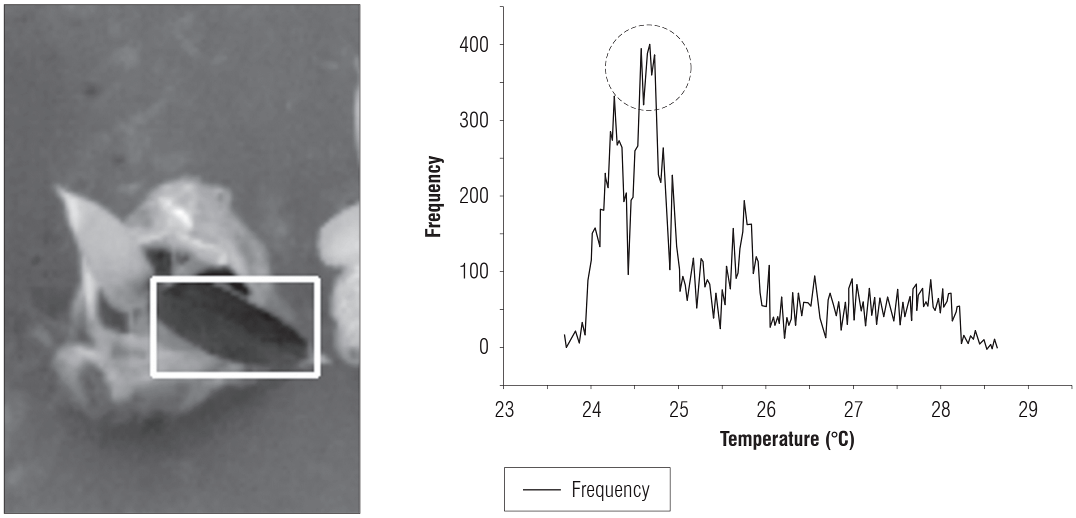

FIGURE 5. T_{wet} reference temperature using the most frequent value of the thermography histogram.

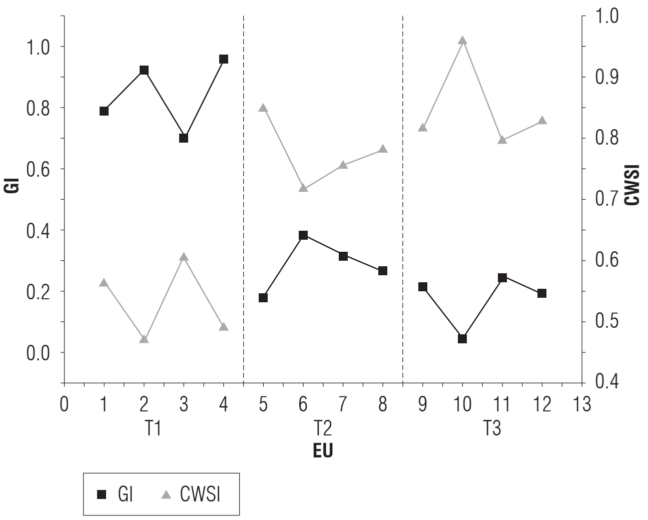

FIGURE 6. CWSI and GI for treatments T1 (100% FC), T2 (50% FC) and T3 (10% FC).

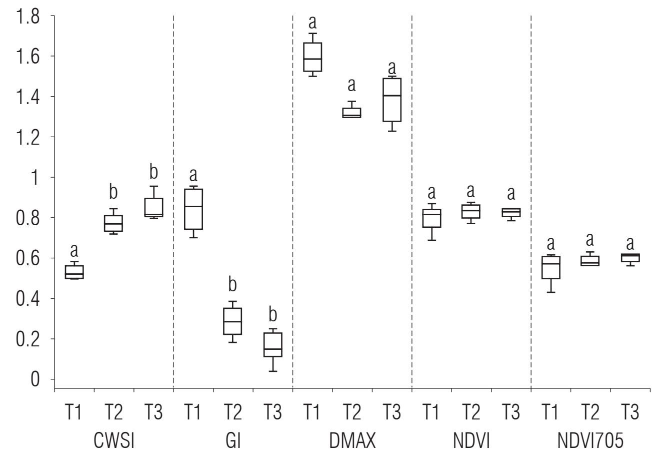

FIGURE 7. Statistical diagram of the CWSI, GI, *D*max, NDVI and NDVI705 parameters. Different letters show statistically significant differences.

TABLE 2. ANOVA and Tukey test results for GI, CWSI, *D*max, NDVI, NDVI705, between the T1 treatment and the T2 and T3 treatments.

Day	*P*-value	HSD	*P*-value	HSD	*P*-value	HSD	*P*-value	HSD	*P*-value	HSD
	CWSI		GI		NDVI705		NDVI		*D*max	
5	0.71	-	0.076	-	0.17	-	0.48	-	0.83	-
9	0.17	-	0.25	-	0.25	-	0.51	-	0.013*	T2, T3
14	8.03 e-5*	T2, T3	1.37e-6*	T2, T3	0.26	-	0.49	-	0.007*	T2, T3
16	1.02 e-6*	T2, T3	1.56e-6*	T2, T3	0.21	-	0.24	-	0.014*	T2, T3
18	3.12 e-6*	T2, T3	9.03e-6*	T2, T3	0.47	-	0.11	-	7.00e-4*	T2, T3
20	3.25 e-5*	T3	3.00e-4*	T3	0.45	-	0.19	-	0.024*	T3

*Significant difference between treatments $P \leq 0.05$.

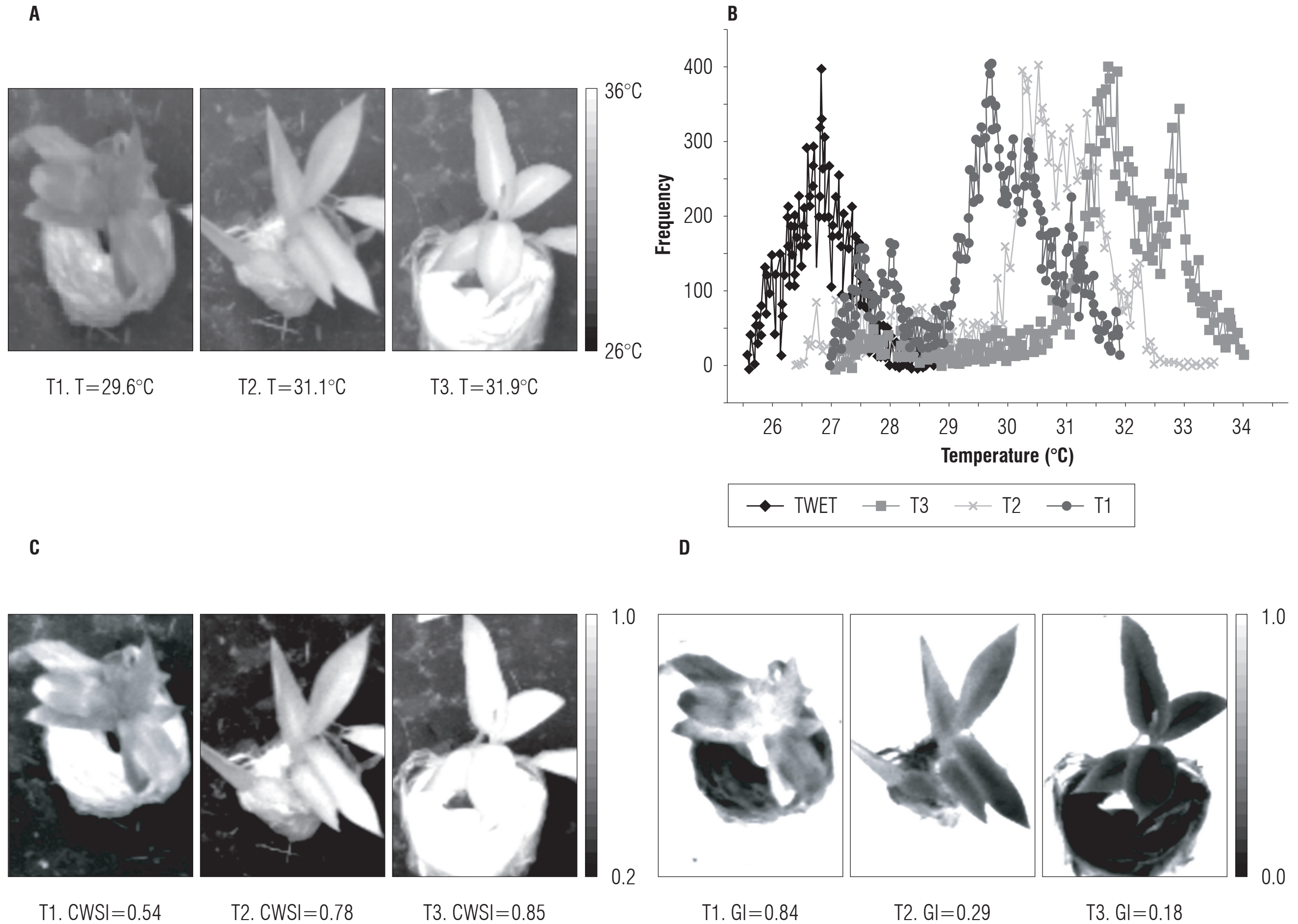

FIGURE 8. Thermal response of *He* to induced water stress in treatments T1 (100% FC), T2 (50% FC) and T3 (10% FC). A) Gray scale image of the temperature of the experimental units, B) Thermography histogram, C) Gray scale image of the CWSI thermal index, and D) Gray scale image of the GI thermal index.

Figure 8B shows the histograms associated with the ROIs of the thermographs shown in Figure 8A, taken on experiment d 14. Information was obtained on the thermal indices of the plant species for each pixel associated with the leaf tissue of the EUs using Equations 4 and 5, whose gray level is proportional to the value of the index in each pixel (Fig. 8 C and D).

From the image analysis, it was found that the higher intensity values in the CWSI images and the lower intensity values in the GI images were associated with the treatments with a water deficit. Similar results were found by Rud *et al.* (2014), Gómez-Bellot *et al.* (2015), and Bellvert *et al.* (2014). The EUs of the T2 treatment, once they were taken to field capacity, had higher stomatal conductance indices and lower water stress indices than those seen when there was a water deficit (Fig. 9).

It was also found that the response of the plants to a water deficit was not uniform. It was observed that the EUs under a water deficit showed an increase in leaf temperature, an increase in the CWSI and a decrease in the GI, initially in the central part and then spreading to the rest of the leaf. Figure 10 shows an EU with a water deficit and non-uniform temperature field, in which the histogram did not have a single defined maximum. Figure 11 shows a control EU and uniform temperature field. The associated histogram had a single maximum in the area of the leaf. The evidence shows that, under water stress, plants have an anisotropic warming in the tissue of their leaves, which, as mentioned above, would be related to the characteristic of thermal instability when there is a low rate of evapotranspiration and stomatal closure as a physiological state of response to water stress.

Faced with an increase in temperature, the plants showed temporary wilting as a natural adaptation mechanism, decreasing the effective area and the temperature in the leaf tissue (Fig. 12).

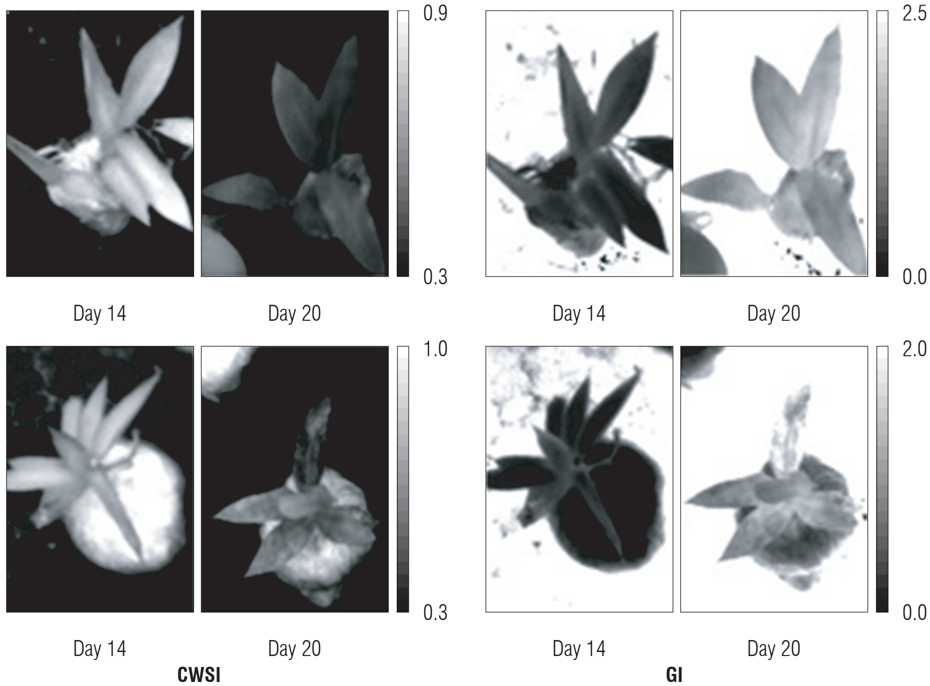

FIGURE 9. CWSI and GI thermal indices of two experimental units of treatment T2 before (d 14) and after (d 20) restoring the moisture of the substrate to field capacity.

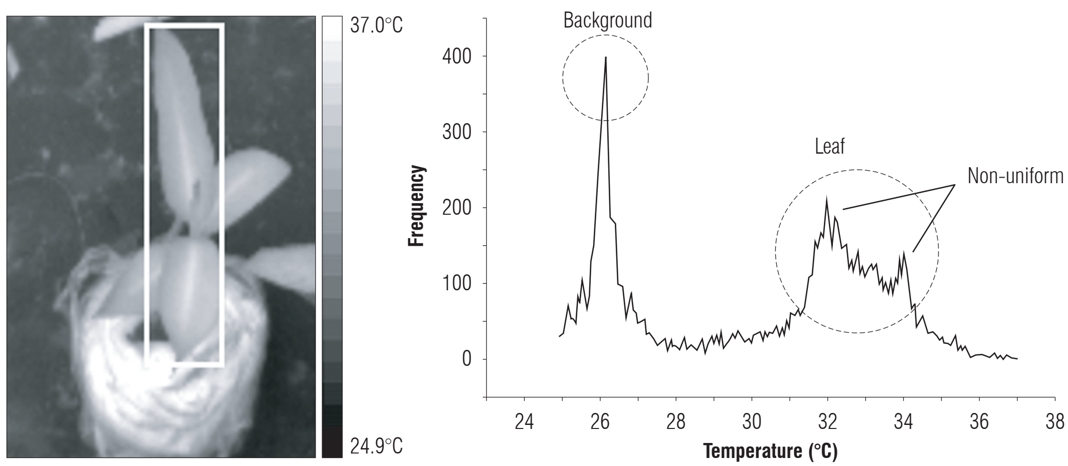

FIGURE 10. Thermal image and ROI histogram. Plant under water stress, non-uniform temperature field.

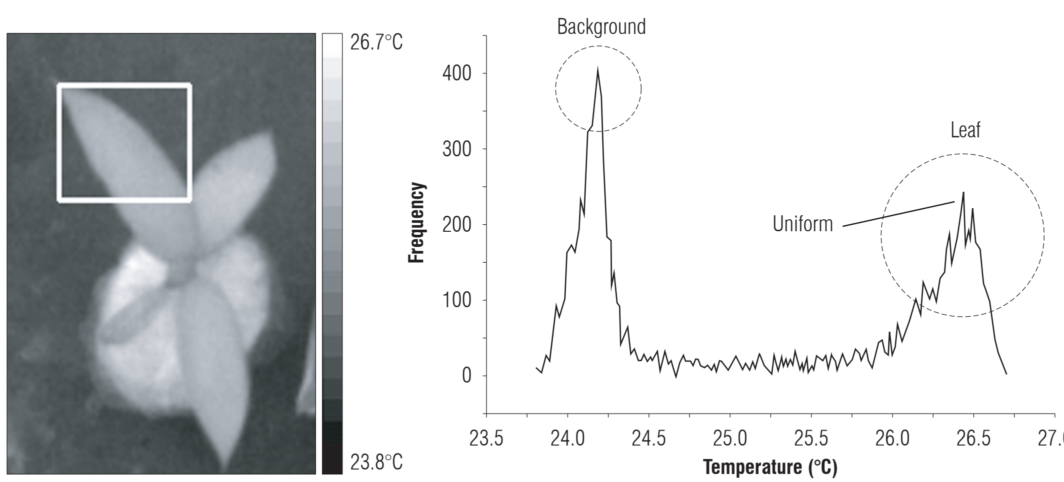

FIGURE 11. Thermal image and ROI histogram. Plant before water stress, uniform temperature field.

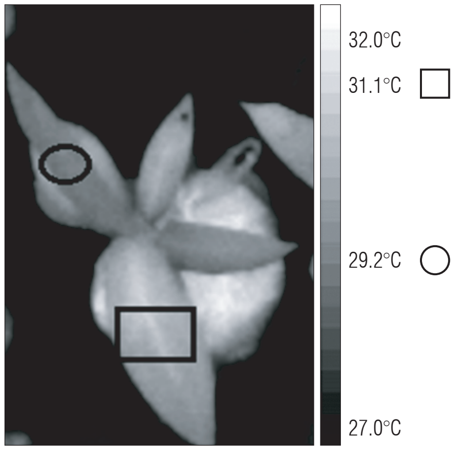

FIGURE 12. Response of the *He* plant to the excessive temperature increase.

The CWSI and GI made it possible to differentiate the EUs at FC and those subjected to a water deficit because of the fact that they have a relationship with the thermoregulation affected by variations in transpiration and *gs*, which, in turn, is associated with the water status of the plant. Bellvert *et al.* (2016), Egea *et al.* (2017) and Santesteban *et al.* (2017) found a relationship between thermal indices and *gs*. Figure 13 shows the average value of the thermal indices between d 5 and 20 for each treatment. For d 19, the moisture was restored to FC for T2.

The maximum values of the CWSI index were associated with the treatments with a water deficit, which is related to the tendency of leaf temperature to approach the reference temperature, inhibiting the transpiration process. On the other hand, the maximum values associated with the GI were related to the EUs at FC. This corresponds, according to Equation 4, to the tendency of leaf temperature to approach the reference temperature, favoring the process of perspiration. The CWSI went from a mean value of 0.9 for d 18 to 0.45 for d 20, after resetting the moisture of the EUs of T2 to FC. Likewise, the GI went from an average value of 0.1 to 1.2, showing that restoring irrigation in the EUs of T2 favored stomatal conductance and decreased water stress in the plants.

Conclusions

The CWSI, GI and *D*max variables showed significant differences between T1 and T2-T3. The thermal indices offered a greater capacity for statistical discrimination between the treatments.

The CWSI and GI were successful indicators of the effect on transpiration caused by the water status of the *He* species. The thermal response in the leaf tissue, when faced with variations in the water status was not uniform for the *He* species. The imaging technique was, therefore, a valuable tool because it provided sufficient spatial resolution to evaluate the response of the plants.

The spectral measurements in the range 350-800 nm did not allow for evaluation of the water status of the *He* species directly. Secondary effects, such as chlorophyll content or changes in the mesophyll structure, may be associated with variations in this range. The NDVI and NDVI705 did not present significant differences between the treatments; however, the maximum value of the first derivative of the reflectance in the 700-750 nm range showed significant differences between the treatment at FC and the treatments under a water deficit.

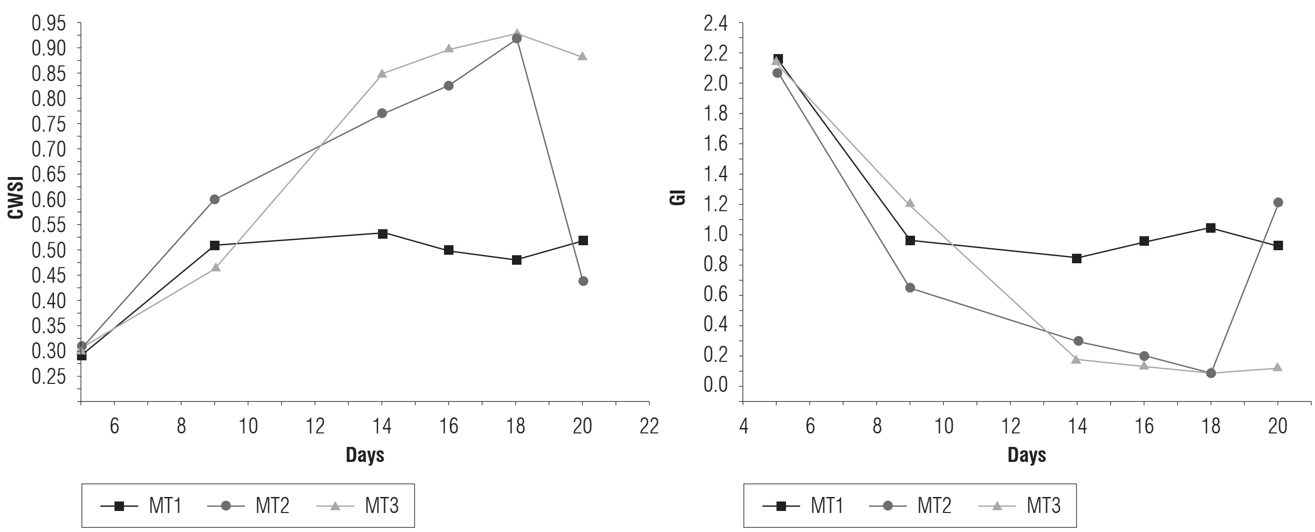

FIGURE 13. Average value of CWSI and GI for treatments T1, T2 and T3.

The thermal imaging technique allowed us to estimate the variations in the transpiration capacity of the *He* species in the face of variations in its water status in a non-invasive way, and, for future studies, it is suggested that an automated thermography processing system be implemented for the calculation of thermal indices in real time. This technique would thereby be strengthened compared to conventional measurements.

Important fieldwork would look at the role of thermal regulation that the evapotranspiration process plays on the receiving surfaces of direct solar radiation (i.e., leaves) and how thermal equilibrium is a necessary condition for good physiological performance, which, in turn, is related to the water state or the adequate presence of water in the substrate.

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Effect of sowing density on yield and profitability of a hybrid corn under tropical conditions

Efecto de la densidad de siembra en el rendimiento y rentabilidad de un híbrido de maíz en condiciones tropicales

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ABSTRACT

A high sowing density in maize is a widely used management practice for increasing crop yield; this method increases intraspecific competition for solar radiation, nutrients and water, so yield per plant is reduced, but a greater number of plants is harvested. However, different corn hybrids present a differential behavior because some are tolerant and some are susceptible to this condition, as determined by their plasticity in adjusting their morphology and phenology. The aim of this study was to identify the optimum sowing density, in technical and economic terms, of a new hybrid corn named 30K73 YG RRFlex since no information is available for tropical conditions. This study was carried out in the province of Tolima, municipality of Valle de San Juan, Colombia, using a completely randomized block design in divided plots; five sowing densities determined by six spatial arrangements, two distances between rows (0.7 and 0.8 m) and three numbers of plants per linear meter (7, 8 and 9) were assessed. The treatments did not generate a nitrogen deficiency in the plants, and the evaluated hybrid developed morphological adjustments at the leaf level in order to maintain constant solar radiation interception. For yield, there were no significant variations, so the yield was similar for all of the evaluated treatments. The best treatment was 87,500 plants ha^{-1}, with a yield of 9,916.66 ± 1,078 kg ha^{-1} and a profitability of 58%.

Key words: seeding density, nutritional status, crop yield, growth.

RESUMEN

El uso de altas densidades poblacionales en cultivos de maíz se considera una práctica de manejo muy usada para incrementar el rendimiento del cultivo; este método de siembra aumenta la competencia intraespecífica por radiación solar, nutrientes y agua, por lo que el rendimiento por planta se ve reducido, pero se compensa por el mayor número de plantas cosechadas. Sin embargo, los diferentes híbridos de maíz tienen un comportamiento diferencial pues algunos son tolerantes o susceptibles a esta condición. Esto es determinado por la plasticidad del material para ajustar su morfología y fenología. El objetivo de este trabajo fue identificar la densidad poblacional óptima en términos técnicos y económicos, para un nuevo hibrido de maíz 30K73 YG RRFlex, del que no se conocía una recomendación a nivel de trópico. Este trabajo fue realizado en el departamento del Tolima, municipio del Valle de San Juan, Colombia, usando un diseño en bloques completos al azar en parcelas divididas; allí se evaluaron cinco densidades poblacionales determinadas por seis arreglos espaciales dados por dos distancias entre surcos (0.7 m y 0.8 m) y tres números de plantas por metro lineal (7, 8 y 9). Allí se encontró que los tratamientos no generaron deficiencia de nitrógeno en las plantas. Además este hibrido desarrolló un ajuste morfológico a nivel foliar para mantener constante la intercepción de radiación solar. En cuanto a rendimiento se encontró que no se presentaron variaciones significativas, por lo que el rendimiento fue similar para los tratamientos evaluados, de tal forma que el mejor tratamiento fue de 87,500 plantas ha^{-1} con un rendimiento de 9,916.66 ± 1,078 kg ha^{-1} y una rentabilidad del 58%.

Palabras clave: densidades poblacionales, estado nutricional, rendimiento de cultivo, crecimiento.

Introduction

Maize (*Zea mays* L.) cultivation is essential for humanity since it is an important source of carbohydrates, which are necessary for obtaining metabolic energy (Morales-Ruiz *et al.*, 2016). Moreover, considering the increased world population in the future, it is necessary to increase corn yield in order to satisfy carbohydrate demands. In the absence of biotic or abiotic stress, solar radiation capture is the determining factor for plant performance (Cox and Cherney, 2001). However, it increases through agronomical practices such as genetic breeding, sowing dates, fertilization and sowing densities (Duvick, 2005; Morales-Ruiz *et al.*, 2016). The use of high sowing densities has been a

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widely studied practice, with constant evolution over time (Tollenaar, 1992). Thus, the implementation of an adequate sowing density can increase the interception of solar radiation and water and nutrient uptake by crops (Duan, 2005).

A high sowing density leads to increased intraspecific competition for light, generating stress as a result of low radiation and limiting photosynthesis; in addition, plant growth is altered by avoiding a response to the shading produced by stalk elongation and petiole and apical dominance. Nevertheless, corn plants respond by adjusting leaf morphology, with narrower and smaller leaves with a more acute insertion angle (Gou *et al*., 2017), resulting in changes in the leaf area index (LAI).

Solar radiation captured by canopies and efficiency in carbohydrate conversion determine dry matter accumulation (Boomsma *et al*., 2009). The solar radiation captured by canopies depends on the fraction of the solar radiation intercepted per day, and this is defined by the LAI and the light extinction coefficient (k) (Shi *et al*., 2016). Therefore, reaching an optimum LAI in the shortest possible time is important for increasing dry matter production (Morales *et al*., 2016). A reduction of 20% in stalk thickness has been reported with the use of high sowing densities, so plants become more susceptible to lodging. In addition, plants change the yield components and morphological characteristics of the cob (Testa *et al*., 2016). Under this condition, yield per plant is reduced, but a higher number of harvested plants compensates for this reduction (Hashemi *et al*., 2005). Furthermore, maximum yield per unit area can be obtained with a density of 100,000 plants ha^{-1} (Huseyin *et al*., 2003).

Nitrogen is an essential macroelement that limits plant performance (Marschner, 2012). It is closely related to photosynthesis since it is an essential part of the Rubisco enzyme and chlorophyll (Imai *et al*., 2008; Lee *et al*., 2011). Nitrogen availability is a yield limiting factor when high sowing densities are used since crops, because of competition, present stress to low contents of this element (Al-Naggar *et al*., 2015). Therefore, it is necessary to increase nitrogen fertilization; however, corn producers cannot perform this practice because of resource limitations, so methodologies that do not generate a nitrogen deficiency stress should be identified (Bänziger *et al*., 1999).

Considering the plant genetic materials of this species, there are hybrids that do not tolerate high sowing densities because of significant yield reductions per unit area. On the other hand, there are hybrids that tolerate this condition because they develop morphological and phenological adjustments (Duvick *et al*., 2004). Therefore, it is important to establish a sowing density for hybrids and for varieties in each agroecological zone in order to reach maximum yield and, therefore, greater profitability. Currently, in Colombia, the genotype that shows this type of behavior is the Impacto® hybrid, which reaches maximum yield with a sowing density of 112,500 plants ha^{-1} (Quevedo *et al*., 2015).

Bearing in mind that the behavior of hybrid 30K73 YG RRFlex under different sowing densities is not known, the aim of this study was to evaluate the effect of different sowing densities on the yield and the profitability of this hybrid and to identify its optimal sowing density.

Materials and methods

This research was carried out between the months of October and March, 2013 in the municipality of Valle de San Juan, province of Tolima, Colombia. The average temperature was 26°C, with 80% relative humidity and average precipitation of 1,552 mm per year. This study site was selected because about 22% of the genetically modified corn is planted in this region (Agronet, 2018). The location coordinates were 4°12’42.8” N and 75°9’38.1” W, at an altitude of 650 m a.s.l. The soil where the experiment was established had a fluvial-volcanic origin and sandy clay loam texture.

The experiment was established in a randomized complete block design in divided plots with four replicates. The main plots had distances between the rows of 0.7 m and 0.8 m, and the subplots were number of plants established per linear meter (7, 8 and 9). This combination generated six treatments that corresponded to the sowing densities that are shown in Table 1.

TABLE 1. Treatments as a function of sowing densities.

Row distance (m)	Number of plants	Sowing density (plants ha^{-1})
0.7	7	100,000
0.7	8	114,286
0.7	9	128,571
0.8	7	87,500
0.8	8	100,000
0.8	9	112,500

Each subplot was comprised of six rows that were 10 m in length, where two plants per site were manually sown. When the plants reached the V3 stage, thinning was carried out, leaving one plant per site. Agronomic management was carried out according to local production practices, and all of the experiment units were subjected to the same agronomic and edaphoclimatic management conditions, so the observed variations were attributed to the population density treatments. The fertilization of the crop was carried out based on soil analysis and crop extraction, which was 120 kg of N ha^{-1}, 80 kg of P_2O_5 ha^{-1} and 120 kg of K_2O ha^{-1}.

To achieve the aim of this study, the following variables were evaluated within the four central rows:

Relative chlorophyll content

The relative chlorophyll content (RCC) in the SPAD units was estimated in the fifth youngest leaf during the vegetative phase (V8) and the beginning of the reproductive phase (VT); The RCC was evaluated in the basal, middle and distal zone of each leaf in four plants per subplot using a portable chlorophyll meter (SPAD 502, Konica-Minolta, Japan).

Plant and cob height and stalk diameter

These variables were evaluated in all plants that were in their R1 stage and throughout four linear meters. The plant height was registered as the length in meters from the base of the stalks to the apex of the cobs. Moreover, the cob height was measured from the base of the stalks to the insertion of the lowest cobs. The stalk diameter was calculated in node one with a digital calibrator and, with this measurement, the stalk area was calculated (Eq. 1) following the methodology described by Testa *et al.* (2016).

$$Stalk\ area = \left(\frac{\left(\frac{D}{2} \times \frac{d}{2} \right)}{100} \right) \times \pi \qquad (1)$$

where D refers to the maximum diameter and d to the minimum diameter.

Solar radiation intercepted per day and light extinction coefficient

Four measurements were taken per subplot when the plants were in the VT stage. The solar radiation intercepted (RI) measurement was carried out in the hours of maximum solar radiation (11:00 to 13:00 h) using a linear ceptometer (AccuPAR Linear Ceptometer, Decagon Devices, Pullman, WA, USA) where the incident radiation in the upper part (IRUP) and in the lower part (IRLP) of the canopy was assessed (Williams, 2012). With this data, the RI was calculated using the formula described in Equation 2 (Shi *et al.*, 2016). Moreover, with these measurements, the light extinction coefficient (k) was also estimated using Equation 3 (Flénet *et al.*, 1996).

$$RI = 1 - \left(\frac{IRLP}{IRUP} \right) \qquad (2)$$

$$k = \frac{-\ln\left(\frac{IRLP}{IRUP} \right)}{LAI} \qquad (3)$$

Performance components and morphological characteristics of the cob

To estimate the prolificacy or the number of cobs per plant, four lineal meters were assessed in each subplot, and the number of cobs was counted. For the other obtained data, the harvest of the two central rows was carried out when the plants reached physiological maturity (R6). From the harvested cobs, 20 were randomly selected, and the number of rows per cob, number of grains per row, seed index, cob height and diameter, percentage of grains and number of grains per cob were established. Subsequently, all of the harvested cobs were manually shelled, and the result of this process was weighed and adjusted to 12% grain moisture.

Profitability

To calculate profitability (Pr), Equation 4, as published by Quevedo *et al.* (2015), was used. For this, a production cost (PC) structure was made for each treatment, where the only variation source was seed cost. In addition, the grain sale price (SP) was calculated according to the price stated by the national agricultural exchange market of Colombia. Letter Y represents yield.

$$Pr = \left(\frac{(Y \times SP) - PC}{Y \times SP} \right) \times 1 \qquad (4)$$

Statistical analysis

A univariate analysis of variance with mean comparison Tukey test ($\alpha = 0.05$) and multivariate analysis of variance with mean comparison Hotelling test ($\alpha = 0.05$) were performed. For these analyses, a preliminary analysis of the assumptions was carried out. The software used for the statistical analyses was R version 3.4.1 (R Core Team, 2017) through RStudio version 1.0.136 (RStudio, USA).

Results and discussion

Relative chlorophyll content

Table 2 shows that the factors of the experiment and their interaction did not generate statistically significant variations for the RCC.

TABLE 2. Summary of the analysis of variance for the RCC evaluated in V8 and VT plants under different sowing density treatments.

Factor	RCC in V8	RCC in VT
Row distance (RD)	ns	ns
Number of plants (NP)	ns	ns
RD x NP	ns	ns

ns: non-statistical significant differences.

Despite not showing statistical differences, Figure 1 shows that the plants in V8 decreased their RCC as the sowing density increased; however, in VT, this trend was not clear despite the finding that the SPAD value was the highest (50) with the lowest sowing density (87,500 plants ha^{-1}).

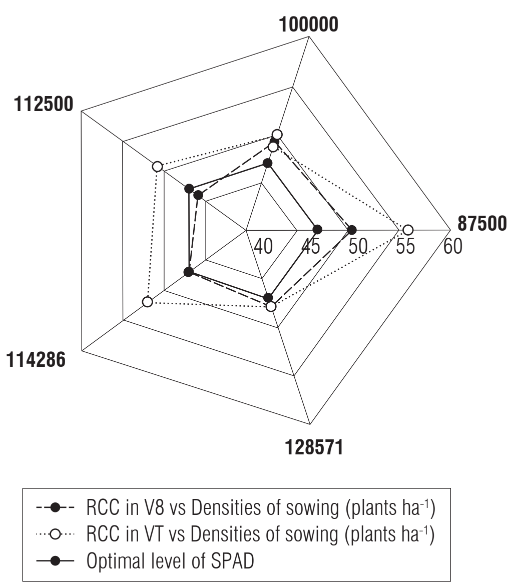

FIGURE 1. RCC in two phenological stages as a function of the effect of six sowing density treatments in corn.

Plant and cob height and stalk diameter

Table 3 shows the effect of each factor on these variables. The interaction of row distance x number of plants was observed. The sowing density did not affect any of the evaluated growth parameters at the statistical level; however, significant effects of individual factors were found. Figure 2 shows that the number of plants generated significant differences in the stalk diameter with an inverse relationship (i.e. increasing the number of plants generated a reduction in stalk diameter). For plant and cob height, the distance between the rows significantly affected this variable. In Figures 3A and 3B, it was observed that the plants and cobs developed a higher height when they were established with a distance between rows of 0.8 m. Nonetheless, Figure 3C shows the ratio observed between the plant and cob height, which established the relative position of the cobs inside the canopy. Additionally, it was observed that the cobs were located in a similar position with both row distances.

TABLE 3. Summary of the analysis of variance for stalk area, plant height and cob height.

Factor	Stalk area	Plant height	Cob height
Row distance (RD)	ns	**	*
Number of plants (NP)	*	ns	ns
RD x NP	ns	ns	ns

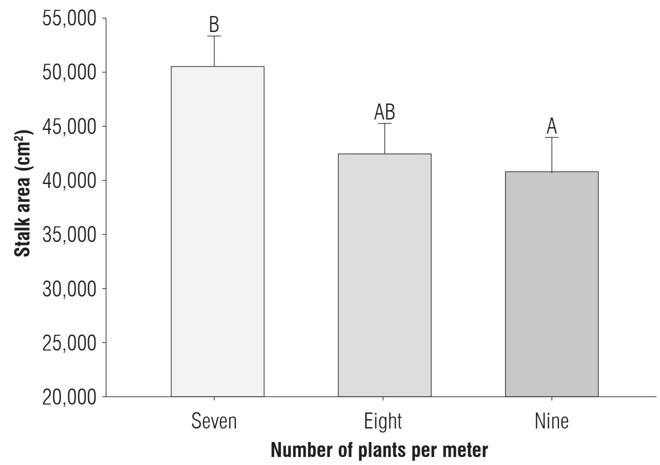

FIGURE 2. Corn stalk area obtained with three different numbers of plants per linear meter. Lines indicate the standard error, and treatments with different letter are significantly different ($P<0.05$).

Morphological cob characteristics

To determine whether the sowing density treatments affected parameters such as number of rows per cob, number of grains per row, seed index, grain percentage, cob height and diameter, a multivariate analysis of variance was carried out (data not shown). This analysis showed that the treatments did not statistically affect the evaluated variables. However, Table 4 shows that the seed index, number of grains per cob and grain percentage showed high variability among the row distances. Therefore, a multivariate analysis of variance was carried out only for these variables. According to the analysis observed in Table 5, there were statistical differences between the two row distances, with evidence that the plants established with a distance of 0.8 m between rows had a greater number of grains per cob as a result of higher grain filling. This was corroborated by a greater grain percentage, plus the fact that grains were heavier.

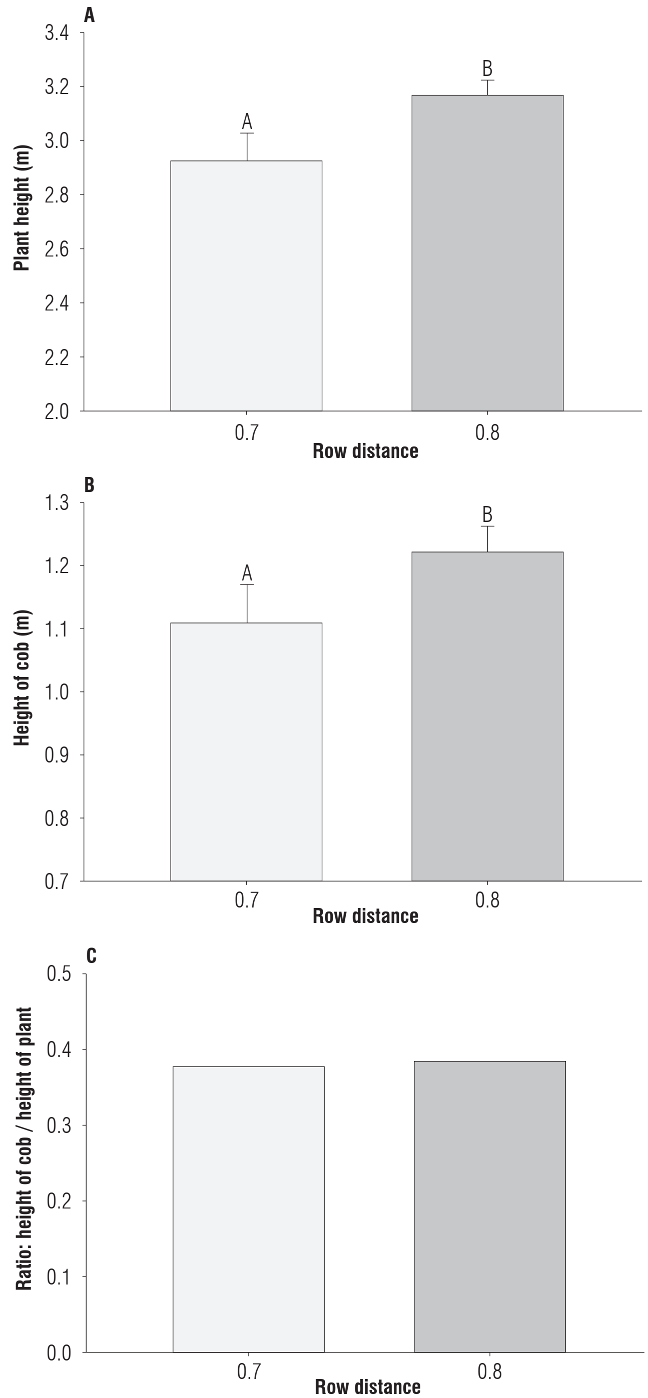

FIGURE 3. Influence of distance between rows on A) Plant height, B) Cob height, C) Cob height/plant height ratio. Lines indicate the standard error, treatments with different letter are significantly different ($P<0.05$).

Leaf area index, light extinction coefficient and intercepted radiation fraction

The results were analyzed with a multivariate analysis of variance, showing that the LAI, *k* and RI were equal at the significant level considered for the effect of the row distance, number of plants and their interaction. Table 6 shows the values of each of these parameters; moreover, it was observed that the LAI did not have a defined trend, ranging between 5-6, as seen with the *k* and RI, which were between 0.15-0.2 and 0.59-0.63, respectively. This suggested that the light distribution inside the canopy was very good; however, the LAI developed by the plants did not capture large amounts of solar radiation.

TABLE 5. Yield components of corn plants established with two different distances between rows.

Row distance (m)	Seed index (g)	No. of grains per cob	Grain percentage (%)	Hotelling grouping
0.7	22.05	390.12	80.65	A
0.8	26.24	398.7	83.2	B

Treatments with different letter are significantly different ($P<0.05$).

TABLE 6. Characteristics of solar radiation interception by corn plants in the VT stage established with different sowing densities.

Row distance (m)	No. plants	Sowing density (plants ha^{-1})	LAI	*k*	RI	Hotelling grouping
0.8	7	87,500	5.78	0.16	0.6	A
0.8	8	100,000	5.65	0.17	0.6	A
0.8	9	112,500	6.17	0.15	0.59	A
0.7	7	100,000	5.25	0.19	0.62	A
0.7	8	114,286	5.8	0.17	0.63	A
0.7	9	128,571	4.97	0.21	0.63	A

Treatments with different letter are significantly different ($P<0.05$).

Yield

Yield is the final expression of plant growth and development regarding yield components. The row distance x number of plants interaction was significant for yield. Table 7 shows that the highest yield was 10,469 ± 765 kg ha^{-1}, obtained with 128,571 plants ha^{-1}; however, this was only

TABLE 4. Morphological characteristics of cobs found in plants sown with two distances between rows.

Row distance (m)	Seed index (g)	Cob diameter (mm)	Cob height	Number of rows	No. of grains per row	No. of grains per cob	Grain percentage (%)	Hotelling grouping
0.7	22.05	36.17	138.4	12.46	31.29	390.12	80.65	A
0.8	26.24	34.94	147.2	12.5	31.9	398.7	83.2	A

Treatments with different letter are significantly different ($P<0.05$).

TABLE 7. Crop yield per plant obtained with different corn sowing densities.

Row distance (m)	Number of plants	Sowing density (plants ha^{-1})	Prolificacy	Yield (kg ha^{-1})	Yield per plant (g)
0.8	7	87,500	1.18 ± 0.1 A	9,914.66 ± 1078 AB	113.31 ± 12.33 B
0.7	7	100,000	1 ± 0 A	7,548.43 ± 567 A	75.48 ± 5.67 A
0.8	8	100,000	1 ± 0 A	8,285.38 ± 230 AB	82.85 ± 2.31 A
0.8	9	112,500	1 ± 0 A	10,082.91 ± 725 AB	89.63 ± 5.96 AB
0.7	8	114,286	1 ± 0 A	10,754.68 ± 342 B	94.1 ± 3.83 AB
0.7	9	128,571	1 ± 0 A	10,469 ± 765 B	81.43 ± 6.45 AB

Treatments with different letter are significantly different (P<0.05); the numbers are means ± standard error.

different at the considered significant level from the density of 100,000 plants ha^{-1}, established in an arrangement with a 0.7 m sowing density and a number of plants of 7, which obtained the lowest yield (7,548.43 ± 567 kg ha^{-1}).

For yield per plant, it was observed that the row distance x number of plants interaction was significant, with evidence that the yield per plant was much higher under 87,500 plants ha^{-1} (lowest evaluated density) than at the other densities. The yield per plant did not exceed 95 g; the high yield per plant in this treatment can be explained by the prolificacy of 1.18, about 18% higher than other densities.

The efficiency evaluation of this practice, in economic terms, is presented in Figure 4 and was elaborated with a purely descriptive analysis. In this figure, it can be observed that, for all of the treatments, the profitability exceeded 30%, and the treatment with 87,500 plants ha^{-1} stood out as the most profitable. For cost per plant, it was observed that, as the sowing density increased, the cost decreased, with $0.127 USD for the treatment with 128,572 plants ha^{-1}.

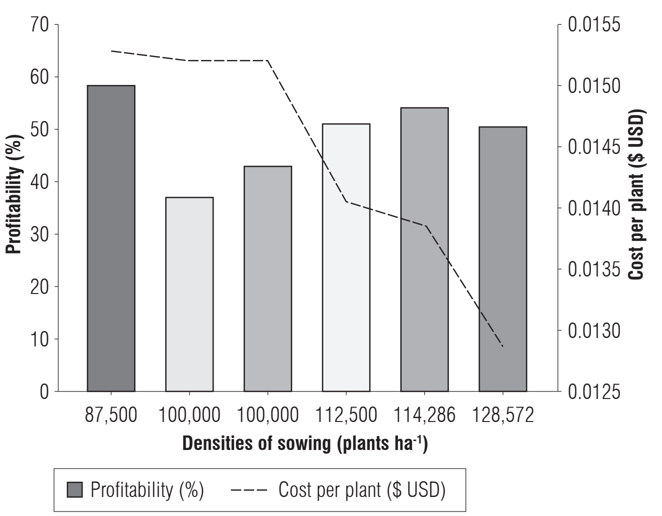

FIGURE 4. Profitability and cost per plant obtained with different treatments as a function of sowing density.

The RCC in monocotyledonous plants has a strong relationship (r^2 = 0.8) with foliar nitrogen contents (Xiong *et al.*, 2015). In corn, the chlorophyllometer is a widely used tool for nitrogen assessment (Yu *et al.*, 2010). In this study, it was observed that, for the hybrid 30K73, the RCC was not significantly affected by the treatments in either of the two evaluated phenological stages. Nonetheless, Figure 1 shows that, in plant stage V8, the treatments with 87,500 plants ha^{-1} and 100,000 plants ha^{-1} exceeded the optimum RCC level (47 SPAD) established for corn (Sainz and Echeverría, 1998); on the other hand, with a higher sowing density, the RCC was very close to this level. Moreover, in the VT stage, the plants in all of the treatments, with the exception of the sowing density 128,571 plants ha^{-1}, exceeded the optimum level, with the sowing density 87,500 plants ha^{-1} obtaining the highest RCC with 55 SPAD. It is noteworthy that, according to these results, the treatments did not produce nitrogen deficiency since none reached 35.3 SPAD (Novoa and Villagrán, 2002). It has been reported that a high sowing density causes stress as a result of a low nitrogen content (Al-Naggar *et al.*, 2015). However, intraspecific competition as caused by high sowing densities did not limit nitrogen availability for the hybrid 30K73 plants. This means that the hybrid can be tolerant to high sowing densities, which agrees with Al-Naggar *et al.* (2015), who stated that there are hybrids that need high amounts of nitrogen when they are established with a sowing density close to 95,200 plants ha^{-1}.

The foliar nitrogen content has a strong relationship with RI and solar radiation conversion to dry matter, i.e. with efficient radiation use, since this element is related to greater foliar expansion and chlorophyll and Rubisco contents (Uhart and Andrade, 1995; Imai *et al.*, 2008; Lee *et al.*, 2011). With the use of high sowing densities, plants can adjust their leaf morphology and adapt to solar radiation competition (Duvick *et al.*, 2004).

The LAI is a variable that is altered with changes in population density; it increases with the sowing density in order to expose more area and increase RI (Morales-Ruiz *et al.*, 2016); however, the development of a higher LAI depends on nitrogen availability (Chen *et al.*, 2017). In the case of the 30K37 hybrid, it was found that it adjusted its leaf morphology and maintained a LAI that did not vary significantly in the various treatments. Likewise, the *k* in this hybrid was very low (0.21-0.15), which means that the light was evenly distributed in the canopy, allowing a large amount of radiation to reach the lower third part of the plants. Therefore, this variable was not affected by the treatments, which contrasts with other studies, where the *k* increased when the sowing density increased (Flénet *et al.*, 1996; Morales-Ruiz *et al.*, 2016). Despite reaching a low *k*, the RI was very low in all of the treatments within a range of 0.59-0.63 and, therefore, there were no statistical differences between the treatments. This could have been because changes in the LAI were not significant, which contrasts with the RI obtained by Morales-Ruiz *et al.* (2016).

The evaluated hybrid was tolerant to a high sowing density since it adjusted its leaf morphology to maintain a constant RI to allow it to grow and develop.

Furthermore, variables, such as plant and cob height and stalk diameter, were related to plant lodging (Tollenaar, 1992; Wang *et al.*, 2016). Studies have reported that the sowing density affects these characteristics (Testa *et al.*, 2016; Gou *et al.*, 2017). The plant height was affected by the row distance; the plants reached their highest height with a row distance of 0.8 m. Usually, the opposite happens with a narrower row distance because the light quality is altered, with a greater amount of far-red light (Rajcan and Swanton, 2001), generating an avoidance response to shading and increasing stalk elongation and, therefore, plant elongation (Gou *et al.*, 2017).

The cob height had a similar behavior to that of the plant height, with the cobs located at a greater height with a row distance of 0.8 m. However, Figure 3C shows that cobs in both treatments were inserted below the center of gravity (0.5) and exactly in the same position (0.39), regardless of height. This indicates that cob insertion in this hybrid is a characteristic that is not affected by sowing density, thereby reducing the possibility of lodging.

The number of plants affected the stalk area in this study; the stalks became thinner as the number of plants increased. This agrees with the findings published by Testa *et al.* (2016), who found that stalk area was reduced by 20% as a result of a high sowing density, meaning this behavior is undesirable since lodging susceptibility increases.

An increase in sowing density is considered a stress factor for plants since it affects yield, which is reduced per plant. However, crop yield can be compensated for with a higher number of harvested plants (Li *et al.*, 2015). In this research, it was found that the seed index and number of grains per cob were reduced by planting this hybrid with a row distance of 0.7 m as a result of an increase in intraspecific competition for carbon, nutrients and water (Liu *et al.*, 2015; Testa *et al.*, 2016). Furthermore, this behavior explains the low yield per plant (<100 g) in all of the treatments, except for the one with 87,500 plants ha^{-1}, which had the lowest sowing density. However, different authors have reported that this is a normal behavior (i.e. compensation resulting from a higher number of harvested plants). This means that hybrid 30K73 YG RRFlex, used in this study, has plasticity because it adjusts its yield components according to the sowing density to maintain a similar crop yield, which would allow each plant to ensure seed availability to establish future generations.

The sowing density for every hybrid and variety that will be planted in any agroecological zone should be adjusted (Sangoi *et al.*, 2002). In a previous research on the same agroecological zones and treatments, the Impacto hybrid increased its yield in response to an increase in sowing density, with 112,500 plants ha^{-1} as the point of inflection for yield. For this reason, this hybrid has been considered as tolerant to high sowing densities (Quevedo *et al.*, 2015) taking into account the previously discussed results, in which it is evident that hybrid 30k73 has an opposite behavior. Furthermore, it is important that each genetic material that is released to the market is accompanied by specific management recommendations so that the best genetic potential of the cultivar can be achieved.

In addition, the management practices of a crop must be considered from a technical and economic viability point of view (Khush, 2015). From an agronomic point of view, for grain production, it is recommended that this hybrid be established with a sowing density of 87,500 plants ha^{-1} in a spatial arrangement with a row distance of 0.8 m and seven (7) plants, which will achieve better stalk development that avoids plant lodging and generates a profitability of 58%, higher than the other treatments. On the other hand, if this hybrid is used for forage production, it is advisable that it be established with a density of 128,571 plants ha^{-1}, a row distance arrangement of 0.8 m and nine (9) plants since the LAI and grain yield remain constant. This would produce a higher amount of forage per area; however, this hypothesis should be evaluated in another experiment.

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Radish (*Raphanus sativus* L.) morphophysiology under salinity stress and ascorbic acid treatments

Morfofisiología del rábano (*Raphanus sativus* L.) bajo estrés salino y tratamientos con ácido ascórbico

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ABSTRACT

The use of saline or low-quality water in agriculture is an alternative to increasing water demand, especially in arid or semi-arid regions. However, the use of water with high levels of salts causes disturbances in plants, which can lead to their death; thus, alternatives to mitigate these effects are relevant in current agriculture. Currently, antioxidants are used to mitigate the effects of salts in plants, and among them ascorbic acid has been frequently mentioned. Therefore, the aim of this study was to evaluate the effect of irrigation with saline water combined with applications of ascorbic acid on the development and photosynthetic activity of radish (*Raphanus sativus* L.) plants. This experiment was carried out in a greenhouse with a randomized block design, with the treatments distributed in a 5×5 incomplete factorial scheme, composed of five electrical conductivities of the irrigation water (ECw): 0.50, 1.30, 3.25, 5.20 and 6.00 dS m^{-1}, and five ascorbic acid (AA) doses: 0.00, 0.29, 1.00, 1.71, and 2.00 mM. The evaluated variables were: shoot height, leaf number, tuberous root diameter, chlorophyll a, b and total content, chlorophyll a/b ratio, initial fluorescence, maximum fluorescence, variable fluorescence and quantum yield of photosystem II. The saline water influenced the analyzed variables in the radish crop regardless of the ascorbic acid application. The ascorbic acid was not efficient in attenuating the deleterious effect of salinity in the irrigation water on the development and fluorescence of the radish. However, it was observed that the concentration of 1.00 mM of ascorbic acid promoted an increase in chlorophyll a, b and total in the salt-stressed radish plants.

Key words: abiotic stress, growth, photosynthesis, vegetable, crop.

RESUMEN

El uso de aguas salinas en la agricultura surge como alternativa al aumento en la demanda de agua, principalmente en regiones áridas o semiáridas. Sin embargo, el uso de agua con alto contenido de sales causa disturbios en las plantas pudiendo llevar hasta la muerte de las mismas. Por esta razón, se buscan alternativas para atenuar tales efectos. Actualmente, los antioxidantes se utilizan para mitigar los efectos de las sales en las plantas; entre estos antioxidantes s el ácido ascórbico ha sido frecuentemente mencionado. Basado en lo expuesto, este trabajo tuvo por objetivo evaluar el efecto del riego con aguas salinas combinado con la aplicación de ácido ascórbico, sobre el desarrollo y actividades fotosintéticas de plantas de rábano (*Raphanus sativus* L.). El experimento fue desarrollado en ambiente protegido en bloques al azar con esquema factorial incompleto 5×5, con cinco conductividades eléctricas del agua de riego (ECw: 0.50, 1.30, 3.25, 5.20 y 6.00 dS m^{-1}) y cinco dosis de ácido ascórbico (AA: 0.00, 0.29, 1.00, 1.71 y 2.00 mM). Las características evaluadas fueron altura de plantas, número de hojas, diámetro de la raíz tuberosa, índice de clorofila a, b, y total, relación de clorofila a/b, fluorescencia inicial, fluorescencia máxima, fluorescencia variable y rendimiento cuántico del fotosistema II. El uso de agua salina influenció todas las variables analizadas en el cultivo del rábano independiente de la utilización del ácido ascórbico. El uso del ácido ascórbico no fue eficiente como atenuante de los efectos de la salinidad del agua de riego sobre el desarrollo y la fluorescencia del cultivo del rábano. Sin embargo, se observó que la aplicación del ácido ascórbico en la dosis de hasta 1.00 mM promueve el aumento en los índices de clorofilas a, b y total del rábano sometido al estrés salino.

Palabras clave: estrés abiótico, crecimiento, fotosíntesis, hortaliza.

Introduction

The world's growing population in recent years has been accompanied by increased food demand, and oleraceous crops are important to meeting this growing demand. In addition, oleraceous crops contribute directly to family farming activities (Noda and Noda, 2016).

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The radish (*Raphanus sativus* L.) is an edible root vegetable of the Brassicaceae family, originated in the Mediterranean region, which presents globular swollen tap root with white or reddish skin. The radish is a fast-growing vegetable that can be harvested 25 to 35 d after sowing. The fast harvest cycle makes it particularly suitable for small and medium oleraceous producers, who can cultivate them along with longer cycle crops (Filgueira, 2008).

The radish contains significant amounts of vitamins A, C and B, and minerals such as calcium, phosphorus, magnesium, iron, sodium and potassium, which are essential nutrients to humans (Vidigal *et al.*, 2007). The radish crop, like other oleraceous crops, requires quality water to fully grow.

Irrigated agricultural systems require water availability and quality. The occurrence of low precipitation rates and saline soil conditions in arid and semi-arid regions around the world prevents salts from leaching, causing their accumulation within soil and water. High salt concentration conditions cause nutritional imbalance, reduced transpiration and photosynthesis rates, lower leaf CO_2 availability, and restricted stomatal conductance, which result in morphologically reduced plant biomass (Silva *et al.*, 2013; Silva *et al.*, 2018).

Salinity stress comprises two main components. The osmotic component, in which a high salt concentration in the soil solution reduces water availability to plants, and the ionic component, which is related to the toxicity of the ions released by the salts (Silveira *et al.*, 2010; Prisco *et al.*, 2017). Taking into account the necessity of using low quality water in irrigation systems, several studies have attempted to find ways to make these waters suitable for plant development and productivity (Oliveira *et al.*, 2016; Rodrigues *et al.*, 2017; Veras *et al.*, 2018).

Ascorbic acid is a major antioxidant found in plants and plays an important role in several cellular processes. The foliar application of ascorbic acid stimulates photosynthetic pigment production and plant growth under stress conditions. Several studies have indicated that ascorbic acid plays a fundamental role in protecting plants from multiple environmental stresses, including salinity stress (Maia *et al.*, 2012; Melo *et al.*, 2014).

In the literature, there are several reports on the use of ascorbic acid for attenuating the deleterious effects of saline stress, such as in *Triticum aestivum* (Abbasi and Fakhani, 2015; Siddiqui *et al.*, 2018), Zea mays (Billah *et al.*, 2017), *Linum usitatissimum* (El-Bassiouny and Sadak, 2015), *Citrus aurantium* (Kostopoulou *et al.*, 2015), and *Hordeum vulgare* (Agami, 2014). Based on this, the aim of this study was to evaluate the mitigating effect of ascorbic acid on the morphophysiological characteristics of salt stressed radish (*Raphanus sativus* L.).

Materials and methods

This experiment was carried out from October to November, 2017 in a greenhouse at the Agricultural Sciences Center of the Federal University of Paraiba (UFPB), located in the city of Areia, Paraiba, Brazil. The soil used in the experiment was collected in the 0-20 cm depth from the Plant Science Department land in the Federal University of Paraiba. This soil was classified as Latossoil (EMBRAPA, 2014), and its chemical analysis is shown in Table 1.

The radish variety used was the hybrid Margaret Queen Kobayashi (ISLA®). Five seeds were sown per plastic bag at a depth of 2 cm, and emergence occurred 5 d later. Then, the treatments with the saline water irrigation, according to the predetermined ECw values, were initiated. Ten d after emergence, the plants were thinned to one plant per bag.

The experimental design was a randomized block with the treatments distributed in a 5x5 factorial scheme, generated with the Central Box experiment matrix (Bortoluzzi and Alvarez, 1997). The factorial design was composed of five electrical conductivities of the irrigation water (ECw): 0.50, 1.30, 3.25, 5.20 and 6.00 dS m^{-1}, and five ascorbic acid (AA) doses: 0.00, 0.29, 1.00, 1.71 and 2.00 mM, using four replicates. Plastic bags with a 3.0 dm^3 capacity, containing one plant each, represented the experiment units.

The water with the ECw of 0.50 dS m^{-1} was obtained from the UFPB water supply system. A mixture of NaCl, $CaCl_2.2H_2O$ and $MgCl_2.6H_2O$ at a 7:2:1 ratio was added to the 0.50 dS m^{-1} water to prepare the saline treatment water with the higher ECw values (1.30, 3.25, 5.20 and 6.00 dS m^{-1}) (Medeiros, 1992). A micro processed portable conductivity meter CD-860 (Instrutherm, Brazil) was used to prepare

TABLE 1. Soil analysis report.

pH (H_2O)	OM (%)	P	K^+	Na^+	Ca^{2+}	Mg^{2+}	Al^{3+}	$H^+ + Al^{3+}$	BS	CEC	BSA (%)
		-----mg dm^{-3}-----		---cmol$_c$ dm^{-3}---							
6.20	2.48	24.85	78.42	0.07	3.90	1.90	0.00	2.43	6.07	8.50	71.46

OM - organic matter, BS - base sum, CEC - cation exchange capacity, BSA - base saturation.

the saline waters. Irrigation management was measured using a drainage lysimeter (Alves *et al*., 2017).

The radish leaves were sprayed with ascorbic acid until completely wet 14 d after seedling emergence. The non-ionic surfactant Tween 80® was added to the ascorbic acid solution for a better absorption effect at a concentration of 0.0002%.

At 15 and 30 d after seedling emergence, the following development variables were evaluated: shoot height, measured from base to leaf apex using a graduated ruler in centimeters; leaf number, counting all the leaves from each plant; tuberous root diameter determined at 2 cm of the soil using a digital pachymeter; chlorophyll a, chlorophyll b, total chlorophyll content and chlorophyll a/b ratio. The chlorophyll contents were determined with a portable electronic chlorophyll meter model CFL 1030, ClorofiLOG® (Falker, Brazil), and one reading was performed on each plant in totally expanded leaves.

The initial fluorescence (F_0), maximum fluorescence (F_m), variable fluorescence (F_v) and quantum yield of photosystem II (F_v/F_m) were measured at 28 d after seedling emergence. Chlorophyll fluorescence measurements (F_0, F_m, F_v and F_v/F_m) were obtained using the modulated fluorometer Plant Efficiency Analyser - PEA II® (Hansatech Instruments Co., UK), and the readings were performed between 09:00 and 10:00 in the morning, on young, fully expanded, non-harmed and well lighted leaves. The fluorescence parameters were measured after a period of 30 min of dark adaptation with foliar tweezers (Konrad *et al*., 2005). The obtained data were submitted to analysis of variance and regression using the software R (R Core Team, 2017).

Results and discussion

It was observed that the saline irrigation water had an effect on the development variables assessed at 15 and 30 d after seedling emergence. The shoot height, leaf number and tuberous root diameter were reduced with the increasing ECw values (Fig. 1). Differences between the two assessment periods on the above-mentioned variables were observed once they were development variables, and over a period of 15 d there was an increase in size.

Aerial development is an important characteristic in salt stressed plant evaluation because it provides important information on plant response to stressful conditions (Jamil *et al*., 2007). For the aerial part development variables, shoot height (Fig. 1A) had a higher value than the one predicted within the confidence interval in the electrical conductivity of 5.20 dS m^{-1}, with plants reaching a mean value of 10.5 cm. At the electrical conductivity of 6.00 dS m^{-1}, the radish plants reached an average value of 8 cm. Different results were observed by Putti *et al.* (2016), who registered no significant difference in radish behavior when submitted to 5.00 dS m^{-1} level of salinity.

At 15 DAE, the leaf number (Fig.1B) remained constant at all ECw levels. At 30 DAE, the leaf number decreased as the salinity increased. A similar result was found by Nascimento *et al*. (2015), who registered reduced leaf numbers in pepper (*Capsicum annum* L.) subjected to increasing salinity levels. Reduced leaf growth is a plant defense mechanism under stress conditions, such as water deficit and salinity, and it diminishes transpirational water loss (Taiz *et al*., 2017). These results showed leaf sensitiveness to salinity stress, which made them reduced both in size and number as the salt concentration increased in the irrigation water.

For tuberous root diameter (Fig. 1C), a slight reduction was observed as the concentration of salts in the irrigation water increased at 15 and 30 DAE. The results obtained in this study corroborate those obtained by Bacarin *et al.* (2007), who studied the salinity effect on the growth and development of radish grown in a nutrient solution, and verified a reduction in leaf area and tuberous root diameter in response to the salinity stress.

Figure 2A shows shoot height as a function of the ascorbic acid treatment. The highest values were 7.8 cm at 15 DAE and 10.6 cm at 30 DAE, obtained in the absence of AA and at the dose of 1.71 mM, respectively. The lowest shoot height values were observed at the highest AA dose (2.00 mM), with 7.3 and 8.8 cm at 15 and 30 DAE, respectively.

For leaf number, a constant number of leaves was observed for all AA doses applied at 15 DAE. At 30 DAE, the radish plants treated with 0.29 mM of AA had a slight increase in the leaf number when compared to the control treatment plants (0.00 mM), showing that, as the concentrations of AA increased, a 13% decrease was observed in the leaf number, as compared to the control treatment (Fig. 2B).

Ascorbic acid is closely related to photosynthesis and respiration processes. The AA concentration in plant tissue varies as a function of plant response and different stress conditions, such as intensity and species sensitiveness to stress (Venkatesh *et al*., 2012). Plants that present a low ascorbic acid synthesis capacity are very sensitive to environmental stress conditions, thus their growth and development are affected and, in some situations, an exogenous AA application is necessary in order to supply this antioxidant deficiency (Gao and Zhang, 2008).

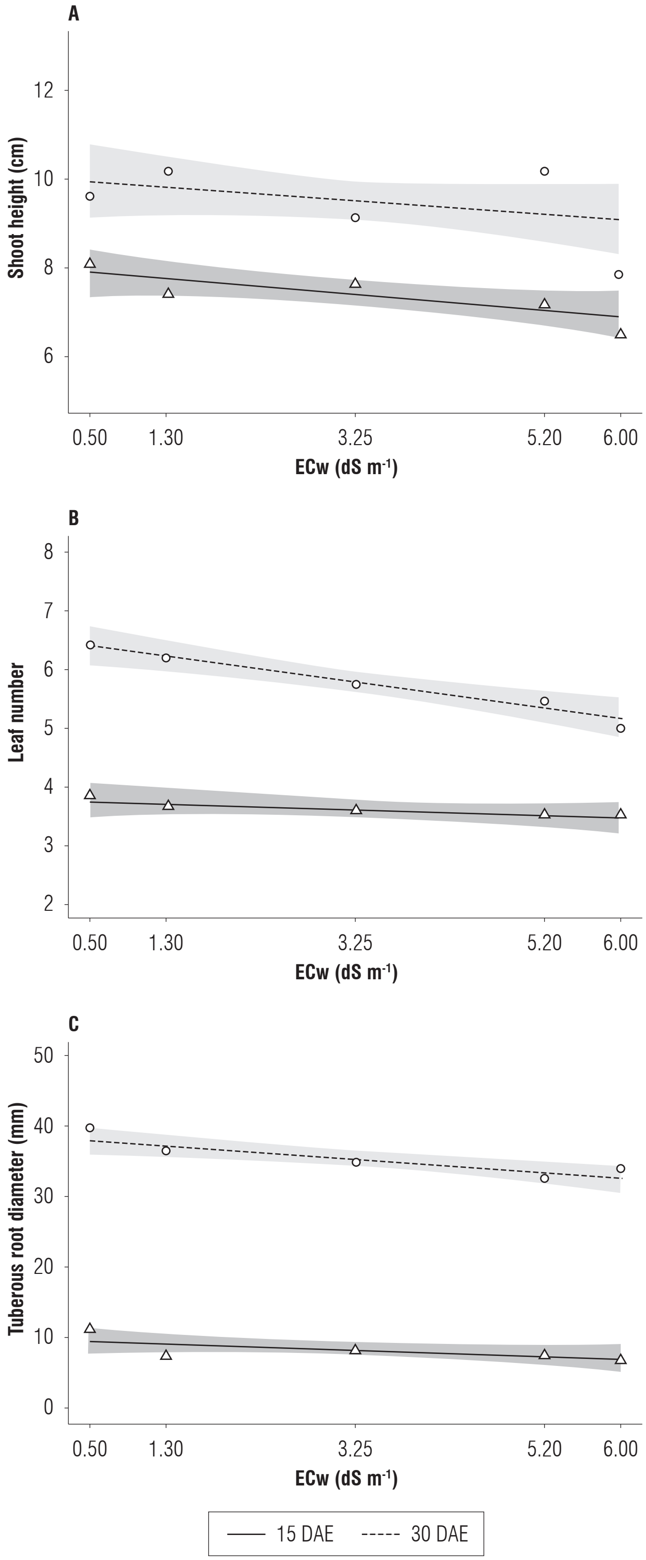

FIGURE 1. Shoot height (A), leaf number (B) and tuberous root diameter (C) of radish (*Raphanus sativus* L.) subjected to different electrical conductivities of the irrigation water (ECw) at 15 and 30 DAE.

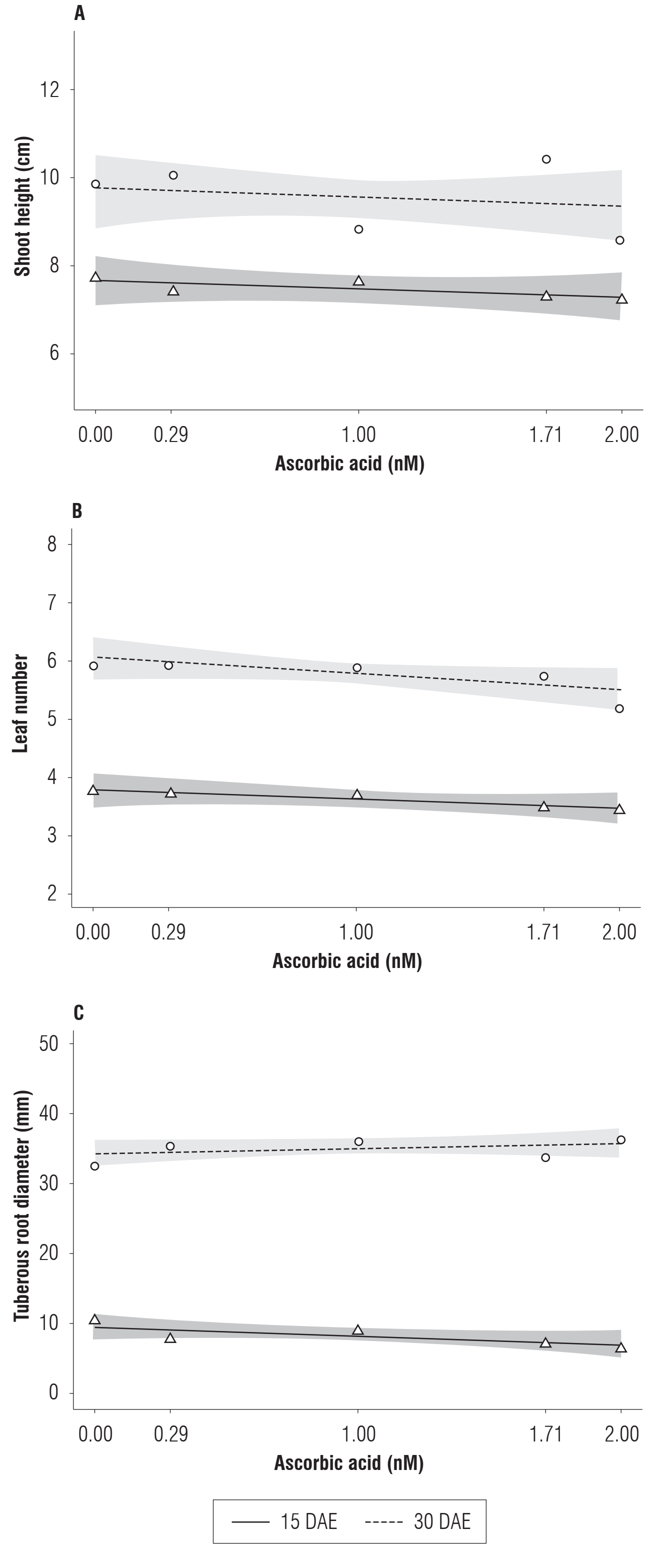

FIGURE 2. Shoot height (A), leaf number (B) and tuberous root diameter (C) of radish (*Raphanus sativus* L.) subjected to different ascorbic acid doses at 15 and 30 DAE.

At 15 DAE, increases in the AA concentrations resulted in a slightly reduced tuberous root diameter. The control plants presented a 0.9 mm tuberous root diameter, whereas the radish plants treated with 2.0 mM of AA presented a 0.7 mm tuberous root diameter. Different results were observed at 30 DAE, when the control plants presented a 34 mm tuberous root diameter, and the plants treated with 2.0 mM of AA reached 38 mm (Fig. 2C).

The foliar a, b, total and a/b chlorophyll indices (FCI) were influenced by the addition of salts in the irrigation water (Fig. 3). In general, the plants subjected to the highest electrical conductivity of the irrigation water (6.0 dS m^{-1}) and evaluated at 30 DAE showed the highest chlorophyll a, b and total values. For the assessment period, no differences were observed for the chlorophyll a or total chlorophyll. However, differences for chlorophyll b in electrical conductivities around 5.2 dS m^{-1} and higher were observed. For the chlorophyll a/b ratio, differences were only registered in the electrical conductivity of 3.25 dS m^{-1}.

No difference was observed in the chlorophyll a content as a function of time; in the first evaluation (15 DAE), the lowest value (24.62) was found in the ECw of 0.50 dS m^{-1}, and the highest value (25.91) was seen in the ECw of 1.30 dS m^{-1}, with a decreasing response from this electrical conductivity. In the second evaluation period (30 DAE), the minimum and maximum values were 23.75 and 27.54 in the ECw of 1.30 dS m^{-1} and 6.00 dS m^{-1}, respectively (Fig. 3A).

The chlorophyll b content (Fig. 3B) presented the highest values of 5.92 at 15 DAE and 7.68 at 30 DAE, both occurring in the ECw of 6.00 dS m^{-1}. The assessment period influenced the chlorophyll b values in relation to the ECw treatment. When comparing the results obtained at 15 and 30 DAE, an increase for all tested ECw was observed,

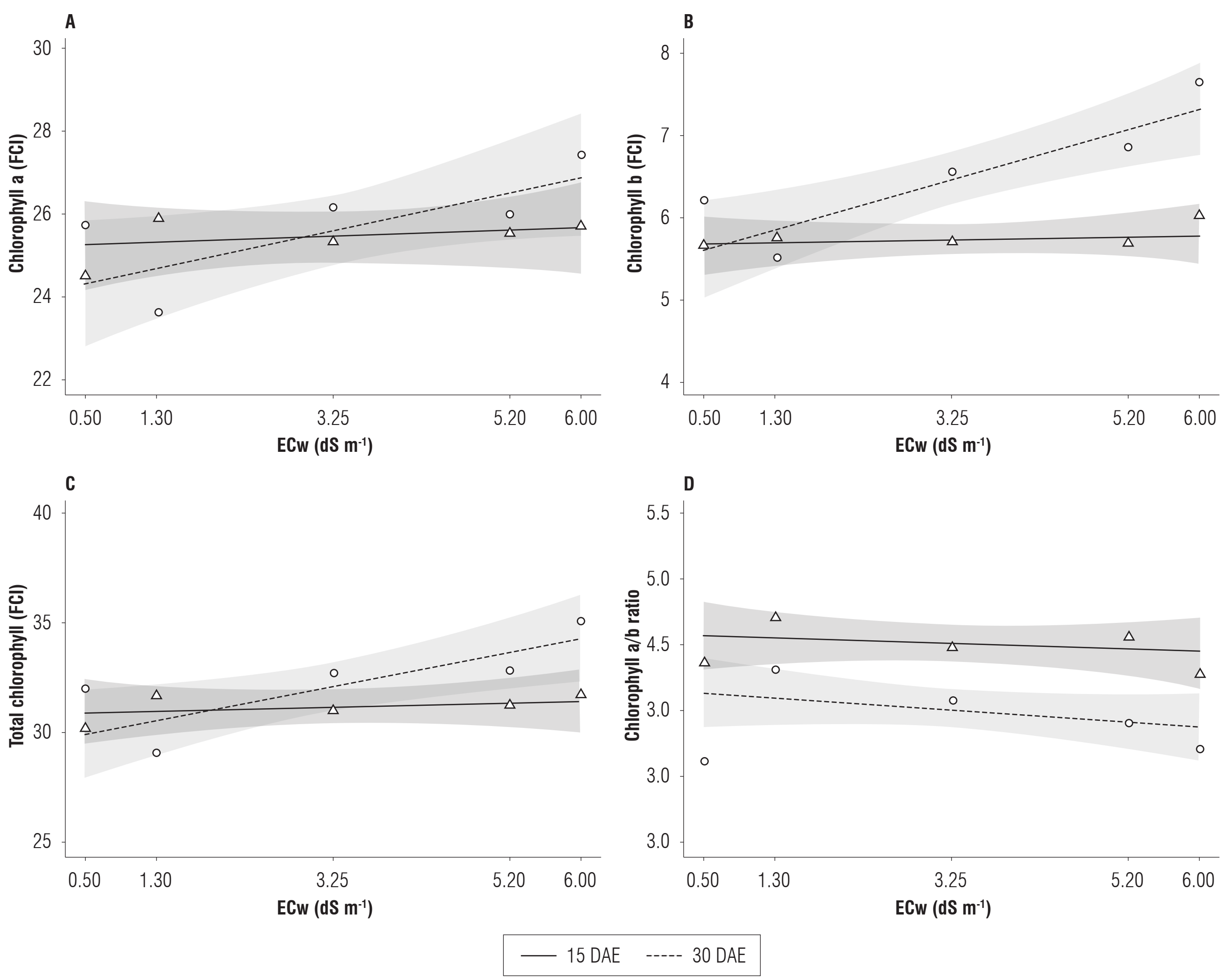

FIGURE 3. Chlorophyll a (A), chlorophyll b (B), total chlorophyll (C) and chlorophyll a/b ratio (D) of radish (*Raphanus sativus* L.) subjected to different electrical conductivities of the irrigation water (ECw) at 15 and 30 DAE.

except for 1.30 dS m^{-1}. These increments were 10.8, 15.1, 20.2 and 32.8% for the ECw of 0.5, 3.25, 5.20 and 6.00 dS m^{-1}, respectively.

Figure 3C shows that the lowest value for the total chlorophyll content at 15 DAE was 30.2, obtained in the ECw of 0.50 dS m^{-1}, while the highest value (32.1) was found in the ECw of 1.30 dS m^{-1}. At 30 DAE, the lowest value was 28.8, obtained in the ECw of 1.30 dS m^{-1}, whereas the highest value of 35.1 was observed in the ECw of 6.00 dS m^{-1}. The chlorophyll a/b ratio presented higher values at 15 DAE, with a maximum and minimum value of 4.7 and 4.25, obtained in the ECw of 1.30 and 6.00 dS m^{-1}, respectively. In the second evaluation (30 DAE), the highest value was 4.28, and the lowest was 3.67, obtained in the ECw of 1.30 and 0.50 dS m^{-1}, respectively (Fig. 3D).

These increases in the plant chlorophyll content when submitted to higher ECw levels are contrary to those found by some authors (Chaparzadeh and Behboud, 2015; Jasim *et al*., 2016; Kasim *et al*., 2016). However, their studies were conducted with higher salinity levels, and they did not clarify the radish variety used. According to Jamil *et al.* (2007), the chlorophyll content is reduced in plants that are sensitive to salinity and increases in tolerant plants.

The content of chlorophyll a, chlorophyll b, total chlorophyll and the chlorophyll a/b ratio were affected by the ascorbic acid treatment. The content of chlorophyll a and total presented no difference in relation to the assessment period. For the chlorophyll b content, there was a difference between 1.0 and 1.71 mM of AA, whereas for the chlorophyll a/b ratio, there was a difference in the 0.29, 1.0 and 1.71 mM of AA (Fig. 4).

At 15 DAE, the maximum value of chlorophyll a was 26.83, obtained when submitted to 0.29 mM of AA. From this AA dose on, the chlorophyll a content decreased and reached

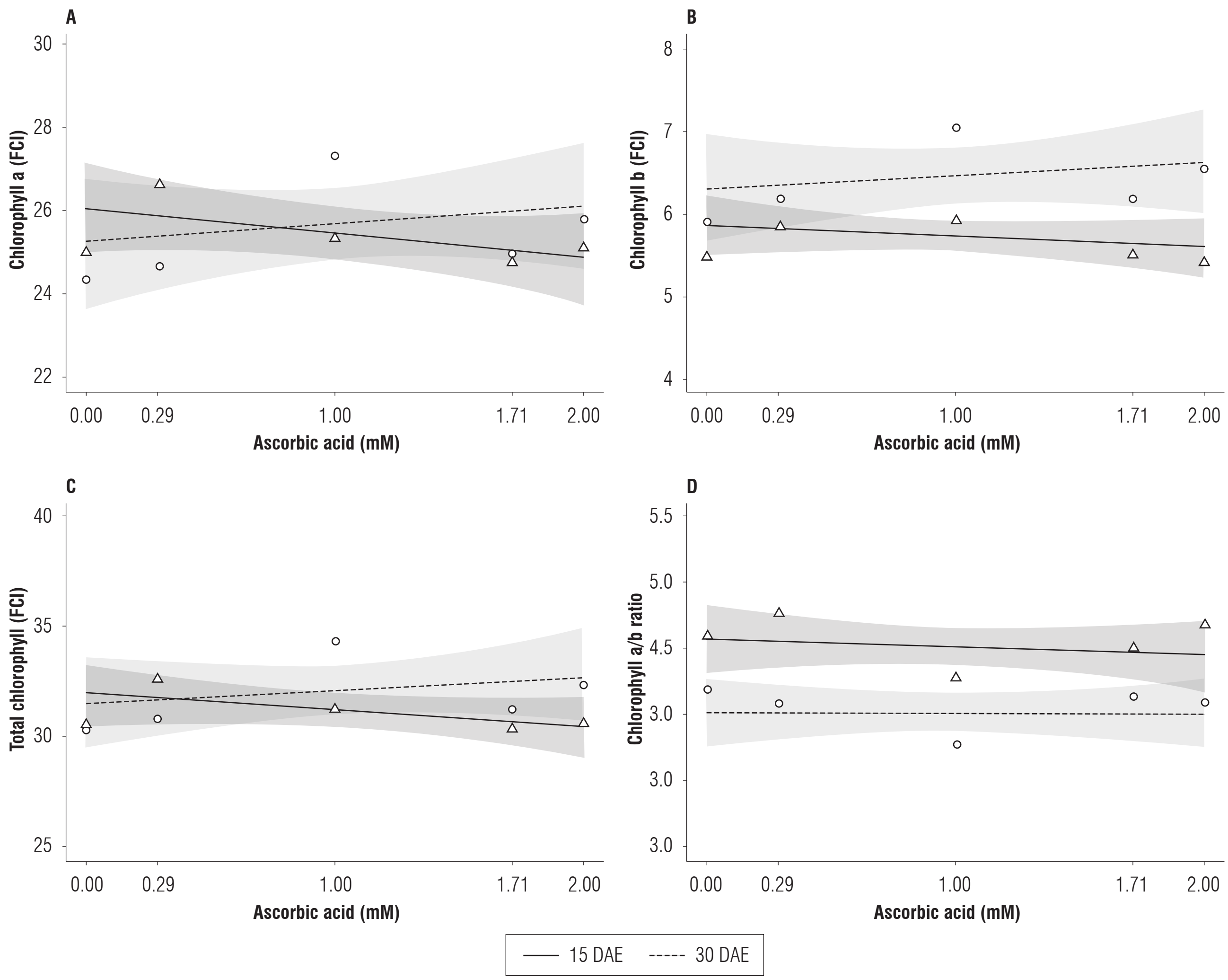

FIGURE 4. Chlorophyll a (A), chlorophyll b (B), total chlorophyll (C) and chlorophyll a/b ratio (D) of radish (*Raphanus sativus* L.) subjected to different ascorbic acid doses at 15 and 30 DAE.

a minimum value of 25.09 at the dose of 1.71 mM. At 30 DAE, the lowest chlorophyll a content value (24.47) was observed in the absence of AA, and the highest (27.62) was obtained with the AA dose of 1.00 mM, with decreasing values from this dose on (Fig. 4A).

Figure 4B shows that the chlorophyll b content was affected during the evaluation periods. At 30 DAE, the chlorophyll b values were higher, with increases in all AA doses in relation to the evaluation performed at 15 DAE, representing increments of 5.6; 11.3; 18.6; 16.3 and 19.2% for the 0.00; 0.29; 1.00; 1.71 and 2.00 mM of AA, respectively.

The total chlorophyll presented little variation in relation to the different doses of AA and the two assessment periods (Fig. 4C). At 15 DAE, the highest value was 32.52, obtained in the AA dose of 0.29 mM, and, from this dose on, the total chlorophyll content started to decrease, with 30.08 being the lowest value at the dose of 1.71 mM. At 30 DAE, the minimum value was 30.07, obtained in the absence of AA with a quadratic effect, and the maximum value was 34.65 with 1.00 mM of AA.

The lowest chlorophyll a/b ratios were observed in the AA dose of 1.00 mM, 4.28 and 3.79 at 15 and 30 DAE, respectively. The highest values for each assessment period were 4.78 at 15 DAE and 4.16 at 30 DAE, obtained with the doses of 0.29 mM and absence of AA, respectively (Fig. 4D). The chlorophyll content increase up to the 1.00 mM dose can be explained by the role of AA in regulating plant biochemical/physiological reactions (Khan *et al.*, 2006). However, higher doses of AA do not provide the same effects.

For initial fluorescence, it was observed that the maximum efficiency (93.6) was obtained under the electrical conductivity of 4.3 dS m^{-1}. The maximum and variable fluorescence presented mean values of 428.25 and 342.68, respectively (Fig. 5A-C). Distinct results were found in

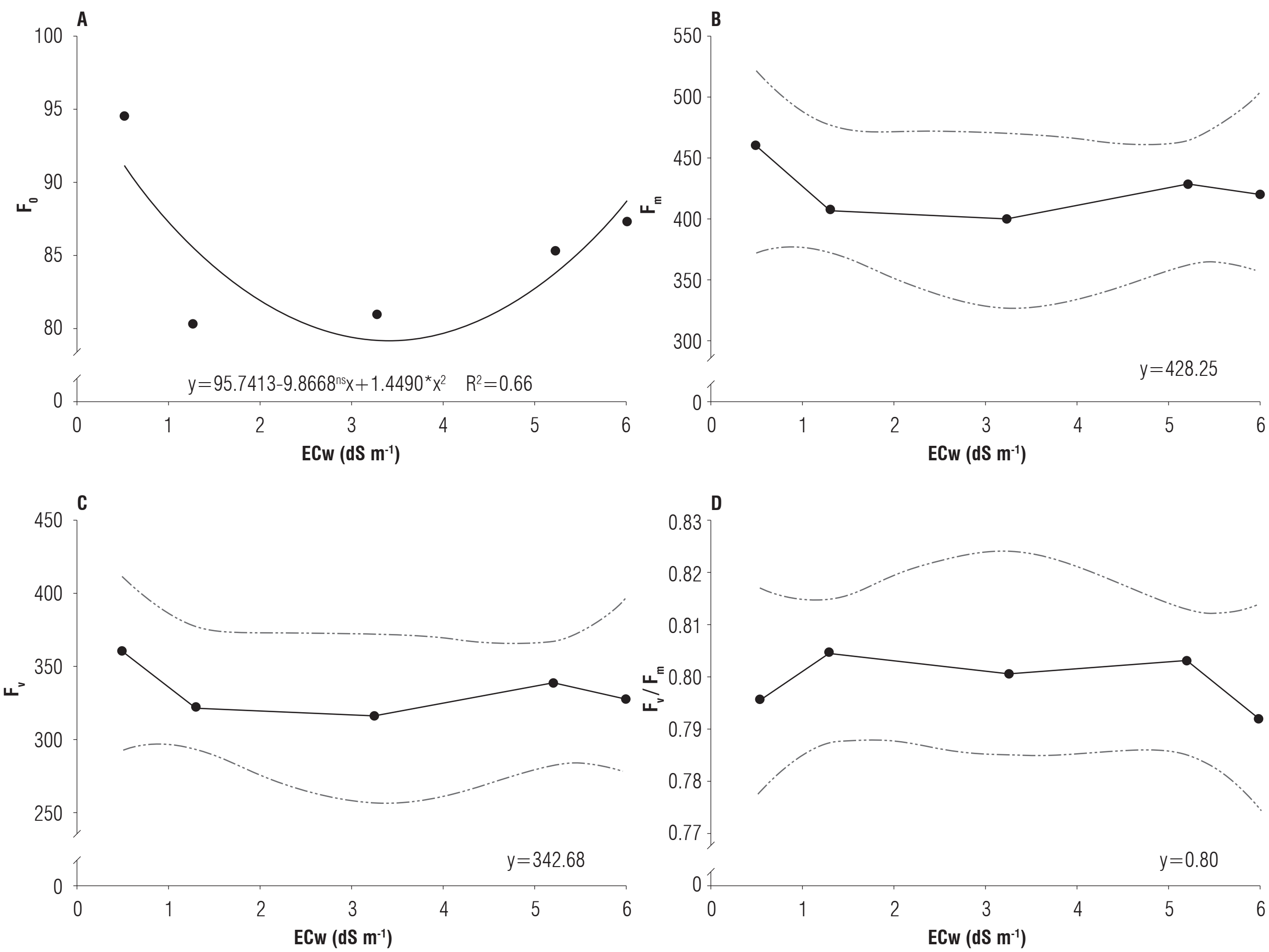

FIGURE 5. A) Initial fluorescence (F_0); B) Maximum fluorescence (F_m); C) Variable fluorescence (F_v); and D) Quantum yield of photosystem II (F_v/F_m) of radish (*Raphanus sativus* L.) subjected to different electrical conductivities of the irrigation water (ECw).

the literature. When studying the NaCl effect, up to the concentration of 220 mM on salt-stressed radish plants, Bacarin *et al.* (2007) found no significant differences in the initial fluorescence as the salinity level increased.

For the quantum yield of photosystem II, the highest value (0.806) was obtained in the ECw of 1.30 dS m^{-1}, while the lowest value was 0.792 in the ECw of 6.00 dS m^{-1} (Fig. 5D). Jamil *et al.* (2007) reported decreases in the quantum yield of photosystem II of radish plants as the salinity level increased.

Conclusions

The saline water influenced the analyzed variables in the radish (*Raphanus sativus* L.) plants regardless of the ascorbic acid application. The ascorbic acid was not efficient in attenuating the deleterious effect of salinity in the irrigation water on the development and fluorescence of the radish plants. However, the concentration of 1.00 mM of ascorbic acid promoted increased chlorophyll a, b and total in the salt-stressed radish plants.

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Evaluation of irrigation in roses in a suitable substrate with three water depths and the percentage of drainage

Evaluación del riego en rosa en sustrato de acuerdo a tres láminas de agua y el porcentaje de drenaje

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ABSTRACT

The municipality of Facatativa, where the research was carried out, is located in the Bogota Plateau (Department of Cundinamarca), an area specialized in the production of flowers that, along with other producing regions of the department, represent around 72% of national production. Establishing the amount of water and the time of application is a key factor that contributes to obtain the quality and production required, according to the requirements in commercial crops, such as rose. The main objective of this study was to evaluate the effect of three irrigation depths on *Rosa* sp. Vendela variety in substrate (80% toasted rice husks and 20% compost), based on evapotranspiration and percentage of drainage, in a greenhouse in the Bogota Plateau. The experiment was conducted in two crop cycles during 2014. The production and quality of the harvest were evaluated with three irrigation depths: Control treatment (T1) with 100% of the crop's evapotranspiration (ETc) and drainage percentage (% D), T2 and T3, equivalent to 80% and 65% of T1, respectively. Irrigation was applied as follows: 15.04, 12.39 and 10.35 m^3 per bed were applied for two cycles in T1, T2 and T3, representing a reduction of 17.6 and 31.2% for T2 and T3, respectively, compared to the control treatment. The production of floral stems, the bud diameter and length did not have significant differences (P<0.05) according to the Duncan test; however, T1 had the highest number of stems. The length and diameter of the stem, the crop coefficient Kc and water use efficiency (WUE) presented significant differences between the treatments. The growth curves showed a continuous increase, which fit the logistic model.

Key words: deficit, evapotranspiration, production, quality, growth curve.

RESUMEN

El municipio de Facatativá, lugar donde se realizó la investigación, se encuentra ubicado en la Sabana de Bogotá (Departamento de Cundinamarca), zona especializada en la producción de flores, y que en conjunto con otras regiones productoras del departamento, representan alrededor del 72% de la producción nacional. Establecer la cantidad de agua y el momento de aplicación es un factor clave que contribuye a obtener la calidad y producción requeridas, conforme con las exigencias en cultivos comerciales como la rosa. El objetivo del presente trabajo fue evaluar el efecto de tres láminas de riego en *Rosa* sp. variedad Vendela en sustrato (80% cascarilla de arroz tostada y 20% compost), con base en la evapotranspiración y porcentaje de drenaje, en invernadero en la Sabana de Bogotá. El experimento se realizó en dos ciclos durante el año 2014. Fue evaluada la producción y calidad de la cosecha, con tres láminas de riego, un tratamiento control (T1) con una lámina del 100% de la evapotranspiración de cultivo (ETc) y el porcentaje de drenaje (% D), un T2 y T3 equivalentes al 80 y 65% de T1, respectivamente. Se aplicaron 15.04, 12.39 y 10.35 m^3 por cama, durante los dos ciclos, en T1, T2 y T3, representando una reducción del 17.6 y 31.2% para T2 y T3, respectivamente, con respecto al tratamiento control. La producción de tallos florares, el diámetro y la longitud del botón no presentó diferencias significativas (P<0.05) de acuerdo con la prueba de Duncan; sin embargo, el T1 tuvo la mayor cantidad de tallos. La longitud y diámetro del tallo, el coeficiente de cultivo Kc y la eficiencia en el uso del agua (WUE) presentaron diferencias significativas entre tratamientos. Las curvas de crecimiento presentaron un incremento continuo, con ajuste al modelo logístico.

Palabras clave: déficit, evapotranspiración, producción, calidad, curva de crecimiento.

Introduction

The water requirements of a crop refer to the amount of water and the timing of its application, with the objective of offsetting the moisture deficit of the soil or substrate and the evaporative demand that occurs during the growing season (Arevalo *et al.*, 2014).

During the early stages of a crop, water is lost mainly due to direct evaporation from the soil; however, as development

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increases and it fully covers the ground, transpiration becomes the most important process (Allen *et al.*, 2006).

There are several factors involved in the process of evapotranspiration: crop characteristics, management, soil or growth medium and atmospheric variables that affect evaporation and transpiration (Allen *et al.*, 2006). The ETc is calculated as the product of the cultivation coefficient (kc) and the evapotranspiration of the reference crop (ETo); with optimum management and adequate water supply, maximum yields are achieved according to climatic conditions. Generally, the correction factor (kc) is required, which depends on the type of crop, variety, age and agronomic management (Allen *et al.*, 2006).

The most appropriate indicator for an irrigation scheduling is measuring the water status of the plant because it best evidences the agronomic response of the crop to the water level that it was submitted to (Vélez *et al.*, 2007). The water status of the plant can be monitored with the leaf water potential or stem potential (Ψt), expressed in Mega Pascals (MPa) and measured with a Scholander pressure chamber (Arévalo *et al.*, 2013).

The cycle of a flower stem is on average 10 to 11 weeks; half of this period is vegetative growth and the other half is reproductive. The growing period is subdivided into bud induction and development of the flower stem, false leaves are closed and most of the time have a reddish color (Rodríguez and Flores, 2006). The reproductive stage starts with the induction of floral primordia (palmiche) coinciding with a color differentiation of the stem and leaves from red to green. The following phenological stages are known as 'rice' (bud diameter around 0.4 cm), and pea (0.5 to 0.7 cm) with fully open leaves and a more rounded bud. Next stages are identified as 'garbanzo' (0.8 to 1.2 cm) with losses of reddish stems and leaves, and 'scratch color' (1.8 to 2.9 cm) the phenology stage when the sepals have begun to spread due to the growth of the bud, further developing the color of the petals. Finally, 'cut' (harvest) indicates when the flowers are ready to be commercialized, but not fully developed (Cáceres *et al.*, 2003).

The growth analysis is based on the development of measurements, such as dry weight, stem length, number of leaves, and number of branches, among others (Arévalo *et al.*, 2013). Furthermore, it can be described from a mathematical point of view through direct measurements, such as total dry mass of the plant, leaf area and time. In addition to derived measurements, there are several growth indexes useful to analyze the plant development rate and state the effect of management strategies on the crop development, such indexes are the relative growth rate (RGR), the crop growth rate (CGR), net assimilation rate (NAR), leaf area duration (LAD), relative leaf area (RLA), leaf area index (LAI), and specific leaf area (SLA) (Melgarejo *et al.*, 2010). The interpretation of the logistic model based on the growth of a parameter that has great commercial value, such as the length of the flower stem, represents a useful tool for programming and agronomic management of commercial rose crops, determining the right time for harvest, according to commercial requirements (Rodríguez and Flores, 2006).

The main objective of this research was to assess the effect of different irrigation levels calculated with the crop evapotranspiration on production and crop quality for the rose (*Rosa* sp.) Vendela variety planted in substrate. Based on the work carried out, it is recommended to evaluate the effect of applying an 80% of the water needs and a correction factor of the drainage percentage between 30 and 40%, seeking to preserve the quality of the flower stems.

Materials and methods

The experiment was conducted between August and November 2014 (week 35 to 48), on the Santa Maria farm (xv property), in a plastic greenhouse with an area of 5,163 m^2, overhead and lateral ventilation with mechanical opening and closing regulated by electric gear motors, structure in galvanized iron pipe, in 5-gauge white polyethylene. During the test weeks, the minimum and maximum average temperatures were recorded as 17.97°C and 20.63°C, respectively. The study area was located in the municipality of Facatativa (Cundinamarca, Colombia), with coordinates 4º46'50.3" N, 74°19'18.0" W and 2,586 m a.s.l. According to the Caldas-Lang model, the area is cold and dry, with an average annual temperature of 13.6°C, and maximum and minimum temperatures of 22°C and 6°C, respectively; the winds usually blow in the south-east direction, with average speeds of 2.6 m s^{-1}; relative humidity of 76%, annual rainfall below 650 mm and actual evapotranspiration between 630 and 640 mm yr^{-1} (IGAC, 2012).

The studied crop was *Rosa* sp. Vendela variety, planted in 2013 in a 32.5 m long and 0.8 m wide beds grown in substrate, composed of 80% burnt rice husk and 20% compost within a framework of continuous production, evaluated at all developmental stages.

A completely randomized design (CRD) was used, with three treatments and three replicates per treatment, each repetition had three beds, considering the bed as the experiment unit, for a total of nine beds per treatment. The treatments consisted of three irrigation depths: control treatment T1 with a depth equivalent to 100% of the crop evapotranspiration (ETc), and T2 and T3, equivalent to 80% and 65% of T1, respectively.

During the experiment, the irrigation was programmed by volume, determined daily as a correction factor of the drainage percentage (FDP).

The reference crop evapotranspiration (ETo) was determined daily, with direct readings with a 1 mm precision atmometer (ET gage), between 7:00 and 7:30 a.m. ET gage consists of a canvas covered ceramic evaporation plate mounted on a water reservoir, the reservoir's capacity is 300 mm deep and the green canvas was #54, used to estimate alfalfa reference. The ET gage was installed at the average height of the canopy. Based on the methodology established by Allen *et al.* (2006), the crop evapotranspiration (ETc) was determined with a fixed crop coefficient value (kc) of 0.94 recommended by Rodríguez (1998) to program irrigation and production optimization with minimal drainage depth.

Planning to assess and monitor the drained volume, nine volume lysimeters were installed, three per treatment, to calculate the drainage percentage (%D), as the ratio of the drawn depth and net applied depth. The crop coefficient (Kc) was calculated from the ETi/ETo ratio, where (ETi) is quantified as the water balance (difference between the volume of water applied by irrigation and drained volume in the lysimeter), taking the changing soil moisture as a constant value.

The growth of the stem and the bud was monitored for two crop cycles, the first beginning at 35 weeks and the second at 39, approximately nine weeks per cycle, obtaining simple sigmoidal type curves, with three growth phases: logarithmic, linear and senescence, as described by Salisbury and Ross (1994). Additionally, the absolute growth rate (AGR) and relative growth (RGR) were evaluated for the bud and stem.

The equations used were mostly adjusted from the model obtained after calculating the AGR of the stem length (Eq.1) and bud diameter (Eq.2). Equations as follow:

$$y = \frac{a \times b \times e^{-b(x-c)}}{(1 + e^{-b(x-c)})^2} \tag{1}$$

$$y = \frac{a \times b \times c \times e^{-cx}}{(1 + e^{-b(x-c)})^2} \tag{2}$$

A second set of equations was adjusted from a model obtained from the RGR of stem length (Eq.3) and bud diameter (Eq.4). These equations were:

$$y = \frac{AGR}{L} \tag{3}$$

$$y = \frac{b \times c \times e^{-cx}}{1 + b \times e^{-cx}} \tag{4}$$

where, y is the absolute rate of stem growth, a is the maximum final dimension, b is day on which the highest growth rate occurs, c is the relative rate of growth at the inflection point, x is time in weeks and L is stem length.

Thirty-six (36) stems per treatment were labeled. The measurement was made in the vegetative phase and in the reproductive phases; "palmiche" is the induction of the floral primordium, followed by "rice" with less than 4 mm in diameter, "pea" with an approximately 0.5 cm long peduncle, "garbanzo" with a peduncle with an average length of 4 cm, and approximately thirteen pairs of leaves, "double garbanzo" with a peduncle approximately 9 to 12 cm long, "scratch color" in which the color of the petals is observed, and "cutoff". The production of the floral stems was measured weekly per bed for each treatment and repetition.

The production results and floral stem growth were analyzed using descriptive statistics, nonlinear regression, analysis of variance (ANOVA) and mean comparison test with the Duncan method and a significance level of 5%, using SPSS® Statistics software (Armonk, New York).

Results and discussion

The potential evapotranspiration (ETo) measured with an atmometer was 1.83 mm, while the ETo for the type A tank was 2.14 mm. Crop's evapotranspiration (ETc) determined with the atmometer ETo from week 35 to 48 was 1.68. The crop evapotranspiration with lysimeter (ETi) for T3 (1.65 mm) was significantly different (P<0.05) to T2 and T1 (1.99 and 2.05 mm, respectively). In the T1 treatment, the ETi and ETo of the atmometer and the type A tank were similar, ranging 2.05, 1.83 and 2.14 mm, respectively; thus, the ETo was adjusted to the water needs of the crop.

The total volume of water applied to treatments T1, T2 and T3 was 15.04, 12.39 and 10.35 m^3 per bed, respectively, in the two cycles, which is equivalent to 3.64, 2.99 and 2.51 mm per day, respectively (Fig. 1). The reductions in the volume of water applied to T2 and T3 compared to T1 were 17.6% and 31.2%, respectively. The volumes were lower than those reported by Patiño (2000) who stated that the irrigation volume must range between 3.70 and 5.29 mm per day.

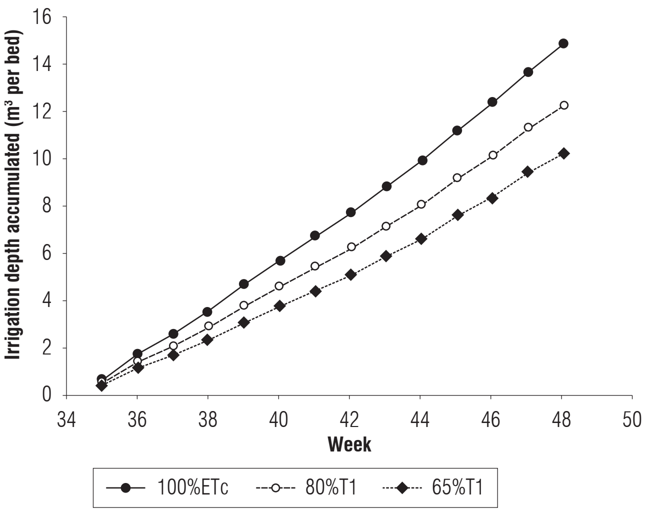

FIGURE 1. Accumulated irrigation depth in m^3 per treatment and per bed in the two crop cycles.

The mean daily values of the drained depth were 1.64, 1.00 and 0.82 mm in T1, T2 and T3, respectively. The drainage percentage (%D) in T2 and T3 was similar, according to the daily average values, 44.30%, 32.83% and 31.09% for T1, T2 and T3, respectively. The %D was adjusted taking into account the preset interval to use the drainage percentage correction factor (DPF) (30-50%) in nine (9) of the fourteen (14) weeks, with averages of 44.30% and 41.60% for the %D and DPF of T1, respectively (Fig. 2). The %D was lower than those reported by Esmeral *et al.* (2011) in rose cv. Charlotte, resulting in 52.65, 63.17 and 63.14% for the three substrates, 100% for burnt rice husks (BRH), 65% for BRH + 35% coconut fiber and 35% for BRH + 65% coconut fiber, respectively. However, it was higher than those proposed by Arreaza (2000) and Meneses (2004) in this study the %D ranged from 5% to 20% for carnations planted in the substrate, and reported by Martínez and Roca (2011) 10 to 30%.

The coefficient of culture medium calculated for T1, T2 and T3 was 1.15, 1.13 and 0.93, respectively, with significant differences (P<0.05) according to the Duncan test for T3 (T2 and T1) and a high Pearson linear correlation (P<0.05) ETi, in T1 and T2, with 0.50 and 0.48, respectively (Tab. 1). The Kc values were not associated with a growth stage and were close to those reported by Esmeral *et al.* (2011), who obtained values of 0.79 and 1.50, in rose cv. Charlotte planted on the Bogota Plateau. The Kc value of T1 (1.15), was similar to that found by Arevalo *et al.* (2013), who reported a value of 1.13, for a treatment watered at 100% ETc in rose cv. Freedom in soil.

The growth showed a continuous behavior (Fig. 3A). In the second measurement cycle starting at week 43, T1 had

TABLE 1. Evapotranspiration of the referenced crop (ETo), the lysimeter (ETi)and calculated crop coefficient (Kc). Values with different letters indicate significant differencesaccording to the Duncan test (P<0.05).

Week	ETo (mm d^{-1})	T1 (100%ETc)		T2 (80%T1)		T3 (65%T1)	
		ETi (mm d^{-1})	Kc	ETi (mm d^{-1})	kc	ETi (mm d^{-1})	Kc
35	1.30	1.59 a	1.17 a	1.42 a	1.01 a	1.14 a	0.80 a
36	1.79	1.66 a	0.95 a	1.82 a	0.99 a	1.61 a	0.90 a
37	1.41	1.53 a	1.14 a	1.64 a	1.17 a	1.35 a	0.99 a
38	1.60	1.85 a	1.18 a	1.84 a	1.17 a	1.55 a	0.99 a
39	1.83	2.47 a	1.34 a	2.27 a	1.24 ab	1.96 a	1.07 b
40	1.64	1.98 a	1.32 a	1.77 a	1.18 a	1.44 a	0.96 a
41	1.50	1.66 a	1.01 a	1.62 a	1.01 a	1.26 a	0.78 b
42	1.84	2.27 a	1.20 a	2.13 a	1.14 a	1.76 a	0.95 a
43	1.75	1.90 a	1.11 a	2.04 a	1.18 a	1.71 a	0.97 a
44	1.73	2.18 a	1.13 a	2.33 a	1.25 a	1.83 a	0.99 a
45	2.43	2.19 a	1.04 a	2.00 a	0.97 a	2.11 a	0.93 a
46	2.07	2.26 a	1.20 a	2.11 a	1.13 ab	1.65 a	0.88 b
47	2.33	2.70 ab	1.21 a	2.62 a	1.14 ab	2.01 b	0.78 b
48	2.25	2.41 a	1.18 a	2.30 ab	1.13 a	1.82 b	0.94 a

the highest values of stem length to harvest after week 47 with 60.37 cm; while T2 and T3 were 55.81 and 55.80 cm respectively, According to the results, there were no significant differences (P<0.05) in treatments. For the diameter of the floral stem (Fig. 3B), at the end of the cycle, the values 5.76, 5.94 and 5.49 mm were obtained for T1, T2 and T3, with coefficients of variation (CV) of 14.60, 10.90 and 13.62%, respectively. No significant differences (P<0.05) were established after assessing the diameter of the floral stem. In the second cycle, the length of the bud was measured since week 42, with initial values of 0.89, 0.95 and 0.81 cm for T1, T2 and T3, with a CV of 32.46%, 28.51% and 34.16%, respectively. At the conclusion of the cycle, in week 47 (Fig. 4A), average bud lengths of 4.01, 4.02 and 4.28 cm, with a CV of 14.08%, 12.68% and 12.85% for T1, T2 and T3 were obtained, respectively. No significant differences where found (P<0.05).

At the end of the second period, the bud diameter was 21.34, 21.96 and 23.26 mm with a CV of 19.10%, 14.31% and 13.53% for T1, T2 and T3, respectively. There were no significant differences between the treatments (P<0.05) (Fig. 4B).

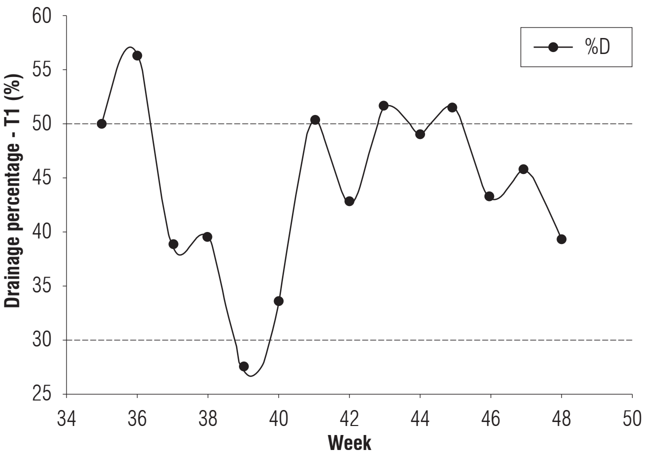

FIGURE 2. Fluctuation in the drainage percentage (%D) in the corresponding range for the DPF (30-50%) in T1.

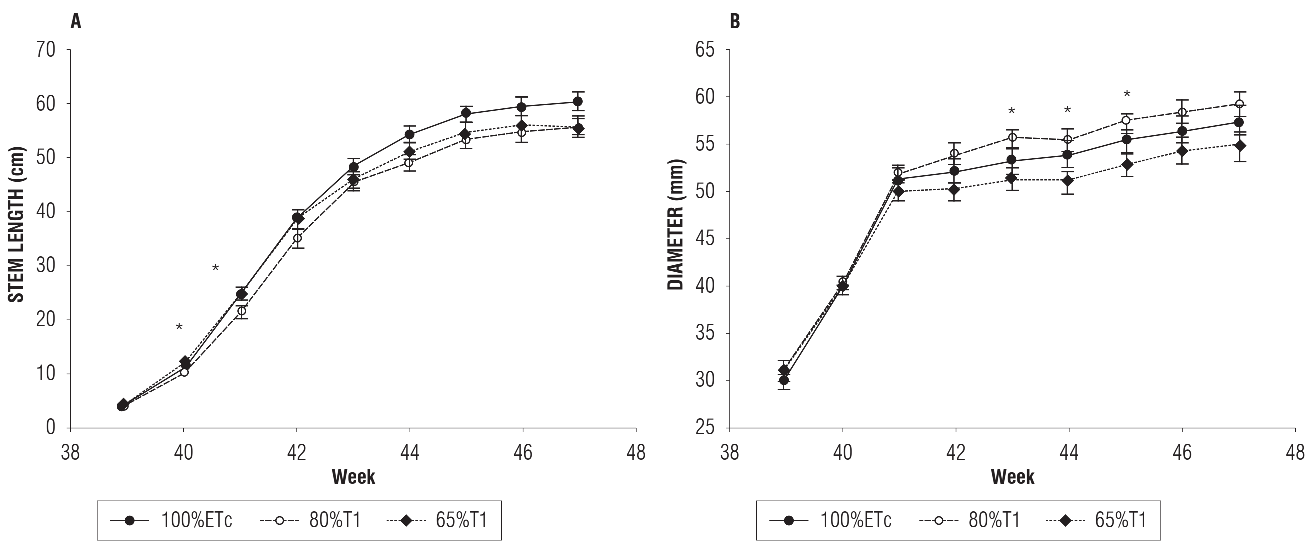

FIGURE 3. Growth curve of floral stem, weeks 39 to 47, A) length and B) diameter. The * indicates a significant difference according to the Duncan test (P<0.05).

TABLE 2. Phenological stages of *Rosa* sp. Vendela, as a function of the bud diameter; weeks 42 to 47. Values with different letters indicate significant differences according to the Duncan test (P<0.05) (week, days after cutting, phenological stages, bud diameter).

Week	Days after cutting (DDC)	Phenological stages	Bud diameter (mm)	Bud diameter (mm)	Bud diameter (mm)
			T1 (100%ETc)	T2 (80%T1)	T3 (65%T1)
42	46	Rice	3.97 a	4.19 a	4.30 a
43	53	Pea	5.46	5.44 a	5.80 a
44	60	Garbanzo	7.78 a	7.69 a	8.41 a
45	67	Double garbanzo	12.51 b	12.61 b	14.21 a
46	74	Scratch color	16.96 a	17.20 a	18.11 a
47	81	Cut	21.34 a	21.96 a	23.26 a

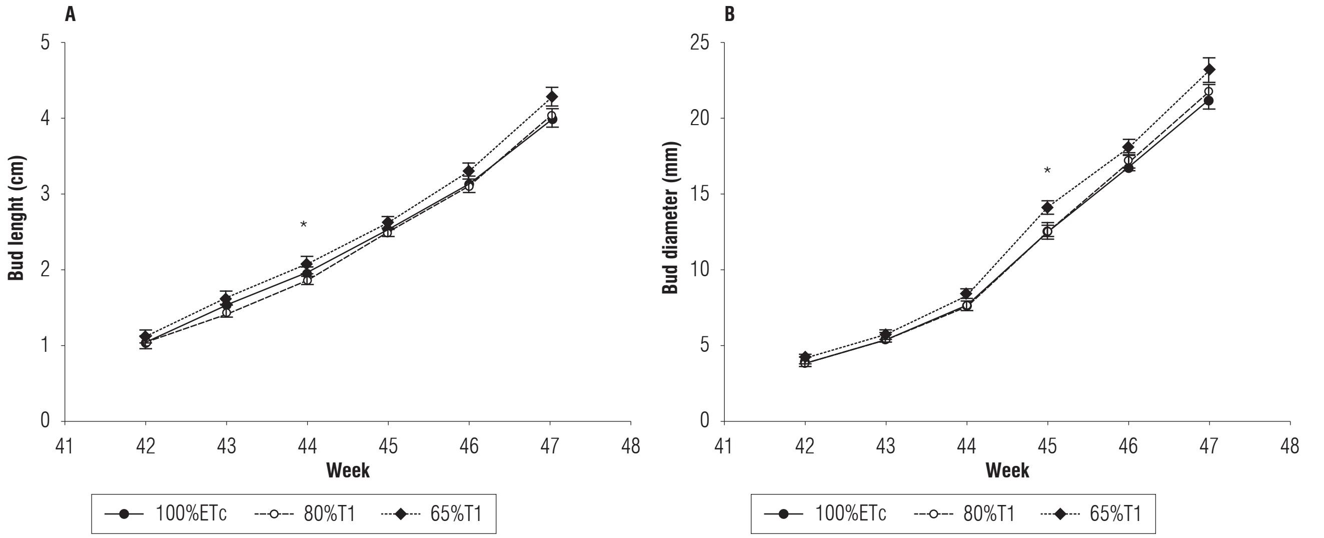

FIGURE 4. Growth curve of floral buds, weeks 39 to 47, A) length and B) diameter. The * indicates a significant difference according to the Duncan test ($P<0.05$).

The length and diameter of the stem and bud did not show significant differences between treatments for the second cycle of measurements, because the stress was moderate and did not cause reductions in the quality of the flower; however, decreases in the length and diameter of the stem confirm what was found by Bolla *et al.* (2010), who stated that under unfavorable conditions of water availability and even with small decreases, there was a cessation of vegetative growth. Although the length of the stem found in the treatments ranged within the commercial requirements (50 to 60 cm for the Vendela variety), the shortest length in T1 influences the commercial value depending on the destination market.

The floral bud had typical weekly phenology stages for the flower stem (Tab. 2), coinciding to the appearance of the bud, identified with the peak of stem elongation at week 37, as reported by De Hoog (2001).

The curves from Table 3 fit the logistic model reported by Flórez *et al.* (2006). According to this logistic model, the larger stem growth length values during the second cycle were 59.74, 55.17 and 55.81 cm for T1, T2 and T3, respectively, achieving this growth after 41 weeks. The slope of the curve for T1, T2 and T3 was 1.008, 1.021 and 1.017, respectively. The growth time is represented by (t). The AGR of the flower stalk in the three treatments showed a typical trend of a bell curve (Fig. 5A), showing a steady growth, and reaching maximum values at week 42 (13.84, 13.13 and 12.37 cm for T1, T2 and T3, respectively). AGR values were zero in the phase of senescence to harvest. The T2 RGR showed a different behavior between weeks 39 and 40 but a similar trend was recorded after that period until harvest, were values ranged close to zero (Fig. 5B).

For the bud diameter in the second cycle, the AGR increased weekly until peaking up at week 46 in the T1 and T2 treatments, with values of 4.71 and 5.04 mm, respectively. The maximum value of T2 was obtained at week 47 (Fig. 6A). The RGR presented a steady decline in all of the treatments until harvest at week 47 (Fig. 6B). There were no significant differences ($P<0.05$) in the cumulative production of floral stems, with 12,393, 9,779 and 11,197 units for T1, T2 and T3, respectively.

TABLE 3. Equations of the logistic model for the stem length; weeks 39 to 47.

Treatment	Stem length	
	Model	R^2
T1 (100%ETc)	$y = \dfrac{59.7375}{1 + e^{-1.0081\ (t-41.4200)}}$	0.9988
T2 (80%T1)	$y = \dfrac{55.1700}{1 + e^{-1.0211\ (t-41.4796)}}$	0.9985
T3 (65%T1)	$y = \dfrac{55.8097}{1 + e^{-1.0169\ (t-41.2636)}}$	0.9981

An average productivity of 1.31, 1.03 and 1.18 stems per plant per month for T1, T2 and T3 were reached, respectively. These results concur to those reported by Arévalo *et al.* (2013), who found values of 1.32, 1.28 and 1.24 stems per plant per month for Rosa cv. Freedom in soil. Bolla *et al.* (2010), found a reduction in the production of marketable

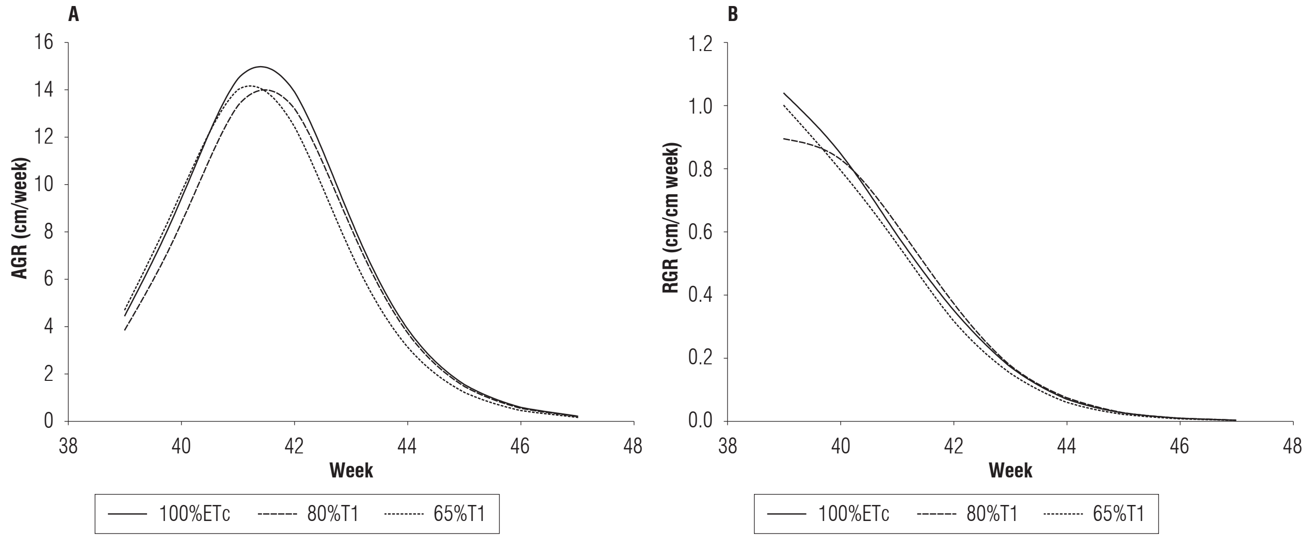

FIGURE 5. Growth curve for the floral stem length, weeks 39 to 47, A) Absolute growth rate (AGR), B) Relative growth rate (RGR).

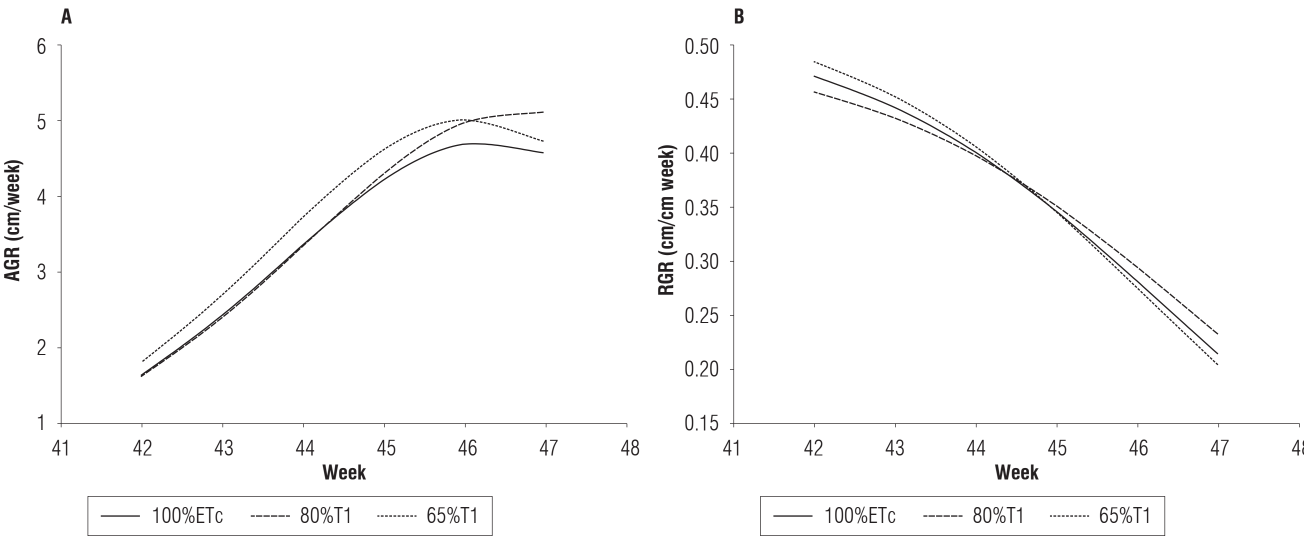

FIGURE 6. Growth curve of the bud diameter, weeks 39 to 47, A) Absolute growth rate (AGR) and B) Relative growth rate (RGR).

rose flower stalks grown in three different substrates, under water deficit conditions, using only 67% of the irrigation needs.

For the WUE, 140.43, 132.00 and 186.61 stems m^{-3} were obtained for T1, T2 and T3, respectively, finding significant differences ($P<0.05$) between T3 and T2 and T1. The treatment with the lowest water supply produced the highest stem number during the first and last five weeks. This finding confirms the reports by Bolla *et al.* (2010), who observed increases in the WUE under a reduced water application. It also coincides to the observations by Raviv and Bloom (2001) in a crop of rose cv. Sonia and Laser, where WUE increased when the water level dropped. This contrasts to what was found in this research for treatment T2, with a lower production of flower stalks with respect to T1.

Conclusions

The crop coefficient values (Kc) obtained from the water balance were close to those reported for other rose varieties grown in a greenhouse on the Bogota Plateau.

The volume of applied irrigation from the potential evapotranspiration (ETo) and drain percentage (%D) did not affect the water status of the plants.

The growth of the floral stem was adjusted to a logistic model, showing the characteristic sigmoidal trend that allows the strategies planning for the crop production. The largest length values were 59.74, 55.17 and 55.81 cm for T1, T2 and T3, respectively, achieving this growth in 41 weeks.

Reductions of 35% in the irrigation depth affected the growth of the stems but not the floral button, leaf area and the accumulated production of flower stems, although a higher quantity of short stems was observed.

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Assessing water demand with remote sensing for two coriander varieties

Determinación de las necesidades hídricas de dos variedades de cilantro mediante sensores remotos

Euseppe Ortiz[1]* and Enrique A. Torres[1]

ABSTRACT

The use of remote sensing to determine water needs has been successfully applied by several authors to different crops, maintaining, as an important basis, the relationship between the normalized difference vegetation index (NDVI) and biophysical variables, such as the fraction of coverage (f_c) and the basal crop coefficient (K_{cb}). Therefore, this study quantified the water needs of two varieties of coriander (UNAPAL Laurena CL and UNAPAL Precoso CP) based on the response of f_c and K_{cb}, using remote sensors and a water balance according to the FAO-56 methodology. A Campbell Scientific meteorological station, a commercial digital camera and a portable spectro radiometer were used to obtain information on the environmental conditions and the crop. By means of remote sensing associated with a water balance, it was found that the water demand was 156 mm for CL and 151 mm for CP until the foliage harvest (41 d after sowing); additionally, the initial K_{cb} was 0.14, the mean K_{cb} was 1.16 (approximately) and the final K_{cb} was 0.71 (approximately).

Key words: UNAPAL, *Coriandrum sativa*, NDVI, water demand.

RESUMEN

El uso de la sensores remotos para determinar las necesidades hídricas ha sido aplicada exitosamente por varios autores en diferentes cultivos, manteniendo como base importante la relación existente entre el índice de vegetación de diferencia normalizada (NDVI) con variables biofísicas como la fracción de cobertura (f_c) y el coeficiente basal de cultivo (K_{cb}). Por esta razón, en este estudio se cuantificaron las necesidades hídricas de dos variedades de cilantro (UNAPAL Laurena CL y UNAPAL Precoso CP) basado en la respuesta de la f_c y K_{cb}, utilizando sensores remotos y empleando un balance hídrico según la metodología de FAO-56. Para la obtención de información de las condiciones ambientales y del cultivo, se utilizaron una estación meteorológica Campbell Scientific, una cámara digital comercial y un espectro radiómetro portátil. Se encontró, por medio de sensores remotos asociados a un balance hídrico, que la demanda hídrica para CL fue de 156 mm y 151 mm para CP, hasta la cosecha de follaje (41 días después de siembra). Ademas el K_{cb} inicial fue de 0.14, el K_{cb} medio 1.16 (aproximadamente) y el K_{cb} final 0.71 (aproximadamente).

Palabras clave: UNAPAL, *Coriandrum sativa*, NDVI, necesidades hídricas.

Introduction

Coriander (*Coriandrum sativa* L.) is an annual herbaceous plant that reaches between 60 and 90 cm in height during the flowering period; it belongs to the family Apiaceae (Umbelliferae) (Vallejo *et al*., 2004). Its fruits are used for the elaboration of oils, fragrances and flavorings, while its leaves are used as a condiment (Usman *et al*., 2003).

In Colombia, eight types of coriander varieties are sown (Estrada *et al*., 1997), and around 2,431 ha are harvested, with Cundinamarca (36%) and Valle del Cauca (23%) being the larger producing departments (MADR, 2016).

In the departments of Valle del Cauca and Cauca, the UNAPAL Precoso (CP) variety is highly accepted because of its short vegetative period (28-35 d after sowing, DAS) and adaptation to climatic conditions (20-27°C) (Estrada *et al*., 2004). On the other hand, the variety UNAPAL Laurena (CL) is a new variety that will be released soon, created from the genetic improvement of the CP variety. This variety preserves the favorable characteristics of its parent, with a capacity to generate a greater number of basal leaves and average yields in foliage of 40 t ha^{-1} (Otero, 2016).

Adequate water availability at each phenological stage is decisive for achieving proper coriander production because it requires shallow sowing (5 mm), has a shallow root system (10-15 cm) and a short vegetative period. These characteristics make it a demanding crop that requires short and frequent irrigation, mainly in the early stages of establishment and development (Vallejo *et al*., 2004). According to Mejía *et al.* (2014), in controlled trials in mesh houses, irrigation with less than 200 mm in the vegetative period reduced the number of leaves, the height, and the yield.

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However, higher irrigation can generate greater evapotranspiration (ET_c) of this crop without significant increases in yield. It is inferred that, in order to maximize the yield of coriander, an adequate estimation (or measurement) of the ET_c is necessary in order to accurately determine the water needs of this crop and the irrigation amount to be applied. Among the methods globally accepted to determine ET_c, two methods of water balance are noteworthy, as described by Allen *et al.* (2006): the single coefficient (Eq. 1) and the dual coefficient (Eq. 2); the latter separately analyzing the plant's transpiration component and soil evaporation (Wright, 1982).

$$ET_c = ET_o \times (K_c) \quad (1)$$

$$ET_c = ET_o \times (K_{cb} + K_e) \quad (2)$$

where ET_c is the evapotranspiration of the crop (mm), ET_o is the reference evapotranspiration (mm), K_c is the coefficient of cultivation or single coefficient, K_{cb} is the coefficient of transpiration and K_e is the coefficient of evaporation.

The implementation of one method or the other differs in terms of the timescale. Approximation with the single coefficient integrates two coefficients; therefore, it is designed for crops whose irrigation demand can be extended weekly or at even higher frequencies (Allen *et al.*, 2006). On the other hand, the dual coefficient is used for estimates of daily ET_c, allowing differentiation between the behavior of K_{cb} and K_e, especially in the days following irrigation or precipitation events (Hunsaker *et al.*, 2005), which makes this method the most suitable for the implementation of high frequency localized irrigation systems.

K_{cb} is directly related to the morphology and physiology of a plant, especially to the loss of water vapor through the opening and closing of stomata in response to environmental conditions, influenced by energetic, physiological and aerodynamic factors (González Piqueras, 2006). K_{cb} recommendations for various crops were given by Allen *et al.* (2006); however, these values are for crops under standard conditions, which on many occasions cannot be replicated because of local conditions.

K_{cb} determinations for coriander have not yet been defined. However, closer approximations have been obtained by Mejía *et al.* (2014) with the determination of K_c under controlled conditions in mesh houses. K_c values of 0.83 for germination, 1.12 for linear growth and 1.40 for fresh harvest (from 27 to 35 DAS) have been obtained.

On the other hand, remote sensing is currently seen as a science of great importance because of its application in different fields, especially agriculture. The possibility of obtaining information from vegetation without coming into direct contact with the crop makes its application conspicuous since it is a non-destructive and low-cost method. The effectiveness of remote sensing is related to the physiological capacity of plants, specifically chlorophylls, xanthophyll and carotene, which interact with radiation from the sun and generate reflectivity spectral responses associated with phenotypic states seen in the field. The measurement of vegetation reflectivity can be done by using a radiometer or multispectral images adjusted to the spectral resolution (Chuvieco, 2008).

With the first satellite observations with multispectral images of the Earth's surface, Rouse *et al.* (1974) showed certain correlations of vegetation biophysical parameters with specific regions of the electromagnetic spectrum, more precisely, the regions included in the spectrum of Red (R) and near infrared (NIR). Since then, the combinations of different bands of the spectrum to identify agronomic variables have been diversified according to the phenomena of interest; these combinations have been called vegetation indices (VI).

Worldwide, the VI with greater impact and expansion in remote sensing is the Normalized Difference Vegetation index (NDVI) (González, 2006). This index is characterized by results between -1 and 1; when photosynthetically active vegetation densities are high, the NDVI is close to 1. However, for bare soil surfaces, this index is close to zero and takes on negative values for water bodies or cloud cover. One of the advantages and reasons for the great acceptance of NDVI is the simplicity and ease of applying it to intermediate canopy covers (Gilabert *et al.*, 2010). Its high correlations with biophysical variables, such as K_{cb} (Tab. 1) and the plant cover fraction (f_c) (González, 2006; Er-Raki *et al.*, 2007; López *et al.*, 2009; Johnson *et al.*, 2012; Zhang *et al.*, 2015), have allowed for its implementation in studies on water balances in large land surfaces.

Although changes in soil surface roughness and humidity affect the spectral response of a soil (Baret *et al.*, 1993), it is important to note that there are no studies that show a clear relationship between K_e and NDVI because K_e depends on the soil moisture content in the first 10 or 15 cm and not just at the surface. Therefore, K_e cannot be deduced from spectral information of the surface and must be calculated with a water balance.

The quantification of K_e is intrinsic to the fraction of coverage (f_c). As vegetation develops through phenological stages, it increases in the ability to intercept incident light and reduces the exposed soil fraction. Quantifications of f_c have been performed at various scales (local, regional and national), yielding good results in schedules and water management. The research conducted by White *et al.* (2000) concluded that the use of images captured with cameras is the easiest and most reliable mechanism for measuring f_c.

On the other hand, the use of satellite images has seen an exponential increase worldwide since the 1970s, with the development of the first LANDSAT missions. These developments have allowed a wide range of Earth-orbiting satellites to provide information at different spectral and temporal resolutions (González, 2006).

Based on multispectral satellite images, Bausch (1995), Er-Raki *et al.* (2007), and Toureiro *et al.* (2017) were able to quantify water requirements and apply irrigation more accurately in maize and wheat crops. However, the biggest constraint for the use of this technology in Colombia is associated with cloudiness, geographical location and topographical conditions of the country (Martínez, 2017). For this reason, research at lower scales with unmanned aerial vehicles or local measurements is considered viable alternatives (García *et al.*, 2015).

The aim of this study was to quantify the water demand of two varieties of coriander (CL and CP), determined with a water balance, integrating biophysical variables (f_c and K_{cb}), and estimated from remote sensors.

Materials and methods

This study was carried out between the months of April and August, 2017 at the CEUNP experiment center of the Universidad Nacional de Colombia, Palmira (3°25'33"N, 76°25'52"W, 930 m a.s.l.). The nearest meteorological station to CEUNP (14 km) is the Alfonso Bonilla Airport (IDEAM station: 26075040), which has a record period of 1972 to 2016. The data show an average annual precipitation of 893.5 mm, distributed over 144 d, reaching a maximum of 89.6 mm in 24 h. The average temperature is 24°C, and the relative humidity is 73.2%, registering 1810 h of total annual solar brightness and an average wind speed of 1.2 m s^{-1}.

The mechanized land preparation was carried out on the upper 40 cm of the surface, making two passes per implement (disc harrow and rotavator). A soil sample was taken to determine the texture (clay loam) and moisture retention curve (field capacity (FC) = 37.86% and wilting point (WP) = 20.84%).

The sowing was carried out on April 22 (day 0 after sowing, DAS) by distributing the seeds manually at a 25 cm distance between furrows. Weeds were manually controlled during the first six weeks. The nutritional management of the crop was carried out following the recommendations of the experiment center, which consisted of foliar applications of potassium nitrate, Cosmocel® and Triple 18, at different concentrations throughout the stages of growth, development and fruit filling. In addition, at the mid-development (27 DAS), granulated urea was broadcast. For the second week of flowering, on June 28 (61 DAS), "boxing" tutoring was installed on double furrows.

A complete block design was implemented with two treatments (CP and CL varieties) and three replicates, working an area of 34 m^2 distributed in six experiment units (EU) of 5 m^2 (2.5 m x 2 m), as shown in Figure 1. Because of the lack of germination uniformity in the third replicate of CP, this EU was ruled out in the follow-up and analysis.

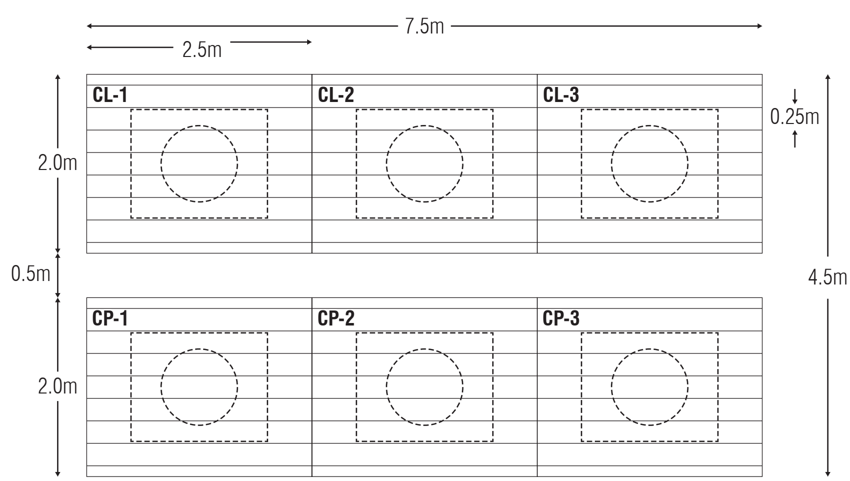

FIGURE 1. Distribution of experiment units (EU) in the field of the two studied varieties. The horizontal lines indicate the rows of the crop. The boxes and circles (dashed line) indicate the area covered by photography and radiometry, respectively.

Calculation of the water demand was based on Equation 2, following the methodology described by Allen *et al.* (2006) in FAO 56, using climatic variables of temperature, relative humidity, solar radiation, wind velocity, precipitation, crop height, f_c and K_{cb}, adapted to local conditions. Drip irrigation system was installed to meet the water requirements of the crop, which was programmed to function according to the level of depletion accumulated in the soil, with the exception of the first week between sowing and germination when the surface was watered following the recommendations of Estrada *et al.* (2004). In general, the irrigation applications ensured that the crop was kept free of water stress.

Climatic information was obtained with the use of a Campbell scientific automatic meteorological station installed on a reference surface of grass or festuca, which recorded data of relative humidity, wind velocity, solar radiation, precipitation and temperature every 10 min. For the days without information from July 2 to 6 (41 and 45 DAS), the information from a near station, belonging to the CENICAÑA automated meteorological network, was used.

Based on the only method recommended to determine ET_o, the FAO Penman-Monteith equation adapted to a festuca reference surface (and daily scale) was used (Allen *et al.*, 2006):

$$ET_o = \frac{0.408 \times \Delta \times (Rn - G) + \gamma \times \frac{900}{T + 273} \times u_2 \times (e_s - e_a)}{\Delta + \gamma \times (1 + 0.34 \times u_2)} \quad (3)$$

where ET_o is the reference evapotranspiration (mm d^{-1}), R_n is the net radiation on the crop surface (MJ M^{-2} d^{-1}), G is the soil heat flux (MJ M^{-2} d^{-1}), T is the average air temperature at 2 m height (°C), u_2 is the wind speed at 2 m height (m s^{-1}), e_s is saturation vapor pressure (kPa), e_a is the actual vapor pressure (kPa), $e_s - e_a$ is the vapor pressure deficit (kPa), Δ is the slope of the vapor pressure curve (kPa °C^{-1}), the psychrometric constant (kPa °C^{-1}).

The height of the plant was measured weekly; additionally, radiometry and photography were taken to determine the NDVI and f_c, respectively. With these parameters, the evolution of the crop was traced, and linear tendencies were plotted with the objective of determining the water demand and scheduling irrigation on the following week (Bausch, 1995; Gonzalez *et al.*, 2004).

The estimation of the f_c was carried out with a Pentax digital camera, model Optio E85, 12 mega pixels, with spectral resolution in the optical range and a field of vision of 53°8' (determined in a laboratory). Three photographs were taken per EU, fixing the camera to a rigid T-shaped structure adjusted to a 1.50 m height. Subsequently, the images were subjected to classifications supervised by algorithms of maximum similarity with the ArcGIS 10.3 software. Six trainings were developed according to the evolution of the crop: (1) germination, (2) mid-exponential growth, (3) maximum coverage, (4) flowering, (5) fruiting and (6) senescence.

For the NDVI (Eq. 4), an Apogee PS100 spectroradiometer with a spectral range between 350-1100 nm was used, with an instantaneous field of view of 23°. For the photographs, three measurements were taken per EU at a 2.1 m height above the ground surface. Two spectral ranges, red (R) and infrared (NIR), based on Landsat 8 bands, were used.

$$NDVI = \frac{NIR - R}{NIR + R} \quad (4)$$

where NDVI is the vegetation index with the standard difference (dimensionless), R is the average reflectivity between 636-673 nm and NIR is the average reflectivity between 851-879 nm.

TABLE 1. Mathematical relationships between the NDVI and the crop basal coefficient (K_{cb}) reported in the literature.

Equation K_{cb}	R^2	Crop	Author
1.181 * NDVI - 0.026	-	Maize*	(Bausch *et al.*, 1987)
1.092 * NDVI - 0.053	0.96	Maize*	(Neale *et al.*, 1989)
1.49 * NDVI - 0.12	0.92	Cotton	(Hunsaker *et al.*, 2003)
1.50 * NDVI - 0.10	0.94	Maize	(González *et al.*, 2004)
1.56 * NDVI - 0.10	-	Various crops	(Calera *et al.*, 2005)
1.46 * NDVI - 0.19	0.93	Maize and wheat	(González, 2006)
1.64 * NDVI - 0.14	0.79	Wheat	(Duchemin *et al.*, 2006)
1.34 * NDVI - 0.14	0.86	Wheat	(López *et al.*, 2009)
1.44 * NDVI - 0.10	0.96	Grape	(Campos *et al.*, 2010)
1.464 * NDVI - 0.253	0.85	Maize	(Toureiro *et al.*, 2017)

Relationship referred to alfalfa as reference surface.

With the NDVI value, and taking into account the NDVI-K_{cb} relationship for various crops (Tab. 1), all the equations were averaged and the K_{cb} of each measurement was determined. Taking into account the response of the NDVI and the f_c, the lengths corresponding to each stage were defined.

The harvest was carried out at 104 DAS, which is equivalent to 98 d after germination (DAG), when the achenes changed from brown to dark brown. The yield was assessed by taking samples of 1 m^2 per EU, collecting the aerial parts of the crop between 8 and 9 a.m. (local time), carefully using pruning shears to avoid making dry achenes fall. At the end of the study, seed samples were randomly taken to determine the moisture content of the seeds at harvest and after drying in the open air.

The samples were left to dry in a covered cellar for 3 d. Afterwards, the seeds were collected manually and, using a sieve, the seeds were separated from the crop residues. For each sample, 5 g of seeds were taken and dried in an oven for 1 h at 130±2°C to determine the moisture content (ISTA, 2016).

After the harvest, the soil was taken to saturation to assess the depth and extent of the roots. Five roots per EU were manually extracted, and the depth and maximum width were measured.

Results and discussion

From the climatic variables measured in the field with the Campbell station, the daily ET_o was calculated along the crop using Equation 3. On average, the ET_o was 3.7 mm d^{-1}. However, as expected, the evapotranspiration of the reference surface had abrupt changes from one day to another because of the variation of weather conditions in the area (Fig. 2).

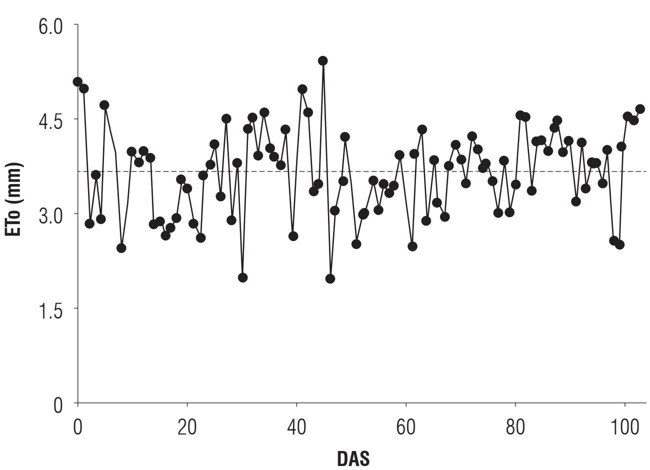

FIGURE 2. Evolution of ET_o during the test. The horizontal line indicates the average ET_o (3.7 mm).

The average plant density in the final stage was 164 and 108 m^{-2} for the CL and CP varieties, respectively. Differences in plant density are considered a statistically non-significant factor in the response of CP seed yield (Puga *et al.*, 2008) and were not evident in the results presented here. However, future studies should focus on evaluating foliage production for green harvest and the influence of stocking density, especially for the CL variety.

Figure 3 shows the evolution of f_c and NDVI for each variety as well as the development stages established under the following criteria: The initial stage (L_{ini}), starts from sowing and ends when f_c is around 0.10, when the crop is in the middle of exponential growth, and its presence begins to exert changes in the response of NDVI. The middle stage (L_{mdc}) is maintained while the f_c is above 0.90; after this point, the NDVI reduces sensitivity to the point of saturation (Odi *et al.*, 2013). As for the final stage (L_{end}), it starts with values of f_c lower than 0.90; the NDVI generates a response to photosynthetically inactive tissues, maintaining distant values for f_c.

The measurements of f_c and NDVI responded adequately to changes throughout the cycle, especially at 59 DAS, where winds stronger than 2 m s^{-1} managed to overturn much of the CP variety and the installation of tutoring considerably reduced f_c in the mid-stage (Fig. 3). However, the NDVI comparisons in CL, before and after tutoring, gave the same response, so the presence of white flowers (about 6%) on the surface of the crop achieved the same effect.

The f_c presented values of 0.02 during the emission of the first true leaves and gradually increased up to 0.99, where it reached the maximum vegetative development shortly before flowering (Fig. 3). The largest standard deviations of f_c were seen in the middle of the development stage and mid-stage, which coincided with the expansion of the crop in search of new spaces of light, until reaching the maximum development where standard deviations were lower. In the mid-stage, there was an increase in the standard deviation because of the tutoring that left fractions of bare soil exposed, increasing the heterogeneity of the crop cover.

The response of the NDVI varied between 0.14 and 0.30 (bare soil) at the initial stage, with 0.14 seen when a low moisture content was present in the soil and 0.3 in the presence of moist soil. In the mid-stage, the response of the NDVI varied between 0.92 and 0.93 when the foliage reached its maximum expression, as shown in Figure 3.

On the other hand, the correlations between NDVI and f_c, before the tutoring, were 0.99 for CL and 0.96 for CP (Fig. 2), according to what was reported by Calera *et al.* (2005),

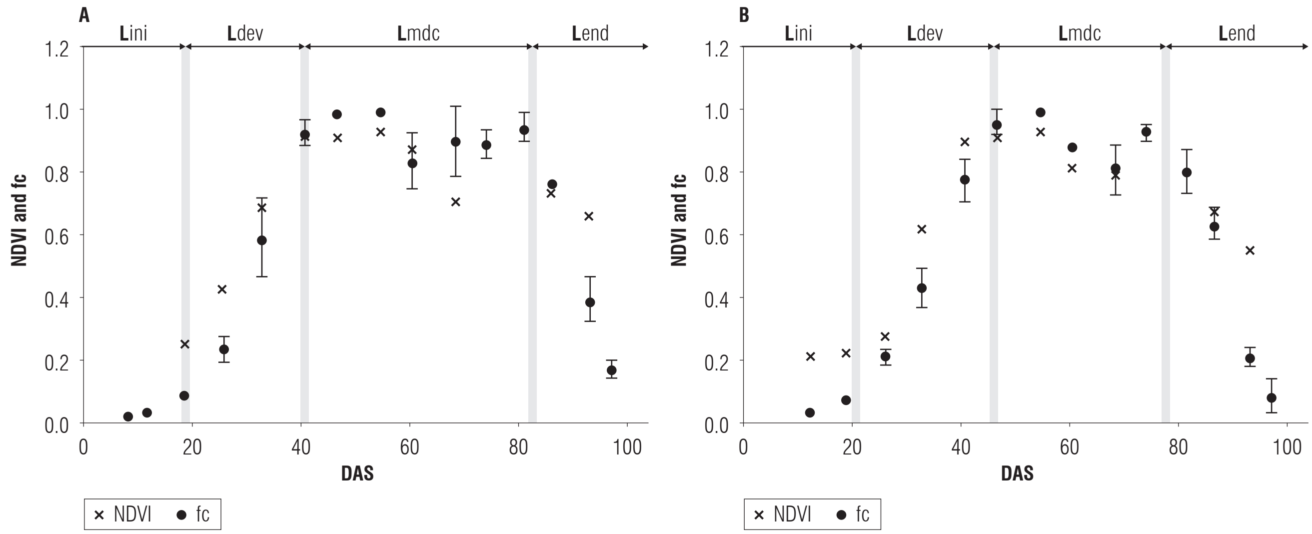

FIGURE 3. Evolution of the f_c and NDVI with demarcation of the stages of development, during the life cycle: A) Laurena and B) Precoso varieties.

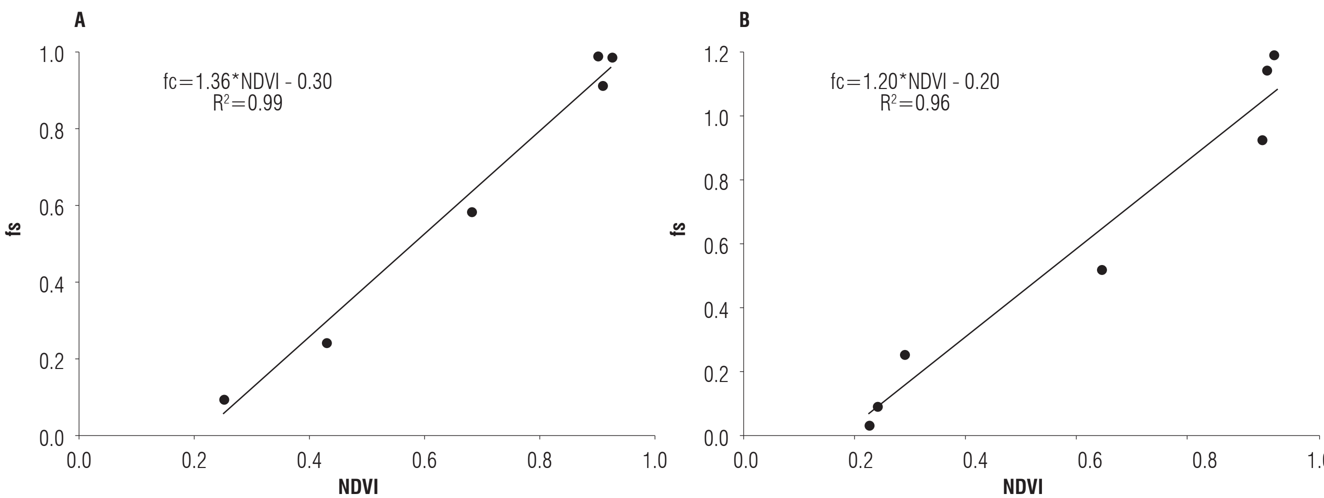

FIGURE 4. Linear relationship found between NDVI and f_c. A) Laurena and B) Precoso varieties.

González (2006), Er-Raki *et al.* (2007), López *et al.* (2009) and Johnson *et al.* (2012).

The duration of the stages of development, without taking the days until germination into account, is presented in Tab. 2. The CP variety showed a 5-day shorter period in the vegetative stages (start, development and mid). This can be an advantage for farmers because it reduces the time until foliage harvest. The final stage may change because the decision to harvest seeds is based on the farmer's experience and the climatic conditions, resulting in a variable final stage.

The values obtained for K_{cb} are shown in Figure 5, with the different NDVI-K_{cb} approximations reported in the literature and presented in the Table 1, using the values

TABLE 2. Length of crop development stages according to the FAO 56 methodology.

Variety	Start	Development	Mid	Final*	Sowing date	Region
	(L_{ini})	(L_{dev})	(L_{mdc})	(L_{end})		
Laurena CL	19	22	42	15	April rainy season	Tropical region
Precoso CP	21	25	32	20		

Values are expressed in days. Lengths corresponds as follow: L_{ini} = From germination until 0.10 f_c, L_{dev} = f_c>0.10 to f_c 0.90, L_{mdc} = fc>0.90 up to beginning of senescence and L_{end} = fc<0.90 up to crop. * Variable stage.

of NDVI obtained in the present study. In this figure, it is observed that the standard deviation of the estimated K_{cb} was small despite the physiological and morphological differences of the various crops of agricultural importance, with values of 0.05 for NDVI near bare soil and 0.11 close to total plant cover.

Based on the K_{cb} average equation, water vapor liberation was maintained through the estomatic aperture in relation to the increase of the exposed plant superficial area and the aerodynamic resistance. For this reason, higher transpiration levels appeared in the mid-stage (Tab. 3).

According to the height measurements, the maximum height for coriander was recorded in the flowering season, with 1.40 m for CL and 1.38 m for CP. Compared to the height achieved with the height reported in the literature, the CP variety significantly exceeded the 0.90 m that was reported by Vallejo *et al.* (2004).

TABLE 3. Basal crop coefficients (K_{cb}) and maximum height adapted from the FAO 56 methodology.

Variety	K_{cb}			Max. height
	Initial	Medium	Final	(m)
Laurena (CL)	0.14	1.17	0.79	1.40
Precoso (CP)	0.14	1.15	0.64	1.38

On the other hand, and as seen in Equations 1 and 2, the behavior of the K_c was the result of the summation of K_e and K_{cb}, where the greatest contributions of K_e were seen in the initial stage or phase of establishment of the crop, and the lowest contributions were seen when the K_{cb} was at the maximum (Fig. 6). However, the strong changes in

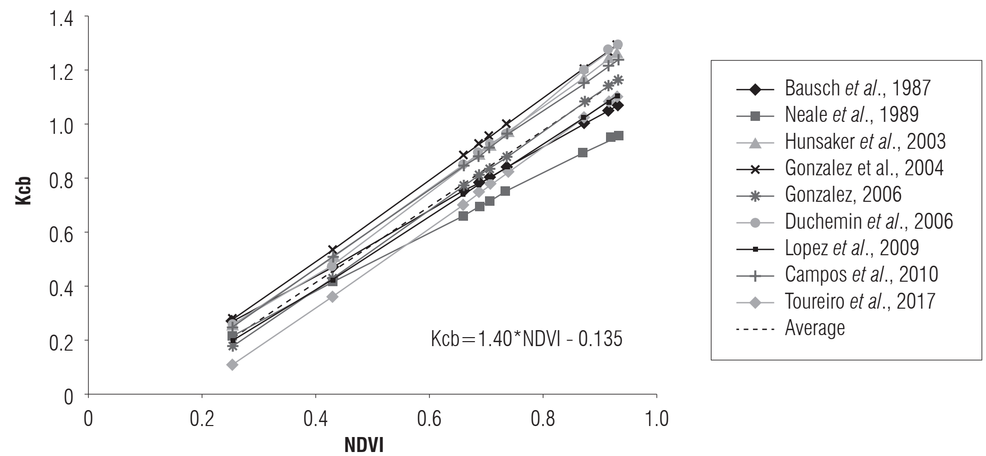

FIGURE 5. K_{cb} approximations calculated with NDVI values.

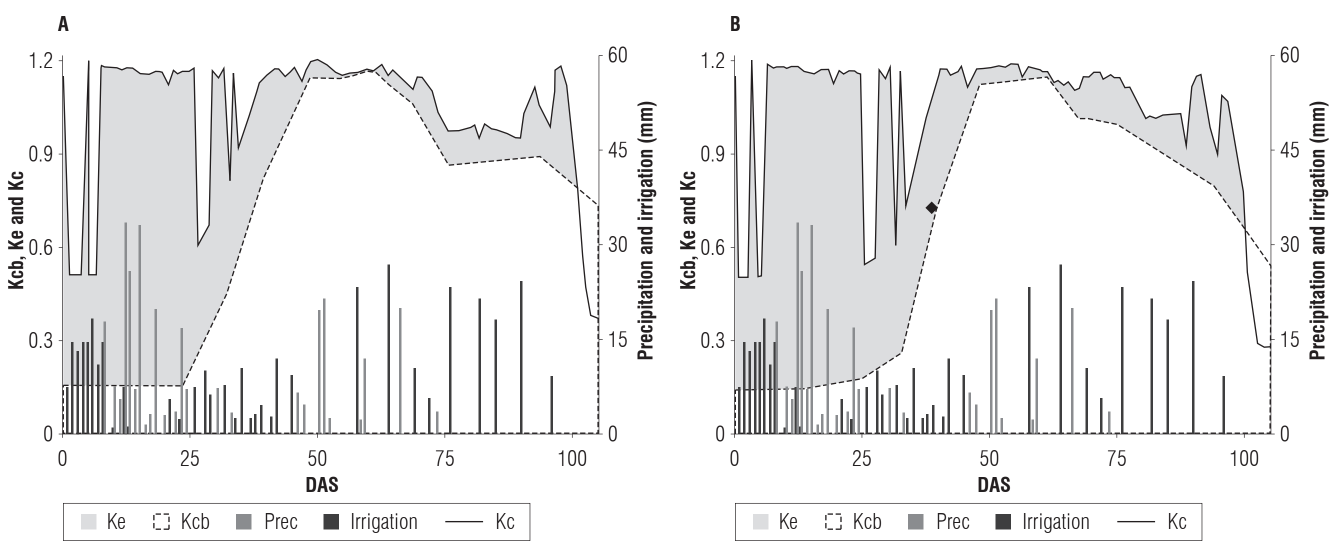

FIGURE 6. K_{cb}, K_e and K_c behavior over time with precipitation and irrigation, A) Laurena and B) Precoso varieties. For the latter variety, the rhombus indicates the appropriate time for foliage harvesting.

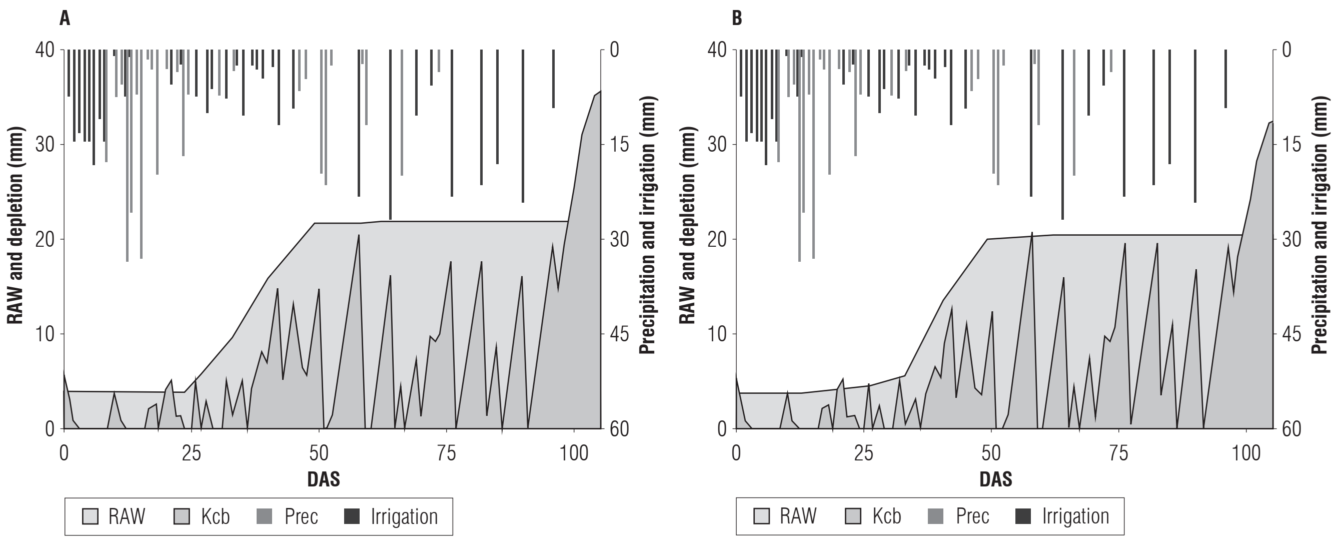

FIGURE 7. Readily available water (RAW) behavior for the crop and water depletion in the soil, in terms of rainfall and irrigation inflows. A) Laurena and B) Precoso varieties.

the behavior of K_e were associated with the capacity of the soil to retain water in the first centimeters and transfer it easily to the atmosphere when it was not covered. For this reason, after a precipitation event, when the total exposed surface was moistened, the K_c presented the maximum values, whereas localized irrigation applications decreased the contribution of evaporation to the K_c by up to 66%. At the end of the cycle, when the availability of water in the soil was considerably reduced, the contribution of K_e was eliminated, and the contribution of K_{cb} decreased with the crop senescence.

According to the water balance, until the middle of the development stage when the fresh foliage was harvested (up to 41 DAS), the crop demand (ET_c) was 156 mm for CL and 151 for CP, indicating that with this methodology, the water requirements were under those reported by Estrada *et al.* (2004) and Mejia *et al.* (2014). Additionally, for both varieties (CP and CL), the water input by precipitation and irrigation was between 208 mm to 187 mm, respectively, whereas losses through drainage (percolation) were 242 mm for CL and 245 mm for CP.

High losses resulting from drainage are a reflection of three factors: the first, the uneven distribution of precipitation throughout the vegetative cycle; the second, the concentration of precipitation in the first days of planting, and third, the application of frequent supplementary irrigation in order to ensure the development of the seed. This is done because, during the initial stage, the pivoting root has not yet been emitted or absorbent hairs have little scope making it impossible to absorb water stored between the porous spaces of the ground surface.

The irrigation schedules, made with estimates of effective depth of 30 cm, yielded positive results, allowing the level of soil depletion to exceed the capacity of readily available water (RAW) by the plant at the end of senescence, which could occur under stress conditions and favor the drying of the fruit (Fig. 7). However, the root analysis at the end of harvest showed that the roots did not exceed a 20 cm depth or a 25 cm width. This suggests that the root-extraction process may underestimate the horizontal and vertical reach of secondary roots.

Under the conditions of this research, the average seed yield was 181 g m^{-2} (±49) for CP and 175 g m^{-2} (±31) for CL, with a seed moisture content of 11% and 12%, respectively. This performance is suitable for both varieties as compared to the yields obtained by Usman *et al.* (2003) and Banda *et al.* (2011) with different methodologies.

Future studies should address the behavior of coriander until foliage harvest as well as the influence of sowing density and a follow-up on evaporation with lysimeters on water requirements, given that, in this study, the behavior of the whole vegetative cycle was assessed.

Conclusions

In the present study, it was observed that water demand management of this crop, based on remote sensors, is viable because of the sensitivity of the images and the radiometry used to determine biophysical variables (f_c and K_{cb}) of the coriander, responding adequately to the natural and human variations that occurred throughout the vegetative cycle.

The transpiration behavior (K_{cb}) in the variety UNAPAL Laurena (CL) and the UNAPAL Precoso (CP) varieties were very similar, differing slightly in the mid-stage (CL = 1.17 and CP = 1.15), with a more accentuated difference in the final stage of senescence (CL = 0.79 and CP = 0.64).

On the other hand, the most contrasting difference between the two varieties was the length of the stages, where CL reached maximum development 5 days before the CP variety. However, CL took 10 d to complete the mid-stage before initiating the stage of senescence, a factor to be taken into account for the drying of seeds in the field.

The calculated ET_c from sowing to harvesting foliage (41 DAS), under field conditions, was 156 mm and 151 mm for CL and CP, respectively. Both varieties demand short and frequent irrigation during the initial stage and at the beginning of the development stage.

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When considered necessary, the authors may acknowledge the researchers or entities that contributed - conceptually, financially or practically - to the research: specialists, commercial organizations, governmental or private entities, and associations of professionals or technicians.

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For books with several editors:

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- ***Book chapters***: author(s). Year. Title of the chapter. Pages (pp. #-#). In: Surnames and names of the editors (eds.). Title of the book. Edition. Publisher, city (and country, if the city is not a capital) of publication. Doi (if applicable)Examples:

  Barringer, S.A. 2004. Vegetables: Tomato processing. pp. 473-490. In: Scott Smith, J., and Y.H. Hui (eds.). Food processing: principles and applications. Blackwell Publishing, New York, USA. Doi: 10.1002/9780470290118.ch29

  Milne, E. and J. Smith. 2015. Modelling soil carbon. pp. 202-2013. In: Banwart, S.A., E. Noellemeyer, and E. Milne (eds.). Soil carbon: science, management and policy for multiple benefits. SCOPE Series Vol. 71. CABI, Wallingford, UK. Doi: 10.1079/9781780645322.0202

  Fukuhara, T. and H. Moriyama. 2008. Endornaviruses. pp.109-116. In: Mahy, B.W.J. and M.H.V. van Regenmortel (eds.). Encyclopedia of virology 3rd ed. Elsiever, Oxford, UK.

- ***Journals***: Author(s). Year. Title of the article. Abbreviated name of the journal. Volume (number), initial page-final page. Doi (if applicable)Examples:

  Laemmli, U.K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227, 680-685. Doi: 10.1038/227680a0

  Lioi, L., F. Sparvoli, I. Galasso, C. Lanave, and R. Bollini. 2003. Lectin related resistance factors against bruchids evolved through a number of duplication events. Theor. Appl. Genet. 107(5), 814-822. Doi: 10.1007/s00122-003-1343-8

- ***Reference to a website***: Author(s). Year. Title of the document. URL (accessed date). Doi (if applicable) Example:

  FAO. 1994. Neglected crops 1492 from a different perspective. URL: http://www.fao.org/docrep/t0646e/T0646E00.htm#Contents (accessed 30 August 2017).

- ***Thesis***: Author. Year. Title of the thesis. PhD thesis (or MSc thesis), Name of the University, City, Country. Doi (if applicable)Examples:

  Maqbool, R. 2013. Nitrogen cycling, optimization of plant nutrition and remote sensing of leaf nutrients in wild blueberries (*Vaccinium angustifolium* Ait.). PhD thesis, Dalhousie University, Halifax, Canada.

  Victoriano, H. 2010. Organogénesis *in vitro* de arándano *Vaccinium corymbosum* L. MSc thesis. Instituto Politécnico Nacional, Jiquilpan de Juárez, México.

- ***Conference proceedings***: Editor(s). Year. Title. Proceedings of the (event), date (year, month, days), city, country. City of publication, Publisher.

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  Harnden P., J.K. Joffe, and W.G. Jones (eds.). 2002. Germ cell tumours V. Proceedings of the 5th Germ Cell Tumour Conference, 2001 September 13-15; Leeds, UK. New York, Springer.

- ***Conference paper***: Author(s). Year. Title of the paper. Pages (pp. #-#). In: Surnames and names of the editors (eds.). Proceedings of the (event), date (year, month days), city, country. City of publication, Publisher.

  Christensen S. and F. Oppacher. 2002. An analysis of Koza's computational effort statistic for genetic programming. pp. 182-91. In: Foster J.A., E. Lutton, J. Mille, C. Ryan, and A.G. Tettamanzi (eds.). Proceedings of the 5th European Conference on Genetic Programming. 2002, April 3-5, Kinsdale, Ireland. Berlin, Springer.

AUTHOR INDEX OF *AGRONOMÍA COLOMBIANA* VOLUME 36, 2018

Author name	Article title	Location
Adailza Guilherme Cavalcante	Organic substrate and organic foliar fertilizer effect on chlorophyll index and growth in Umbuzeiro seedlings	No. 2, pp. 152-157
Adriana Rojas	Screening of Colombian soybean genotypes for Agrobacterium mediated genetic transformation conferring tolerance to Glyphosate	No. 1, pp. 24-34
Aldemar Reyes-Trujillo	Spectral and thermal response of Heliconia psittacorum species to induced water stress	No. 3, pp. 237-247
Alejandro Chaparro-Giraldo	Screening of Colombian soybean genotypes for Agrobacterium mediated genetic transformation conferring tolerance to Glyphosate	No. 1, pp. 24-34
Alfonso Parra-Coronado	Post-harvest quality of pineapple guava [*Acca sellowiana* (O. Berg) Burret] fruits produced in two locations at different altitudes in Cundinamarca, Colombia	No. 1, pp. 68-78
Alian Cássio Pereira Cavalcante	Organic substrate and organic foliar fertilizer effect on chlorophyll index and growth in Umbuzeiro seedlings	No. 2, pp. 152-157
Álvaro Monteros-Altamirano	On-farm conservation of potato landraces in Ecuador	No. 3, pp. 189-200
Álvaro Parrado	How to improve smallholder market access: Evaluation of Mercados Campesinos in Colombia	No. 1, pp. 79-87
Amparo Iris Zavaleta	Optimization of fermentation process conditions for chili pepper (Capsicum frutescens) fruit using Response Surface Methodology	No. 1, pp. 88-96
Ana Carolina Viviani Pagenotto	Interferencia inicial de Cyperus rotundus L. en plántulas pre-brotadas de caña de azúcar cultivares RB985476 e IACSP95-5000	No. 3, pp. 210-216
Ana Gabriela de Sousa Basílio	Radish (Raphanus sativus L.) morphophysiology under salinity stress and ascorbic acid treatments	No. 3, pp. 257-265
Ana Ligia Giraldeli	Interferencia inicial de Cyperus rotundus L. en plántulas pre-brotadas de caña de azúcar cultivares RB985476 e IACSP95-5000	No. 3, pp. 210-216
Nardy Diez	Two-dimensional gel electrophoresis to identify arcelins from Phaseolus vulgaris with inmuno-proteomic analysis	No. 2, pp. 114-119
Anderson Carlos de Melo Gonçalves	Radish (Raphanus sativus L.) morphophysiology under salinity stress and ascorbic acid treatments	No. 3, pp. 257-265
André Felipe Moreira Silva	Interferencia inicial de Cyperus rotundus L. en plántulas pre-brotadas de caña de azúcar cultivares RB985476 e IACSP95-5000	No. 3, pp. 210-216
Andrea Reyes	Effect of irrigation suspension on the growth, water state and production of spinach (Spinacia oleracea L.) plants	No. 2, pp. 120-125
Andrés Rodríguez	Preferences of Scirtothrips dorsalis Hood 1919 (Thysanoptera: Thripidae) for different structures of cotton (Gossypium hirsutum L.) plants in the Magdalena warm valley of Colombia	No. 1, pp. 42-50
Antonio Alberto da Silva	Interaction between timing of foliar fertilizer application and different Metribuzin doses in carrot	No. 1, pp. 35-41
Arturo Goldarazena	Preferences of Scirtothrips dorsalis Hood 1919 (Thysanoptera: Thripidae) for different structures of cotton (Gossypium hirsutum L.) plants in the Magdalena warm valley of Colombia	No. 1, pp. 42-50
Bernardo Navarrete	Development of a new maize hybrid for the Ecuadorian lowland	No. 2, pp. 174-179
Braulio Zarzoso	Optimization of fermentation process conditions for chili pepper (Capsicum frutescens) fruit using Response Surface Methodology	No. 1, pp. 88-96
Buenaventura Monje	Preferences of Scirtothrips dorsalis Hood 1919 (Thysanoptera: Thripidae) for different structures of cotton (Gossypium hirsutum L.) plants in the Magdalena warm valley of Colombia	No. 1, pp. 42-50
Carlos Vegas	Optimization of fermentation process conditions for chili pepper (Capsicum frutescens) fruit using Response Surface Methodology	No. 1, pp. 88-96
Carlos Yanez	Development of a new maize hybrid for the Ecuadorian lowland	No. 2, pp. 174-179
Carolina Bernal	Two-dimensional gel electrophoresis to identify arcelins from Phaseolus vulgaris with inmuno-proteomic analysis	No. 2, pp. 114-119
Cesar Baquero	Characterization and selection of Citrus sinensis Osbeck cv. Margaritera parental trees for repopulation in the Mompox depression region, Colombia	No. 2, pp. 103-113
Christiane Augusta Diniz Melo	Interaction between timing of foliar fertilizer application and different Metribuzin doses in carrot	No. 1, pp. 35-41
Daniel Alarcón-Cobeña	Development of a new maize hybrid for the Ecuadorian lowland	No. 2, pp. 174-179
David Revelo-Luna	Spectral and thermal response of Heliconia psittacorum species to induced water stress	No. 3, pp. 237-247

Continúa

Author name	Article title	Location
Daynet Sosa	Two-dimensional gel electrophoresis to identify arcelins from Phaseolus vulgaris with inmuno-proteomic analysis	No. 2, pp. 114-119
Eddie Zambrano-Zambrano	Development of a new maize hybrid for the Ecuadorian lowland	No. 2, pp. 174-179
Edgar Varón	Preferences of Scirtothrips dorsalis Hood 1919 (Thysanoptera: Thripidae) for different structures of cotton (Gossypium hirsutum L.) plants in the Magdalena warm valley of Colombia	No. 1, pp. 42-50
Eduardo Barragán-Quijano	Effect of sowing density on yield and profitability of a hybrid corn under tropical conditions	No. 3, pp. 128-256
Edwin Restrepo	Yield heterosis and average fruit weight as a function of inbreeding in Cucurbita moschata Duch. ex Poir.	No. 1, pp. 5-12
Elcio Ferreira Dos Santos	Boron-zinc interaction in the absorption of micronutrients by cotton	No. 1, pp. 51-57
Eliana Suárez	Empowerment and associative process of rural women: a case study of rural areas in Bogotá and Cundinamarca, Colombia	No. 2, pp. 158-165
Ender Correa	Characterization and selection of Citrus sinensis Osbeck cv. Margaritera parental trees for repopulation in the Mompox depression region, Colombia	No. 2, pp. 103-113
Enrique A. Torres	Assessing water demand with remote sensing for two coriander varieties	No. 3, pp. 266-273
Érica de Oliveira Araújo	Boron-zinc interaction in the absorption of micronutrients by cotton	No. 1, pp. 51-57
Erika Sánchez-Betancourt	Fertility recovery of anther-derived haploid plants in Cape gooseberry (Physalis peruviana L.)	No. 3, pp. 201-209
Euseppe Ortiz	Assessing water demand with remote sensing for two coriander varieties	No. 3, pp. 266-273
Everth Ebratt	Preferences of Scirtothrips dorsalis Hood 1919 (Thysanoptera: Thripidae) for different structures of cotton (Gossypium hirsutum L.) plants in the Magdalena warm valley of Colombia	No. 1, pp. 42-50
Federica Romagnoli	How to improve smallholder market access: Evaluation of Mercados Campesinos in Colombia	No. 1, pp. 79-87
Felipe Carrara de Brito	Interferencia inicial de Cyperus rotundus L. en plántulas pre-brotadas de caña de azúcar cultivares RB985476 e IACSP95-5000	No. 3, pp. 210-216
Franco Vallejo	Yield heterosis and average fruit weight as a function of inbreeding in Cucurbita moschata Duch. ex Poir.	No. 1, pp. 5-12
Francy Garcia-Arias	Fertility recovery of anther-derived haploid plants in Cape gooseberry (Physalis peruviana L.)	No. 3, pp. 201-209
Gabriella Daier Oliveira Pessoa Carneiro	Interaction between timing of foliar fertilizer application and different Metribuzin doses in carrot	No. 1, pp. 35-41
Gerhard Fischer	Post-harvest quality of pineapple guava [Acca sellowiana (O. Berg) Burret] fruits produced in two locations at different altitudes in Cundinamarca, Colombia	No. 1, pp. 68-78
Gerhard Fischer	Pre-harvest factors that influence the quality of passion fruit: A review	No. 3, pp. 217-226
Giovani Apolari Ghirardello	Interferencia inicial de Cyperus rotundus L. en plántulas pre-brotadas de caña de azúcar cultivares RB985476 e IACSP95-5000	No. 3, pp. 210-216
Gustavo Soares da Silva	Interaction between timing of foliar fertilizer application and different Metribuzin doses in carrot	No. 1, pp. 35-41
Helena Brochero	Preferences of Scirtothrips dorsalis Hood 1919 (Thysanoptera: Thripidae) for different structures of cotton (Gossypium hirsutum L.) plants in the Magdalena warm valley of Colombia	No. 1, pp. 42-50
Helena Jaramillo	Molecular characterization of Potato virus Y (PVY) and Potato virus V (PVV) isolates naturally infecting cape gooseberry (Physalis peruviana) in Antioquia, Colombia	No. 1, pp. 13-23
Iván Galindo-Castro	Two-dimensional gel electrophoresis to identify arcelins from Phaseolus vulgaris with inmuno-proteomic analysis	No. 2, pp. 114-119
Javier Álvarez-Herrera	Effect of irrigation suspension on the growth, water state and production of spinach (Spinacia oleracea L.) plants	No. 2, pp. 120-125
Javier Orlando Orduz-Rodriguez	Characterization and selection of Citrus sinensis Osbeck cv. Margaritera parental trees for repopulation in the Mompox depression region, Colombia	No. 2, pp. 103-113
Javier Restrepo	Yield heterosis and average fruit weight as a function of inbreeding in Cucurbita moschata Duch. ex Poir.	No. 1, pp. 5-12
Javier Vélez	Evaluation of irrigation in roses in a suitable substrate with three water depths and the percentage of drainage	No. 3, pp. 274-283
Jessica Vargas	Physicochemical characteristics of strawberry (Fragaria x ananassa Duch.) fruits from four production zones in Cundinamarca, Colombia	No. 3, pp. 227-236
Jesús Hernán Camacho-Tamayo	Post-harvest quality of pineapple guava [Acca sellowiana (O. Berg) Burret] fruits produced in two locations at different altitudes in Cundinamarca, Colombia	No. 1, pp. 68-78
Joana Gomes de Moura	Radish (Raphanus sativus L.) morphophysiology under salinity stress and ascorbic acid treatments	No. 3, pp. 257-265

Continúa

Author name	Article title	Location
Joel Girón	Ham salting in plastic bags: A way to reduce salt use?	No. 2, pp. 166-173
John Acuña	Physicochemical characteristics of strawberry (Fragaria x ananassa Duch.) fruits from four production zones in Cundinamarca, Colombia	No. 3, pp. 227-236
John Arévalo	Evaluation of irrigation in roses in a suitable substrate with three water depths and the percentage of drainage	No. 3, pp. 274-283
Jorge Valencia	Evaluation of irrigation in roses in a suitable substrate with three water depths and the percentage of drainage	No. 3, pp. 274-283
José Eguez	Development of a new maize hybrid for the Ecuadorian lowland	No. 2, pp. 174-179
José Flavio Cardoso Zuza	Organic substrate and organic foliar fertilizer effect on chlorophyll index and growth in Umbuzeiro seedlings	No. 2, pp. 152-157
José Isidro Beltrán	Effect of sowing density on yield and profitability of a hybrid corn under tropical conditions	No. 3, pp. 128-256
José L. Zambrano	Development of a new maize hybrid for the Ecuadorian lowland	No. 2, pp. 174-179
José Manuel Barat	Ham salting in plastic bags: A way to reduce salt use?	No. 2, pp. 166-173
José Sebastião de Melo Filho	Radish (Raphanus sativus L.) morphophysiology under salinity stress and ascorbic acid treatments	No. 3, pp. 257-265
Joseph Cutler	Pre-harvest factors that influence the quality of passion fruit: A review	No. 3, pp. 217-226
Josinaldo da Silva Henrique	Organic substrate and organic foliar fertilizer effect on chlorophyll index and growth in Umbuzeiro seedlings	No. 2, pp. 152-157
Juan Molina	How to improve smallholder market access: Evaluation of Mercados Campesinos in Colombia	No. 1, pp. 79-87
Júlia Pereira de Moraes	Interferencia inicial de Cyperus rotundus L. en plántulas pre-brotadas de caña de azúcar cultivares RB985476 e IACSP95-5000	No. 3, pp. 210-216
Julio Galindo	Growth response surface for optimizing fertilization in Andean blackberry (Rubus glaucus Benth.) nurseries	No. 2, pp. 135-142
Laura Katalina González	Effect of different sources of nitrogen on the vegetative growth of Andean blueberry (Vaccinium meridionale Swartz)	No. 1, pp. 58-67
Laura Natalia Rugeles	Effect of different sources of nitrogen on the vegetative growth of Andean blueberry (Vaccinium meridionale Swartz)	No. 1, pp. 58-67
Leonardo Vieira de Sousa	Radish (Raphanus sativus L.) morphophysiology under salinity stress and ascorbic acid treatments	No. 3, pp. 257-265
Luz M. Melgarejo	Pre-harvest factors that influence the quality of passion fruit: A review	No. 3, pp. 217-226
Manuel Vergara	Physicochemical characteristics of strawberry (Fragaria x ananassa Duch.) fruits from four production zones in Cundinamarca, Colombia	No. 3, pp. 227-236
Marcelo Rodrigues dos Rei	Interaction between timing of foliar fertilizer application and different Metribuzin doses in carrot	No. 1, pp. 35-41
Marco Heli Franco-Valencia	Life plan for the Yaquivá indigenous reservation in the municipality of Inza, Cauca Colombia, from the perspective of agroecology	No. 2, pp. 143-151
Marcos Antonio Camacho	Boron-zinc interaction in the absorption of micronutrients by cotton	No. 1, pp. 51-57
María Ramírez	Growth response surface for optimizing fertilization in Andean blackberry (Rubus glaucus Benth.) nurseries	No. 2, pp. 135-142
Marina Sánchez de Prager	Life plan for the Yaquivá indigenous reservation in the municipality of Inza, Cauca Colombia, from the perspective of agroecology	No. 2, pp. 143-151
Marlon Caicedo	Development of a new maize hybrid for the Ecuadorian lowland	No. 2, pp. 174-179
Marlon Yacomelo	Characterization and selection of Citrus sinensis Osbeck cv. Margaritera parental trees for repopulation in the Mompox depression region, Colombia	No. 2, pp. 103-113
Martha Bolaños	Growth response surface for optimizing fertilization in Andean blackberry (Rubus glaucus Benth.) nurseries	No. 2, pp. 135-142
Mauricio Marín	Molecular characterization of Potato virus Y (PVY) and Potato virus V (PVV) isolates naturally infecting cape gooseberry (Physalis peruviana) in Antioquia, Colombia	No. 1, pp. 13-23
Mauricio Martínez	Characterization and selection of Citrus sinensis Osbeck cv. Margaritera parental trees for repopulation in the Mompox depression region, Colombia	No. 2, pp. 103-113
Miguel Peña-Varón	Spectral and thermal response of Heliconia psittacorum species to induced water stress	No. 3, pp. 237-247
Murielle Magda Medeiros Dantas	Organic substrate and organic foliar fertilizer effect on chlorophyll index and growth in Umbuzeiro seedlings	No. 2, pp. 152-157

Continúa

Author name	Article title	Location
Natalia Álvarez	Molecular characterization of Potato virus Y (PVY) and Potato virus V (PVV) isolates naturally infecting cape gooseberry (Physalis peruviana) in Antioquia, Colombia	No. 1, pp. 13-23
Nubia Murcia	Characterization and selection of Citrus sinensis Osbeck cv. Margaritera parental trees for repopulation in the Mompox depression region, Colombia	No. 2, pp. 103-113
Oscar Alvarado	Effect of irrigation suspension on the growth, water state and production of spinach (Spinacia oleracea L.) plants	No. 2, pp. 120-125
Pablo Andrés Gutiérrez	Molecular characterization of Potato virus Y (PVY) and Potato virus V (PVV) isolates naturally infecting cape gooseberry (Physalis peruviana) in Antioquia, Colombia	No. 1, pp. 13-23
Paúl Villavicencio-Linzan	Development of a new maize hybrid for the Ecuadorian lowland	No. 2, pp. 174-179
Raúl Grau	Ham salting in plastic bags: A way to reduce salt use?	No. 2, pp. 166-173
Raunira da Costa Araújo	Organic substrate and organic foliar fertilizer effect on chlorophyll index and growth in Umbuzeiro seedlings	No. 2, pp. 152-157
Ricardo Limongi-Andrade	Development of a new maize hybrid for the Ecuadorian lowland	No. 2, pp. 174-179
Ricardo Victoria Filho	Interferencia inicial de Cyperus rotundus L. en plántulas pre-brotadas de caña de azúcar cultivares RB985476 e IACSP95-5000	No. 3, pp. 210-216
Sara Del Castillo	Empowerment and associative process of rural women: a case study of rural areas in Bogotá and Cundinamarca, Colombia	No. 2, pp. 158-165
Silvio Lopez-Pazos	Screening of Colombian soybean genotypes for Agrobacterium mediated genetic transformation conferring tolerance to Glyphosate	No. 1, pp. 24-34
Stanislav Magnitskiy	Effect of different sources of nitrogen on the vegetative growth of Andean blueberry (Vaccinium meridionale Swartz)	No. 1, pp. 58-67
Teresa Mosquera	Empowerment and associative process of rural women: a case study of rural areas in Bogotá and Cundinamarca, Colombia	No. 2, pp. 158-165
Thiago Jardelino Dias	Radish (Raphanus sativus L.) morphophysiology under salinity stress and ascorbic acid treatments	No. 3, pp. 257-265
Toshik larley da Silva	Radish (Raphanus sativus L.) morphophysiology under salinity stress and ascorbic acid treatments	No. 3, pp. 257-265
Valesca Pinheiro Miranda	Interaction between timing of foliar fertilizer application and different Metribuzin doses in carrot	No. 1, pp. 35-41
Víctor Núñez	Fertility recovery of anther-derived haploid plants in Cape gooseberry (Physalis peruviana L.)	No. 3, pp. 201-209
William Cardona	Growth response surface for optimizing fertilization in Andean blackberry (Rubus glaucus Benth.) nurseries	No. 2, pp. 135-142
Yeison Mauricio Quevedo	Effect of sowing density on yield and profitability of a hybrid corn under tropical conditions	No. 3, pp. 128-256
Ygor Henrique Leal	Radish (Raphanus sativus L.) morphophysiology under salinity stress and ascorbic acid treatments	No. 3, pp. 257-265
Yuliana Gallo	Molecular characterization of Potato virus Y (PVY) and Potato virus V (PVV) isolates naturally infecting cape gooseberry (Physalis peruviana) in Antioquia, Colombia	No. 1, pp. 13-23

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