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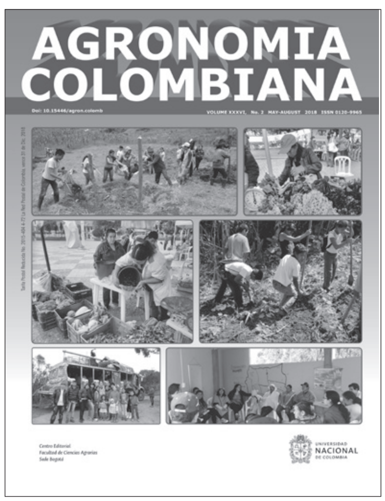

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Characterization and selection of *Citrus sinensis* Osbeck cv. Margaritera parental trees for repopulation in the Mompox depression region, Colombia

Caracterización y selección de árboles madre de naranja cv. Margaritera, *Citrus sinensis* Osbeck, para repoblamiento en la depresión momposina, Colombia

Marlon Yacomelo[1], Cesar Baquero[1], Mauricio Martínez[2], Nubia Murcia[2], Ender Correa[1], and Javier Orlando Orduz-Rodriguez[3]*

ABSTRACT

At the Mompox depression region, located in the lower valley of the Magdalena River, citrus trees and especially the orange cultivar Margaritera (*Citrus sinensis* Osbeck) are cultivated by small producers. The effects of the rainy season during 2010 and 2011 caused the loss of ca. 80% of the planted area in some municipalities. The main objective of this study was to select outstanding genotypes according to fruit quality attributes based on the Colombian Technical Standard NTC4086. This selection was carried out by collecting and characterizing 120 Margaritera orange genotypes represented by three clones: Margarita, Azúcar and Criollo. The characterization was performed using 14 fruit descriptors regulated by the parameters of the International Plant Genetic Resources Institute. Outstanding clones were chosen using a selection index based on attributes such as juice percentage and maturity index. The fruit characterization showed variability for all the descriptors evaluated, especially those related to size, weight and acidity (explained 51.76% of the total variability). Furthermore, these contributed mostly to comprise three phenotypic groups conformed by 79, 23 and 18 genotypes, respectively. Eight outstanding genotypes were selected for quality attributes: four Margarita clones, two Azúcar clones and two Criollo clones; these became a source of guaranteed propagation material in the region.

Key words: Margarita clone, Azucar clone, Criollo clone, selection index, quality attributes.

RESUMEN

En la depresión momposina-valle bajo del Río Magdalena, Colombia, los cítricos y especialmente el cultivar de naranja Margaritera (*Citrus sinensis* Osbeck) son cultivados por pequeños productores. La ola invernal en 2010-2011 ocasionó la pérdida aproximada del 80% del área sembrada en algunos municipios. El objetivo de esta investigación fue seleccionar genotipos sobresalientes según atributos de calidad de fruta con base en la Norma Técnica Colombiana NTC4086. Para ello se colectaron y caracterizaron 120 genotipos de naranja Margaritera representados por tres clones: Margarita, Azúcar y Criollo. En la caracterización se utilizaron 14 descriptores de fruto siguiendo los parámetros del Instituto Internacional de Recursos Fitogenéticos. Los clones sobresalientes se escogieron mediante un índice de selección basado en atributos de porcentaje de jugo e índice de madurez. La caracterización del fruto mostró variabilidad en los descriptores evaluados, especialmente los relacionados con tamaño, peso y acidez (explicaron el 51,76% de la variabilidad total). Igualmente, contribuyeron a la conformación de tres grupos fenotípicos de 79, 23 y 18 genotipos, respectivamente. Se seleccionaron ocho genotipos sobresalientes según atributos de calidad: cuatro del clon Margarita, dos del Azúcar y dos del Criollo; estos se convierten en una fuente de material de propagación garantizado en la región.

Palabras clave: clon Margarita, clon Criollo, clon Azúcar, índice de selección, atributos de calidad.

Introduction

Citrus plants are native to the tropical and subtropical regions of Asia and the Malay Archipelago, and their commercial production is concentrated between 20° and 40° latitude in both hemispheres (Roose *et al*., 2015) incorporating an uncertain number of species (Albert *et al*., 2018). According to FAO, citrus plants are the main fruit crop cultivated worldwide reaching an approximate production of 139,796,997 metric t and a planted area of 9,080,780 ha in 2014 (FAO, 2004; FAOSTAT, 2014). During this same year in Colombia, the area planted with citrics was 97,275 ha with a production of 1,206,856 t per year, being orange the main cultivated citrus species with 54,711 ha of cultivated area that produced 669,187 t (ASOHOFRUCOL, 2016; ENA-DANE, 2016). Orange cultivation relies on the

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use of different clones such as Valencia and Sweety, and in smaller areas clones such as Salustina, Hamlin and various selections of Criollo materials are used (Orduz and Mateus, 2012; Ramírez *et al.,* 2014).

Among the Criollo materials the Margaritera orange stands out; it is grafted on bitter orange (*Citrus aurantium* L.) tree rootstock. Margaritera orange has been bred according to the necessities of the region's producers, finding three clones: Margarita, Azúcar and Criollo. These bred lines differ in their acidity concentrations and total soluble solids, being the Azúcar clones the ones that showed the lowest acidity and the highest total soluble solids. These clones are adapted to the edaphoclimatic conditions of the Mompox depression region and its surroundings, where average monthly temperatures exceed 25°C all year round, daily minimum temperatures are rarely less than 20°C, and the maximums frequently exceed 32°C. The average bimodal annual rainfall (first peak in May and a second peak in October) decreases from 2,200 mm in the south and southeast, and in Cauca and Magdalena rivers to less than 1,000 mm where Loba and Mompox converge (García, 2001). Margaritera orange is cultivated mainly by small-scale producers in the municipalities of Santa Cruz de Mompox, San Fernando and Margarita in the province of Bolívar, and in Guamal, Santa Ana, San Zenón and San Sebastián in the south of the province of Magdalena; this orange has outstanding attributes of 35.22% juice and good taste, making it attractive for domestic and export markets; however, despite the high acceptance of these clones by producers, basic aspects of their phenotypic variability are still unknown.

During the last winter season in 2010-2011, ca. 80% of the orange trees in some municipalities of the Mompox region were affected or destroyed by floods (Huertas *et al.,* 2014; Agronet, 2017).This situation placed the genetic resource of the region at risk. Therefore, the objective of this study was to characterize and select high value Margaritera orange clones, with the purpose of providing propagation material for nurseries, so basic gardens can be established. Once these gardens are established, they can be used as a basis for repopulation in the region as they are part of the producers' subsistence economic strategy. Furthermore, the constant supply of plant breeding can increase the fruit consumption in the cities of the Caribbean coast.

Materials and methods

Phenotypic characterization of fruits from the selected genotypes

A recognition track was established in the Mompox depression in the municipalities of San Fernando, Margarita and Santa Cruz de Mompox in the province of Bolívar,

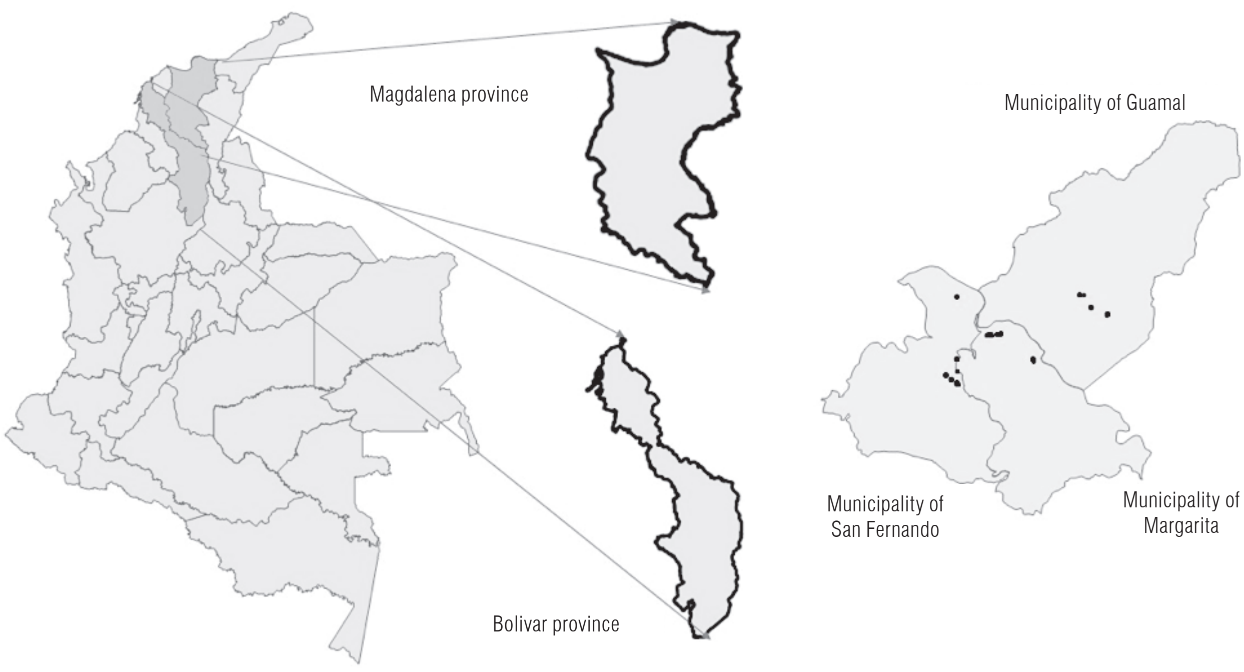

FIGURE 1. Sampled municipalities and geographic location of the genotypes characterized.

and in the municipalities of Guamal and San Sebastián in the province of Magdalena, assessing the citrus fruits productive areas and identifying the crops lines that survived the harsh rainy season occurred in 2011. After analyzing the location data, a focused sampling was carried out, considering Margaritera orange trees as the bred line with higher productivity, better fruit quality, more vigorous and longer-life cycle. Twenty-five farms were visited selecting 120 tree genotypes (Fig. 1), and from these, five fruits of the middle third of the tree crown (one for each cardinal point and a randomly selected one) were chosen per tree. During collection, the fruits were selected following the quality criteria registered in the Icontec NTC4086 regulation (Icontec, 1997). Under these statements, whole fruits with specific features of the Valencia orange were selected. Such traits included: presence of the calyx, healthy tissues, and whole fruits without any symptoms of abnormal external humidity caused by poor postharvest management. The fruits should not have any external and strange smell and/or taste (stemming from other products, packages or recipients and/or agrochemicals that might have been in contact with the fruits). Fresh fruits should also have a firm consistency and be free of any visible strange materials (such as dirt, dust, agrochemicals and foreign objects). Each genotype was codified according to the producer's criteria as follows: a Margarita (Mar) clone, an Azúcar (Azuc) clone or a Criollo (Crio) clone. The fruits were then analyzed in the laboratory using 14 quantitative descriptors for *Citrus* spp. published by IPGRI (2000). For the information analysis, a completely randomized design with 120 treatments and five replicates was used, in which treatments corresponded to the trees sampled and the replicates corresponded to the fruits per tree.

Selection of outstanding clones

A selection index (SI) with standardized variables (mean = 0 and variance = 1) that included variables such as juice percentage (JP) and maturity index (MI) was established; this last one is a variable obtained from the relation between total soluble solids (TSS) and titratable acidity (TA); these are the main attributes considered when orange is commercialized in the agro-industrial markets. According to the Colombian Technical Standards Institute (Icontec) regulation (NTC4086), for the commercialization of Valencia orange (maturity stage I), the desired TSS concentration is 8.2 °Brix, TA of 1.5% citric acid, MI of 5.5 and a JP higher than 40%. Therefore, and after fulfilling the required attributes, the SI (described in equation 1) was established. According to the SI, 7% of the genotypes that comprise the groups obtained in the phenotypic characterization were selected in order to maintain the best clones with the highest variability.

Equation 1:

$$SI = [(JP \times 10) - (|\, 5.5 - MI \,| \times 8)]$$

where:

SI: standardized selection index

JP: juice percentage (%)

MI: maturity index

Statistical analysis

Data obtained in the morpho-agronomic characterization were analyzed using the statistical program SAS® Enterprise Guide® version 5.1 (2012). A descriptive analysis (mean, standard deviation, standard error, coefficient of variation, minimum and maximum values) was carried out to establish the variability of the quantitative characteristics at the level of the selected and characterized trees; an ANOVA and Tukey test of multiple comparisons were also applied with the evaluated variables. A principal component analysis (PCA) was further carried out to establish the descriptors with the highest contribution to total variability. Next, the 120 genotypes were grouped with a cluster analysis obtaining relatively homogeneous groups (Franco and Hidalgo, 2003).

Results and discusion

Phenotypic characterization

The descriptive statistics such as averages, standard deviation, coefficient of variation and minimum and maximum values of the 120 genotypes of Margaritera orange collected are shown in Table 1. In general, the quantitative morphological characteristics showed a low coefficient of variation (CV<26%), except for the titratable acidity descriptors (CV = 40.32%), number of seeds per fruit (CV = 39.49%), resistance to penetration (CV = 34.72%) and fruit peel weight (CV = 34.17%); these are important when discriminating the variability of Margaritera orange genotypes. Moreover, the descriptors that showed lower coefficient of variation were fruit diameter length with 8.09% and 9.55% respectively. The characteristics that showed the highest dispersion values compared to their sampling means were: fruit weight that showed a minimum value of 94.10 g and a maximum value of 472.50 g; fruit peel weight with a minimum value of 23.30 g and 157.90 g as its maximum value; and fruit content with a minimum value of 37 g and a maximum value of 151 g. These values agreed with the ones reported

TABLE 1. Descriptive statistics for 14 quantitative variables of 120 genotypes of the orange Margaritera cultivar (*Citrus* spp.) collected in the Mompox depression.

Descriptors	Mean	SD	Minimum	Maximum	VAR	CV
Fruit weight (g)	205.46	50.96	94.10	472.50	2,597.36	24.80
Fruit diameter (mm)	73.35	5.93	56.20	96.10	35.21	8.09
Fruit length (mm)	71.61	6.84	53.60	100.30	46.79	9.55
Peel thickness (mm)	4.85	0.97	2.60	8.50	0.93	19.99
Fruit axis diameter (mm)	7.20	1.84	4.30	12.30	3.40	25.62
Vesicle length (mm)	14.38	1.83	10.10	19.30	3.35	12.75
Vesicle thickness (mm)	2.06	0.31	1.50	2.80	0.09	15.18
Fruit peel weight (g)	54.82	18.73	23.30	157.90	350.96	34.17
Juice content (g)	72.38	17.05	37.00	151.00	290.85	23.56
Titratable acidity (% citric acid)	0.62	0.25	0.11	1.20	0.06	40.32
pH	3.97	0.58	3.30	5.60	0.33	14.61
Resistance to penetration (kg cm^{-2})	5.75	1.99	2.90	16.00	3.97	34.72
Seeds/fruit	7.50	2.96	0.00	18.00	8.77	39.49
Total soluble solids (°Brix)	9.97	1.15	7.26	13.58	1.31	11.51

SD: standard deviation; VAR: variance; CV: coefficient of variation.

by Duran and Luz (2013), who found fruit weights between 64.6 and 365 g in Valencia oranges cultivated in Chimichagua, department of Cesar.

Correlation analysis

The correlation matrix indicates associations that are statistically significant ($P\leq0.05$) and highly significant ($P\leq0.01$). The most outstanding correlations were fruit weight (FW) compared to: fruit length (FW vs. FL = 0.92**), fruit diameter (FW vs. FD = 0.87**), fruit peel weight (FW vs. FPW = 0.81**) and juice content (FW vs. JC = 0.76**). Meanwhile, the fruit peel weight reported an important correlation (FPW) with peel thickness (FPW vs. PT = 0.81**), fruit length (FWP vs. FL = 0.77**) and fruit diameter (FPW vs. FD = 0.74**). Equally important was the correlation between fruit diameter and fruit length (FD vs. FL = 0.88**), and fruit diameter with juice content (FD vs. JC = 0.67**). Finally, the correlation between fruit length with juice content (FL vs. JC = 0.70**), and titratable acidity with pH (TA vs. pH = -0.7**) were representative as well (Tab. 2).

High correlations between the FW with fruit dimensions (FL and FD) and filling (FPW and JC) were expected. To support this statement, higher fruit dimensions, filling rates, and higher weight, were assessed as the physiological response of the accumulation of metabolites in the fruit development. This effect may be limited whether by the inability of the fruit to accumulate metabolites or by the lack of them within the plant (Agustí and Almela, 1991). Likewise, Bain (1958) characterized the general development pattern of the Valencia orange fruits in the subtropical conditions of Australia, as a simple sigmoidal curve from anthesis to maturation. The same trend was also described by several authors such as Pérez de Camacaro and Jiménez (2009) for the same species in a vegetable garden in the State of Portuguesa in Venezuela, Garzón *et al.* (2013) in the foothills of Llanos Orientales in Colombia, and Orduz *et al.* (2009) in Arrayana mandarin in foothill conditions in Meta.

Furthermore, the titratable acidity and pH showed a negative correlation; when higher juice pH was found, a lower titratable acidity was also found. These variables are related, as the titratable acidity measures the total acid concentrations which are mostly organic acids such as citric, malic, lactic and tartaric acids that influence flavor, color and juice stability. Moreover, pH quantifies H_3O^+ concentration which can be considered as active acidity. In this regard, this value is of great importance for food industries, because it determines the use and control of microorganisms and enzymes driving processes, such as the clarification and stabilization of fruit and vegetable juices, as well as fermented byproducts. In Table 2, Pearson's correlation data for 14 quantitative fruit characteristics in Margaritera orange are presented.

Principal component analysis

PCA showed that 78.82% of the variability was explained by five main or principal components (PC) (Tab. 3). The first PC explains most of the variability with 38.41%, followed by the second 13.50%, the third PC 11.19%, the fourth 8.24% and the fifth PC 7.48%.

TABLE 2. Pearson's correlation results for 14 quantitative fruit characteristics in Margaritera orange (*Citrus* spp.).

Descriptors	FD	FL	PT	FAD	VL	VT	FPW	JC	TA	pH	RP	SF	TSS
Fruit weight (FW)	**0.87****	**0.92****	0.59**	0.37**	0.41**	0.32*	**0.81****	**0.76****	-0.20*	0.16ns	0.05ns	-0.01ns	-0.28*
Fruit diameter (FD)		**0.88****	0.57**	0.37**	0.38**	0.37**	**0.74****	**0.67****	-0.27*	0.19*	0.03*	0.07ns	-0.20*
Fruit length (FL)			0.57**	0.38**	0.37**	0.39**	**0.77****	**0.70****	-0.16ns	0.13ns	0.11ns	0.00ns	-0.35**
Peel thickness (PT)				0.22*	0.30*	0.10ns	**0.81****	0.32*	-0.19*	0.07ns	0.24ns	-0.10ns	-0.05ns
Fruit axis diameter (FAD)					0.079ns	0.40**	0.48**	0.12ns	0.06ns	0.02ns	0.24*	-0.18*	-0.03ns
Vesicle length (VL)						0.01ns	0.39**	0.47**	-0.28*	0.13*	-0.08ns	-0.0ns	-0.21*
Vesicle thickness (VT)							0.26*	0.33*	0.02ns	0.11ns	0.02ns	-0.07ns	-0.13ns
Fruit peel weight (FPW)								0.57**	-0.14ns	0.03ns	0.21*	-0.17*	-0.19*
Juice content (JC)									-0.19*	0.08ns	-0.18*	-0.02ns	-0.45**
Titratable acidity (TA)										**-0.70****	0.24*	0.04ns	-0.12ns
pH											-0.0ns	-0.00ns	0.19ns
Resistance to penetration (RP)												-0.12ns	0.00ns
Seeds/fruit (SF)													0.05ns
Total soluble solids (TSS)													1

** Highly significant ($P \leq 0.01$); * significant ($P \leq 0.05$); ns: not significant ($P > 0.05$); bold values indicate highly significant correlations ($\leq$-0.6 and $\geq$0.6).

TABLE 3. Contribution of quantitative variables to the conformation of the first five main components (PC) of the Margaritera orange (*Citrus* spp.) collected at the Mompox depression.

Variables	PC1	PC2	PC3	PC4	PC5
Fruit weight	0.40*	0.03	-0.06	-0.031	0.09
Fruit diameter	039*	-0.02	-0.33	0.03	0.21
Fruit length	040*	0.07	-0.06	0.02	0.10
Peel thickness	0.30	0.01	0.23	-0.41	0.06
Fruit axis diameter	0.19	0.24	0.35	0.34	0.01
Vesicle length	0.22	-0.19	-0.19	-0.25	-0.23
Vesicle thickness	0.18	0.15	0.06	0.69*	0.06
Fruit peel weight	0.38	0.10	0.15	-0.19	-0.01
Juice content	0.33	-0.03	-0.36	0.09	-0.11
Titratable acidity	-0.12	0.63*	-0.11	-0.05	0.08
pH	0.11	-0.54*	0.30	0.19	-0.03
Penetration resistance	0.04	0.31	0.47*	-0.26	0.02
Seeds/fruit	-0.03	-0.07	-030	-0.06	0.82*
Total soluble solids	-0.14	-0.21	0.43*	-0.02	0.40
% Variance	38.41	13.50	11.19	8.24	7.48

The descriptors with the greatest contribution to phenotypic variability in the first three principal components are mainly associated with fruit dimensions (fruit weight, diameter and length) and the chemical characteristics of the juice (titratable acidity, pH and total soluble solids); This results confirm what was stated by Goldenberg *et al.* (2018) and Agustí *et al.* (2003), in which the fruit size of a variety can vary between a wide margin and is regulated by a set of endogenous factors (genetic factors, fruit position, competition between different growing organs) and exogenous factors (environmental factors and cultural practices); Lado *et al.* (2018) and Reuther and Rios-Castaño

(1969) described that juice acidity and total soluble solids are regulated by environmental conditions, mainly temperature. However, authors like Nii *et al.* (1970), Reuther (1973) and Yamanishi (1994) mention that the influence of temperature on total soluble solids (TSS) is not so clear; for example, in Valencia orange the highest TSS contents are obtained in fruits from subtropical areas, but the conclusion of many studies, combining thermal regimes, climates and crop zones in different fruit development states, is the lack of systematic response of the TSS content to the thermal variations.

Cluster analysis

Multivariate-cluster analysis generated three phenotypic groups (GI, GII and GIII) (Fig. 2). Group GI comprises 79 genotypes of which 93.6% belong to the Margarita clone and 6.4% belong to genotypes of the Criollo clone. Group GII is comprised by 23 genotypes of which 60.86% belong to the Margarita clone and 39.14% genotypes belong to the Criollo clone. Finally, group GIII comprises 18 genotypes, of which 80.33% are identified as Azúcar clones, 11.11% belong to genotypes of the Margarita clone, and one (1) genotype belongs to the Criollo clone (see Supplementary material 1).

Comparing characteristics of the groups comprised, significant differences were found (ANOVA≤0.05) with the exception of attributes: number of seeds per fruit and resistance to penetration. The GI genotypes are mainly characterized by having smaller fruits (FD = 70.92 mm and FL = 68.88 mm) and lower weight (183.73 g), lower MI (15.13) and higher JP (36.80%). On the other hand, the genotypes of the GII have larger fruits (FD = 79.59 mm and FL = 79.41 mm), lower weight (FW = 270.79 g) and lower JP (33.35%). Lastly, the GIII shows fruits with an intermediate size and weight compared to fruit characteristics found in GI and GII; this highlights the fact that the main attribute that identifies the genotypes of this group are the low titratable acidity of the juice and higher pH (TA = 0.21% citric acid and pH = 5.23), which is evidenced by the high maturity index (MI = 66.78) (Tab. 4).

The results corroborate, to a great extent, the appreciations mentioned by producers, confirming the existence of three clones selected by organoleptic characteristics, also emphasizing that these attributes are maintained when asexual propagation is carried out. Quality attributes reported for the Margaritera orange cultivar are characteristic of Valencia orange cultivated in the tropical zone below 300 m a.s.l.; with these conditions fruits develop a green color when ripe, with high concentrations of soluble solids and low acidity (González, 2014). These features differentiate it from the attributes of the Valencia orange cultivated in the subtropical zone and make it difficult to export to markets in developed countries because these characteristics do not meet the optimum requirements for agro-industrial processing (Aguilar, 2012). However, Valencia orange and especially the Margaritera have an assured market niche for fresh consumption, since there is great local demand with greater marketing opportunities and better prices than those offered by the agroindustry (MADR, 2005). Additionally, in Colombia a large part of the citric demands are met with imports from other countries, and oranges represent the highest percentage with 50.6% as orange juice and 17% as fresh fruit (Aguilar, 2012).

It is important to indicate that quality attributes demanded by fruit processing industries are difficult to meet for the Margaritera orange, considering that citrus fruits are native from subtropical zones and in those latitudes annual growth and development cycles of citrics are regulated by climatic seasons (winter, spring, summer and autumn).

TABLE 4. Descriptive analysis of fruit characteristics from the groups obtained in the phenotypic characterization.**GI (79):** group 1 with 79 genotypes; **GII (23):** group 2 with 23 genotypes; **GIII (18):** group 3 with 18 genotypes; CV: coefficient of variation.

Descriptors		FW (g)	FD (mm)	FL (mm)	PT (mm)	FAD (mm)	VL (mm)	VT (mm)	FPW (g)	JC (g)	TA (%citric acid)	pH	PR (km cm^{-2})	SF (u)	SST (ºBrix)	MI	JP (%)
GI (79)	Mean	183.73 c	70.92 c	68.9 c	4.52 b	6.69 b	13.9 b	2.01 a	47.4 c	67.1 b	0.72 a	3.72 b	5.73 a	7.75 a	10.1 b	15.1 b	36.8 a
	SD	29.87	4.58	5.08	0.79	1.64	1.81	0.33	11.22	12.89	0.19	0.19	2.27	3.00	1.01	4.38	5.81
GII (23)	Mean	270.79 a	79.59 a	79.41 a	5.69 a	8.59 a	15.7 a	2.15 a	76.7 a	89.6 a	0.63 a	3.83 b	5.74 a	6.7 a	8.92 c	15.3 b	33.4 a
	SD	53.64	5.08	5.77	0.98	1.79	1.49	0.25	21.91	18.49	0.16	0.19	1.44	2.58	0.73	4.67	4.72
GIII (18)	Mean	217.38 b	76 b	73.63 b	5.22 a	7.64 a b	14.9 a b	2.19 a	59.6 b	73.4 b	0.21 b	5.23 a	5.83 a	7.44 a	10.8 a	66.8 a	34.12 a
	SD	47.38	5.43	6.7	0.92	1.82	1.38	0.28	18.2	17.3	0.13	0.4	1.2	3.2	1.28	27.4	5.75

Means with distinct letters indicate significant difference after the Tukey test (*P*≤0.05).

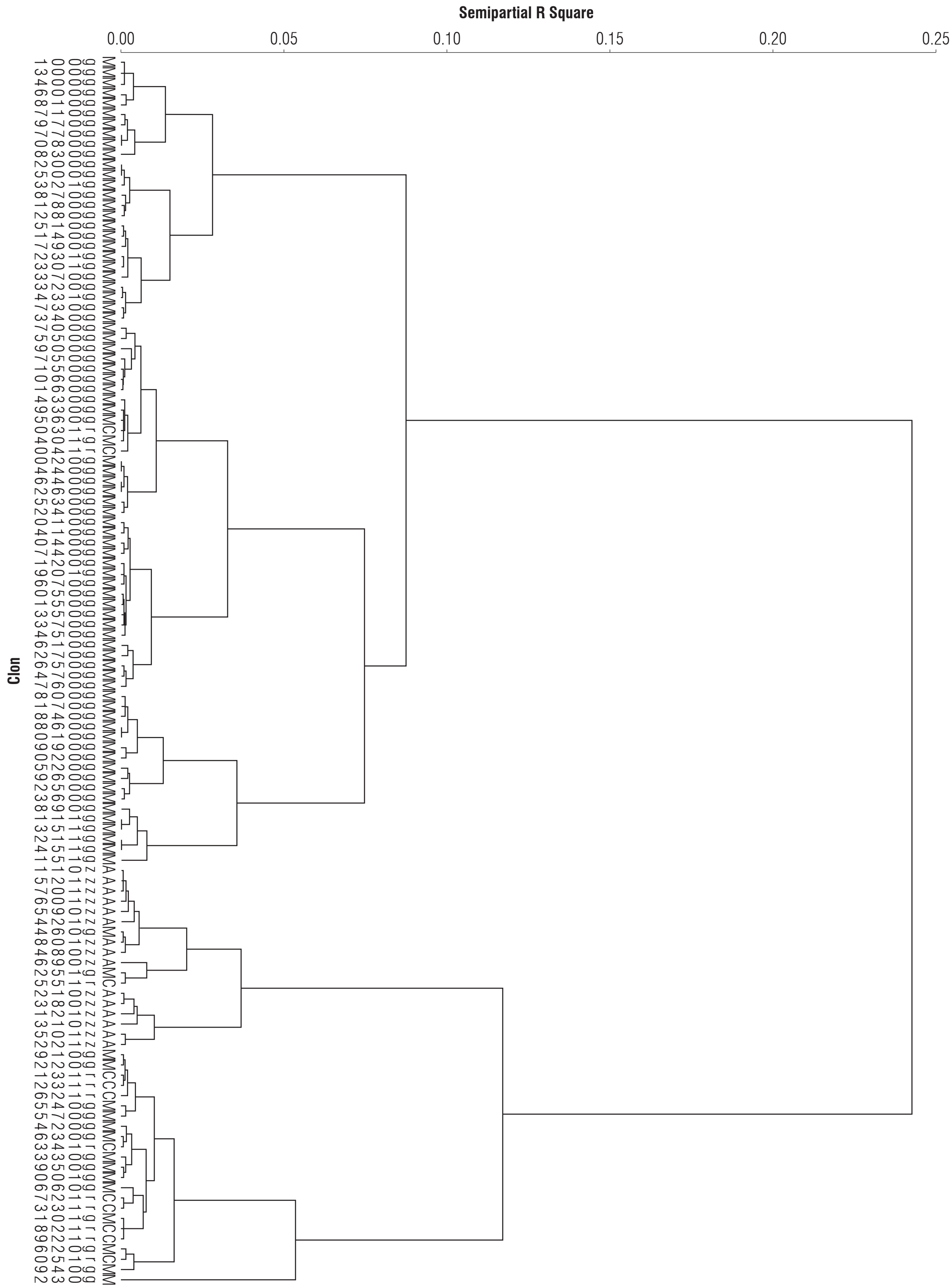

FIGURE 2. Dendrogram analysis of the cluster classification of 120 Margaritera orange genotypes collected at the Mompox depression. Data were obtained from PCA.

According to Orduz *et al.* (2010), seasonal changes related to rainy and dry seasons increase the environment average temperature and light radiation rates; a further decrease in these environmental ranges affects several physical properties such as growth, phenology (Guadalupe *et al.*, 2018) and development behavior, and some quality characteristics, including the juice color range, sugar content and the acidic ratio (that establishes if it is pleasant for the palate) (Vélez *et al.*, 2012).

Armadans *et al.* (2014) and Hernández *et al.* (2014) stated that citric quality is influenced, in addition, by numerous factors such as soil, fertilization, cultural practices, phytosanitary treatments and the graft-holder, which suggests that fruit quality can be improved under tropical conditions.

Selection of outstanding clones based on the Colombian technical standard NTC4086

According to the NTC4086 parameters, GI genotypes were classified as fruits caliber D (FD between 62-71 mm) and GII and GIII genotypes were classified as fruits caliber C (DF between 72-83 mm). Fruit quality ranges were assigned according to Orduz *et al.* (2011), who reported that first quality oranges are those fruits above 230 g. GI and GII genotypes (183.73 g and 217.38 g, respectively) were classified as small fruits, while genotype GIII (270.79 g) was considered as first quality line. All three groups show JP<40%, TSS>8.2 ºBrix, TA<1.5% citric acid, and MI>5.5. Despite the fruit quality is not appropriate to many international industrial markets due to the low juice acidity, the non-fulfillment of many requirements demanded in the NTC4086 is not an impediment for the fruit commercialization in the region.

Taking into account the afore-mentioned, we used the selection index proposed in equation 1 to select within each group the genotypes that are closest to comply with the requirements registered in NTC4086. As a result, approximately 7% of the genotypes of each group were selected among the genotypes that show the highest SI. The selected genotypes were: group I (Mar015, Mar037, Mar063 and Mar070), group II (Cr126 and Cr132) and group III (Az108 and Az122).

Moreover, the selected genotypes from group I belong to the traditional clone known as orange Margarita; these clones are characterized by showing small fruits (Caliber D) with a juice percentage higher than 40%, TSS>8.2 ºBrix and TA>0.80% citric acid. The two genotypes selected from group II belong to the Criollo clones produced by seed, and are characterized by showing larger fruits (Caliber C) and higher weights, with a JP higher than 39%, TSS>8.2 ºBrix and TA>0.64% of citric acid. Finally, the genotypes selected from group III belong to the clone Azúcar range farther from the requirements demanded in the NTC4086. However, it is a highly demanded clone by local producers and general consumers for fresh consumption and is characterized mainly by its high sugar concentration (TSS>9 ºBrix) and low titratable acidity (TA<0.2% citric acid) (Tab. 5). The selected genotypes from groups I and II are those suggested to be offered to nurseries of the region as these show features that comply with the attributes reported in the NTC4086. Furthermore, the selected genotypes from group III are suggested to be considered for special fruit orders that require high TSS concentrations and low TA. It is important to note that the suggested genotypes still show a MI higher than the reported in the NTC4086. However, considering that Margaritera orange is mainly produced to supply fresh consumption markets, and following the statements by Sartori *et al.* (2002), the desired MI for this fruit ranges between 8.8 and 15.4, highlighting the fact that the MI reached by the genotypes of the GI and GII corresponds mainly to fruits in a maturation state 4-5 (MI = 9.8-11.8).

In addition to the genotypes selected for quality attributes, the Marg032 genotype was identified as seedless, making it an alternative for improvement processes, considering the great interest of some markets to acquire seedless fruits.

It is important to indicate that the genotypes characterized in the research, except for the Criollo genotypes that are produced by seed, are all grafted onto a bitter orange (*Citrus aurantium* L.) rootstock, which is a condition for the genotypes to show the features identified in the characterization. In this regard, the rootstock influences more than 20 horticultural characteristics of the grafted variety such as yield, longevity, nutrient adsorption, size, shape, color, internal and external fruit quality, tolerance to diseases, and adaptation to soil and climate conditions (Davies and Albrigo, 1994). Moreover, it might contribute more than any other factor to the success or failure of the citrus industry in any region of the world (Wutscher and Bistline, 1988). In addition, studies carried out by Chaparro-Zambrano *et al.* (2015) on the behavior of Valencia orange grafted in different rootstocks in the low tropics of Colombia showed as a result that the Sunky x English pattern contributed to a high maturity index ratio (TSS/TA = 15.82ab) compared to rootstocks C-35 (13.74ab), Carrizo (IM = 14.39ab), Swingle (IM = 14.83ab), Citrumelo or CPB4475 (IM = 13.2ab), Cleopatra (IM = 13.19b), Volkamerian (IM = 11.44b), Webberi (IM = 12.51b) and Yuma (IM = 12.85b). Additionally, in the year 2017 the same authors found statistically significant differences between TSS and MI in juice of Arrayana tangerine grafted onto six rootstocks (Carrizo, Citrumelo Swingle, Cleopatra, Sunky x English, Sunky x Jacobson and Volcameriana). Other studies carried out in Mexico established that among a group of 20 rootstocks, Flying Dragon, Mandarina Cleopatra, Sacatón, Yuma, Morton, Rubidoux and Troyer confer the same quality to the fruit of Valencia orange than the one achieved with the bitter orange tree (Uribe-Bustamante *et al.,* 2013). Simultaneously, Parameshwar *et al.* (2018) report that Rough Lemon (*C. limón* Linn. Burn) rootstocks have an effect on tree growth and the quality of fruit in Valencia Late orange. Rootstock effects on lemon fruit production and quality were assessed by Legua *et al.* (2018) reporting that the rootstock Forner-Alcaide 2324, Forner-Alcaide 418 and Forner-Alcaide 5 influenced fruit quality in lemon cultivars ('Fino 49' F49, 'Verna 50' V50 and 'Fino Elche' FE). In contrast, Da Silva *et al.* (2018) reported that the quality of navel orange "caracara" was not influenced by any of the evaluated rootstocks (Rangpur lime, 'Florida' rough lemon, 'Volkamer' lemon, 'BRS Tropical Sunki' mandarin, 'Indio' citrandarin, 'Vangasay' rough lemon). The above mentioned allows us to consider the possibility of multiplying the selected clones and grafting them on other rootstocks that can improve the internal and external fruit quality.

Conclusions

Significant differences were found between morphological and fruit quality characteristics of the Margaritera orange. One-hundred and twenty genotypes of Margaritera oranges were selected at the Mompox depression, 92 belonging to the Margarita clone, 15 to the Azúcar clone and 13 to the Criollo clone. The genotypes were morphologically characterized and grouped based on 14 quantitative characteristics, forming three different phenotype sets, grouped with a dissimilarity value of 10. Five main components were established, which explain 78.82% of the variability of the cultivars, being the first component the one that explains the greatest percentage of variability with 38.41%.

Among three Margaritera orange clones studied, eight outstanding genotypes were selected, which became the main source of propagation material for nurseries in the region when carrying out Margarita orange multiplication processes, thus, guaranteeing the origin of the material produced.

The three selected clones become an alternative for nurseries in the region to start the establishment of basic orchards for propagation material, in accordance with resolution ICA 0004215 of 2014.

It is necessary to evaluate the behavior of the selected Margaritera orange clones with other rootstocks, which confer better productivity characteristics, fruit quality and tolerance to excess moisture.

Acknowledgments

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TABLE 5. Characteristics of the selected genotypes with the highest IS.

GROUPS	Clone	IS	FW (g)	FD (mm)	FL (mm)	PT (mm)	FAD (mm)	VL (mm)	VT (mm)	FPW (g)	JC (g)	TA %CA	pH	PR (kg cm^{-2})	SF (U)	TSS (°Brix)	MI (TSS/TA)	JP (%)
GROUP I	Mg015	31.66	179.43	72,00	71.00	3.84	7.70	12.39	2.25	35.55	90.00	0.93	3.62	4.00	7.00	9.68	10.41	50.16
	Mg070	28.32	143.36	64.00	64.00	3.27	9.90	15.99	2.31	25.81	70.00	0.81	3.74	5.20	6.00	8.86	10.94	48.83
	Mg063	19.29	179.50	68.80	69.30	5.50	5.20	13.90	1.60	59.50	75.00	0.94	3.60	10.90	6.00	8.68	9.23	41.78
	Mg037	17.70	170.80	69.80	64.40	3.80	5.10	11.60	2.30	36.40	72.00	1.01	3.60	5.10	6.00	10.60	10.50	42.15
GROUP II	Cr126	22.49	261.70	76.40	79.00	5.10	7.90	18.00	2.30	73.00	112.00	0.64	3.80	5.10	4.00	8.82	13.78	42.80
	Cr132	21.89	247.30	72.00	80.30	4.50	8.70	15.80	1.80	66.20	98.00	0.81	3.70	4.10	8.00	8.36	10.32	39.63
GROUP III	Az122	41.96	212.10	74.70	71.10	7.20	9.40	16.60	2.10	104.60	89.00	0.11	5.10	6.30	2.00	9.12	82.91	41.96
	Az108	38.24	188.30	71.60	68.40	4.60	6.10	15.50	2.00	49.70	72.00	0.12	4.30	4.40	9.00	11.12	92.67	38.24

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SUPPLEMENTARY MATERIAL 1. List of genotypes (codes) belonging to each phenotypic group.

Group 1:

Mg001, Mg002, Mg003, Mg004, Mg005, Mg006, Mg007, Mg008, Mg009, Mg010, Mg014, Mg015, Mg016, Mg017, Mg018, Mg020, Mg021, Mg023, Mg025, Mg034, Mg035, Mg037, Mg038, Mg039, Mg040, Mg041, Mg042, Mg043, Mg044, Mg046, Mg047, Mg048, Mg050, Mg051, Mg052, Mg053, Mg054, Mg055, Mg056, Mg057, Mg058, Mg060, Mg061, Mg062, Mg063, Mg065, Mg067, Mg068, Mg069, Mg070, Mg071, Mg072, Mg073, Mg074, Mg076, Mg077, Mg078, Mg079, Mg080, Mg081, Mg082, Mg097, Mg098, Mg099, Mg102, Mg103, Mg104, Mg109, Mg110, Mg111, Mg112, Mg120, Mg123, Mg151, Mg153,Mg154, Cr130, Crio.134 and Cr140

Group 2:

Mg019, Mg022, Mg024, Mg026, Mg032, Mg033, Mg036, Mg045, Mg049, Mg059, Mg066, Mg075, Mg100, Mg101,Cr126, Cr127, Cr128, Cr129, Cr131, Cr132, Cr133, Cr143 and Cr150.

Group 3:

Az012, Az013, Az083, Az084, Az096, Az105, Az106, Az107, Az108, Az011, Az121, Az122, Az125, Az095, Az124, Marg.64, Marg.152 and Crio.155

Two-dimensional gel electrophoresis to identify arcelins from *Phaseolus vulgaris* with inmuno-proteomic analysis

Electroforesis bidimensional para la identificación de arcelinas en *Phaseolus vulgaris* mediante análisis inmuno proteómico

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ABSTRACT

Arcelins are lectin-like proteins present in the common bean *Phaseolus vulgaris* that are believed to play a role in bruchid resistance. The present study used proteomics to analyze the expression of lectin-like proteins, specifically arcelins, in *P. vulgaris* cultivar varieties from Venezuela. A PAGE-SDS analysis of 30 commercial accessions of *P. vulgaris* showed significant differences in the molecular weight range of lectin-like proteins (arcelins). Eight different accessions were selected based on their electroforetic mobility for the proteomic analysis. Arcelin immuno-detection of two dimentional electrophoresed proteins was used to easily display the different arcelin proteomic profiles of the studied accessions. Mass spectrometry analysis confirmed the arcelin nature of these proteins. This is the first report on arcelin evaluation of the Venezuelan germoplasm of *P. vulgaris* with the aim of enhancing breeding programs by identifying accession materials with resistance to bean storage pests.

Key words: common bean, immunoassay, proteomics, lectin-like proteins, bidimensional gels, mass spectrometry.

RESUMEN

Las arcelinas son proteínas tipo lectinas, presentes en el frijol común *Phaseolus vulgaris* que se piensa que están implicadas en la resistencia a bruquideos. El presente trabajo utiliza la proteómica para analizar la expresión de proteínas tipo lectinas, específicamente arcelinas, en variedades de cultivares de *P. vulgaris* en Venezuela. El análisis mediante electroforesis SDS-PAGE de 30 accesiones de *P. vulgaris,* mostró diferencias importantes en el intervalo de peso molecular de las proteínas tipo lectina (arcelinas). Se seleccionaron ocho accesiones diferentes en base a su movilidad electroforética para el análisis proteómico. Se utilizó la inmuno-detección de proteínas separados mediante electroforesis bidimensional, para mostrar fácilmente los diferentes perfiles proteómicos de las arcelinas entre las accesiones estudiadas. El análisis de espectrometría de masas confirmó la naturaleza de las arcelinas presentes. Este es el primer informe sobre la evaluación de las arcelinas en el germoplasma de *P. vulgaris* venezolano con el objetivo de colaborar con los programas de mejoramiento mediante la identificación de materiales con resistencia a las plagas de frijol almacenado.

Palabras clave: frijol común, inmunoensayo, proteómica, proteínas tipo lectina, geles bidimensionales, espectrometría de masas.

Introduction

The common bean (*Phaseolus vulgaris* L) is a widely cultivated legume that originated in the New World, which has been domesticated in Meso and South America (Chacon *et al*., 2005). Dry beans remain the primary source of dietary protein carbohydrates, minerals and fiber in most developing countries, making them the third most important food legume crop worldwide (Singh and Muñoz, 1999).

The small-scale agriculture of the common bean is constrained by the multiplicity of biotic and abiotic factors that affect crop productivity and quality during the growing season. In addition, one of the most important biotic factors is predation of dry bean seeds by post-harvest pests (bruchids), which largely affect the overall food grain production and consumption. It is estimated that 13% out of a total loss of 37% in global agricultural production is caused by pests and diseases (Obeng-Oferi, 2007).

A cost effective strategy is required in order to provide an efficient and sustainable control of bean infestation by bruchids. A combination of genetic resistance and biological and cultural control methods presents a sustainable way

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to deal with this problem. In efforts to develop bruchid-resistant beans, researchers have identified and introgressed different genetic resistance factors from wild relatives into common bean. The principal resistance factors in mature bean seeds are arcelins (Osborn *et al*., 1988a; Osborn *et al*., 1988b; Zaugg *et al*., 2013).

Arcelins (Arcs) belong to the lectin-like family described in eight allelic variants in various accessions of the wild common bean, *P. vulgaris,* which exhibit different levels of resistance to bruchid insect pests. They are seed storage proteins associated with lectin or the arcelin-phytohaemagglutinin-alpha amylase inhibitor (APA) complex locus (Zaugg *et al*., 2013).

Although homologous to lectins, arcelins have a different intrinsic specificity for complex sugars, creating a mechanism of toxicity to bruchids, as opposed to the monosaccharide-binding affinity of true lectins (Fabre *et al*., 1998; Blair *et al*., 2010). The toxic properties of arcelins may be related to their ability to recognize and interact with glycoproteins and other constituents of the digestive tract membrane, as well as to their capacity to directly bind to the intestinal cells of insects (Minney *et al*., 1990; Fabre *et al*., 1998; Paes *et al*., 2000).

One or two dimensional sodium dodecyl sulfate gel electrophoresis (SDS-PAGE) techniques have been used for seed protein characterization to distinguish Andean bean genotypes from those of Mesoamerica according to the electrophoretic mobility of phaseolins (Gepts *et al*., 1986; Gepts and Bliss, 1986; Kami *et al*., 1995).

Additionally, in spite of the genetic studies that have been carried out and the approaches that have been used to study the *P. vulgaris* proteome (Natarajan *et al*., 2013; Parreira *et al*., 2016), the unique identification of arcelins in the different varieties is complicated.

The present study provides the first inmuno-proteomic approach used for the characterization of arcelins from the common bean, *P. vulgaris,* as a preliminary germplasm characterization.

Materials and methods

Seed material

Mature seeds from eight common bean (*P. vulgaris*) accessions from the germplasm bank of INIA-CENIAP (Maracay-Venezuela) (10º17'14'' N, 67º36'02'' W, and 480 m a.s.l.) were used. Information about the germplasm used in the present study (passport data) was published by Gamero *et al*. (2010). The seeds were selected from different states of Venezuela, showing dissimilar commercial values, consumer preferences and seed color. Commercial seeds corresponded to the states of Victoria, Tacarigua, Montalban, and Tenerife, and study cultivars NAG-7S, DOR-500, MGM-08-02-026 and MGM-10-02-108 were also selected.

Protein extraction and samples preparation

Dry beans were milled, and 100 mg of flour were suspended in 20 mM Borate buffer pH 9 with 30 µg mL^{-1} of a protease inhibitor mix (Amersham Biosciences, UK) and stirred for 1 h at 37ºC. The samples were then centrifuged for 15 min (14,000 *g*) at 4ºC, and the supernatant was used for the assays.

Protein determination

The protein concentration was determined with the Bradford method (1976) using the Bio-Rad assay reagent (Bio-Rad, USA) and bovine serum albumin as the standard.

Polyacrylamide gel electrophoresis

The proteins were separated with SDS-PAGE according to the method of Laemmli (1970). Briefly, 20 ug of proteins were dissociated with heating at 90°C for 5 min in the presence of a denaturing buffer (20 mM Tris-HCl pH 8.6 containing 1% SDS, 8.3% glycerol and 0.5% 2-mercaptoethanol) and separated with electrophoresis in 12% polyacrylamide gels. The proteins were stained with Coomassie Brillant Blue and destained in 7% acetic acid.

Two-dimensional electrophoresis (2-DE)

2-DE was performed as described by O'Farrell (1975); 1-D isoelectric focusing (IEF) was conducted in a ZOOM® IPGRunner™ (Invitrogen, USA), whilst the SDS-PAGE was carried out using a Mini-protean II (BioRad, USA). Each sample was run in triplicate. The protein samples were diluted in a rehydration solution (De Streak™, Amersham Biosciences, UK) with 1% IPG buffer pH 3-10 to a final volume of 155 µl (ZOOM® IPGRunner™ Cassettes, Invitrogen, USA) in a 7 cm IPG strip (Amersham Bio-Sciences, UK), pH 3-10 overnight; the proteins were then focused at a maximum voltage of 2,000 V. After IEF, the proteins were reduced and alkylated by soaking the IPG strips in the equilibration buffer (6M urea, 2% SDS, 30% glycerol, 50 mM Tris-HCl, pH 8.8) containing 1% DTT for 15 min at room temperature and then equilibrated in 4% iodoacetamine (IAA) for 15 min. The equilibrated IPG strips were sealed on top of the SDS-PAGE gel (12% polyacrylamide) using 0.5% agarose. The 2-D SDS-PAGE was carried out using a Tris-glycine-SDS buffer system at 200 V until the dye front reached the bottom edge of the gel. The protein spots were visualized with staining using PlusOne™ silver staining kit (Amersham Bio-Sciences, UK).

Immunoassays

The proteins from the 2-D SDS-PAGE were transferred at 100 V for 1 h to a nitrocellulose membrane for Western blot analysis. The membrane was Ponceau stained for 1.5 min to visualize the transferred protein and air dried. The membranes were re-wetted in a blocking solution composed of 2% non-fat dry milk in tris-buffered saline (TBS) with 0.5% Tween-20 (TTBS) and finally incubated for 1 h. The membrane was then probed for lectin-like proteins during 1 h of incubation with rabbit anti-lectin polyclonal antibodies (Sparvolli and Bollini, 1998) diluted at 1:6000 in a blocking mixture at 1:5 in TTBS. The immune serum was pre-absorbed overnight with 100 ng of bean flour from arcelin-null *P. vulgaris* in 1 mL of TBS to eliminate any unspecific cross-reactivity (Lioi *et al*., 2003). The bound antibodies were detected with horseradish peroxidase conjugated anti-rabbit IgG antibodies (1:2000 in TTBS) using the ECL™ Western Blotting Detection Reagents (GE Healthcare Life Sciences, UK), following the manufacturer's instructions. The image of the chemiluminescent signals was recorded on an X-ray film and developed according to standard procedures in a darkroom.

Image analyses

2-DE protein patterns were obtained as digitalized images using a high-resolution system (ProXCISION, PerkinElmer, Inc., USA). Spot detection, quantitation, and analysis were performed using Phoretix 2D (Nonlinear Dynamics, UK).

Protein identification

Spots of interest were excised, picked and placed in a 96-well plate using the protein picking workstation ProXCISION (PerkinElmer, Inc., USA). The MultiPROBE II PLUS HT EX (PerkinElmer, Inc., USA) Robotic Liquid Handling System was used for enzymatic digestion. Briefly, the method involves the following steps: (i) reduction with 10 mM DTT; (ii) alkylation with 55 mM IAA; (iii) overnight tryptic digestion (6 ng μL^{-1} enzyme); (v) sample loading onto MALDI target using 1 µl of the tryptic digests mixed at 1:1 with a matrix solution (a saturated solution of α-cyano-4-hydroxyxinnamic acid in 0.1% TFA-acetonitrile 50:50).

A MALDI prOTOF™ 2000 (PerkinElmer, Inc., USA) was used for peptide analysis. Calibration was performed using a commercial mixture of peptides, PepMix (Perkin Elmer, Inc., USA), of known molecular masses. TOFworks™ 1.0 (PerkinElmer, Inc., USA) was used for the data processing and for the peak list generation. The most intense peaks of the obtained spectra were identified with MASCOT PMF searching (http://www.matrixscience.com) using the Swiss-Prot and NCBI databases.

Results and discussion

Different methods have been used for the identification and characterization of seed storage proteins in beans, including protein chemistry for purification of storage proteins for further structural and functional studies, proteomics protocols, and immunoassay techniques (Osborn *et al*., 1988a; Osborn *et al*., 1988b; Fabre *et al*., 1998; Sparvolli and Bollini, 1998; Paes *et al*., 2000; Lioi *et al*., 2003; Zaugg *et al*., 2013).

Moreover, in a previous study (Bernal *et al*., 2006), an approximation of the comparative immunoassay technique with antibodies against the lectin family was used; however, the technique was nonspecific for the identification of proteins with mass spectrometry. The present study proposes a combination of proteomics and immunoassays techniques to successfully display the entire Arcelin catalogue of *P. vulgaris* for comparative purposes.

From the lectin family, arcelins were described as proteins associated with the inhibition of some bruchid species development. Although initially found in a limited number of wild common bean accessions from Mexico, approximately 10% of wild common bean accessions from Mesoamerica express Arcs. Until recently, Arcs were thought to be restricted to wild accessions of common bean, but arcelin-like sequences were obtained from other *Phaseolus* species (Osborn *et al*., 1988a; Osborn *et al*., 1988b).

Proteins of 30 *P. vulgaris* commercial accessions from a germplasm bank were first analyzed with SDS-PAGE to select the varieties that presented greater differences in the regions corresponding to the proteins of interest, in this case arcelins; these differences were visualized with image analysis programs (Fig. 1). Figure 1 shows the first selection of seeds with important differences in the 25-40 kDa range, where the majority of arcelins resolved (dotted boxes on the image, Fig.1).

After this analysis, only 8 accessions (Fig.1: 1, 2, 4, 8, 15, 18, 22 and 25) were selected and further analyzed with two-DE. The comparative analysis of the proteome images using Phoretix 2D revealed an average of 104 spots on the different proteomes from *P. vulgaris* (Fig. 2 B-I). These experiments were performed in triplicate for each accession.

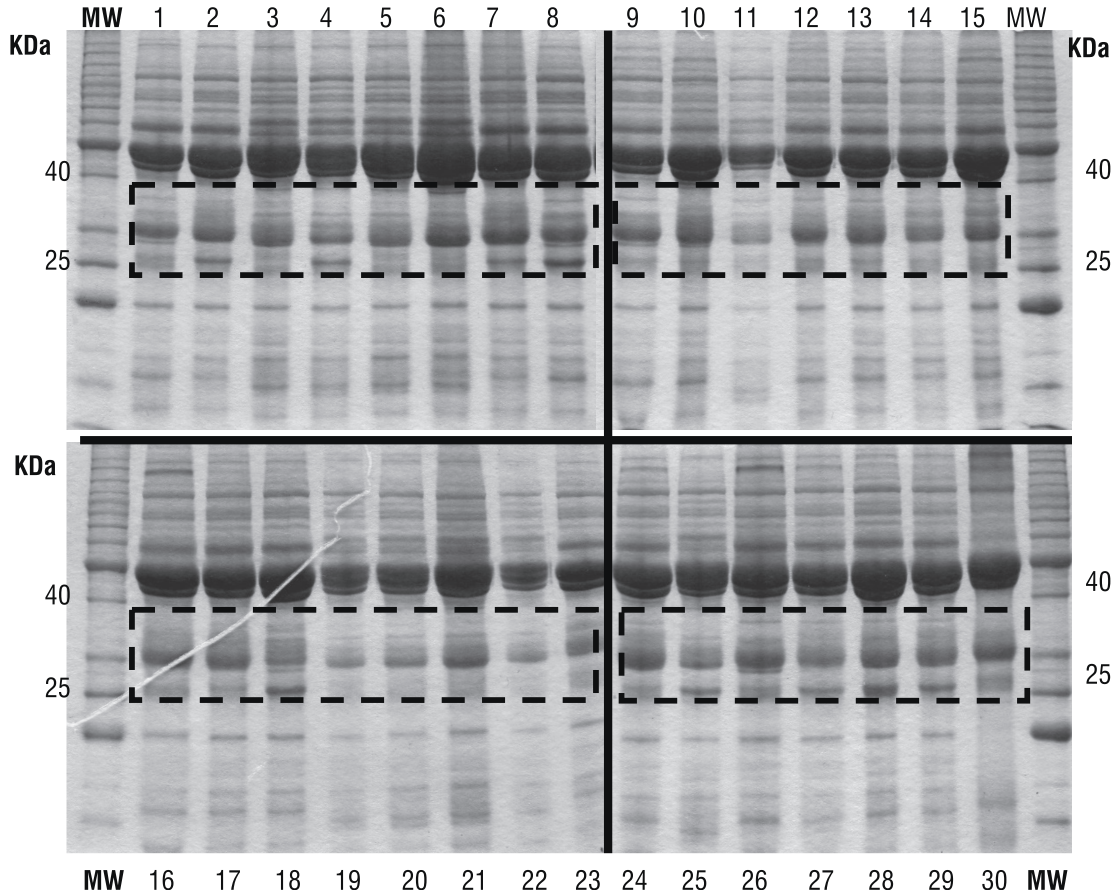

FIGURE 1. SDS-PAGE of extracted proteins from 30 *P. vulgaris* accessions.

The immuno-detection of the arcelins was obtained from the 2-DE gels of extracted proteins from a commercial variety by using a specific immune serum against this family of proteins. Figure 2A shows the results of the immunoblot, with only a few spots detected in the range of 33-35 kDa and 5.5-5.7 pH, which correspond to the characteristics of arcelins. The immunoblot image was used as a reference to identify the same group of proteins in each proteome with Phoretix 2D (Fig. 2 B-I). The protein spot patterns were different in each *P. vulgaris* proteome, suggesting the presence of different arcelins in each case, although it was not possible to distinguish between subgroups of arcelins with this approach. We observed the presence of two, three, four and five spots related to the range of molecular weight and inmuno-detection of arcelins (Fig. 2).

Some authors have stated that the more promising arcelin variants that confer insect resistance are arcelin-1, arcelin-5 and arcelin-8 (Osborn *et al*., 1988a; Zaugg *et al*., 2013); however, there is not much information on the presence of other variants and the role of these proteins in bruchid toxicity is not clear. Moreover, genetic studies suggest that resistance to storage pests in fact depends on a combination of proteins from the APA locus (arcelin, phytohemagglutinin and α-amylase inhibitor), in which case the participation of the arcelin family can play a fundamental role (Blair *et al*., 2010).

More profound mass spectrometry analysis is needed to elucidate which arcelin variants are expressed in these common bean accessions. It is worth noting that assayed commercial varieties have shown spots related to arcelins (Fig. 2). It is interesting to observe this high frequency of arcelins in the Venezuelan commercial varieties of *P. vulgaris*.

As shown in Table 1, it was possible to identify a group of peptides related to arcelins (98% identity) with mass spectrometry.

Despite the tools described in this paper, it was not possible to determine the type of arcelins present in each variety. These results further confirmed the arcelin nature of the immune-detected spots.

The large-scale study of the *P. vulgaris* proteome has been described in previous studies (Natarajan *et al*., 2013;

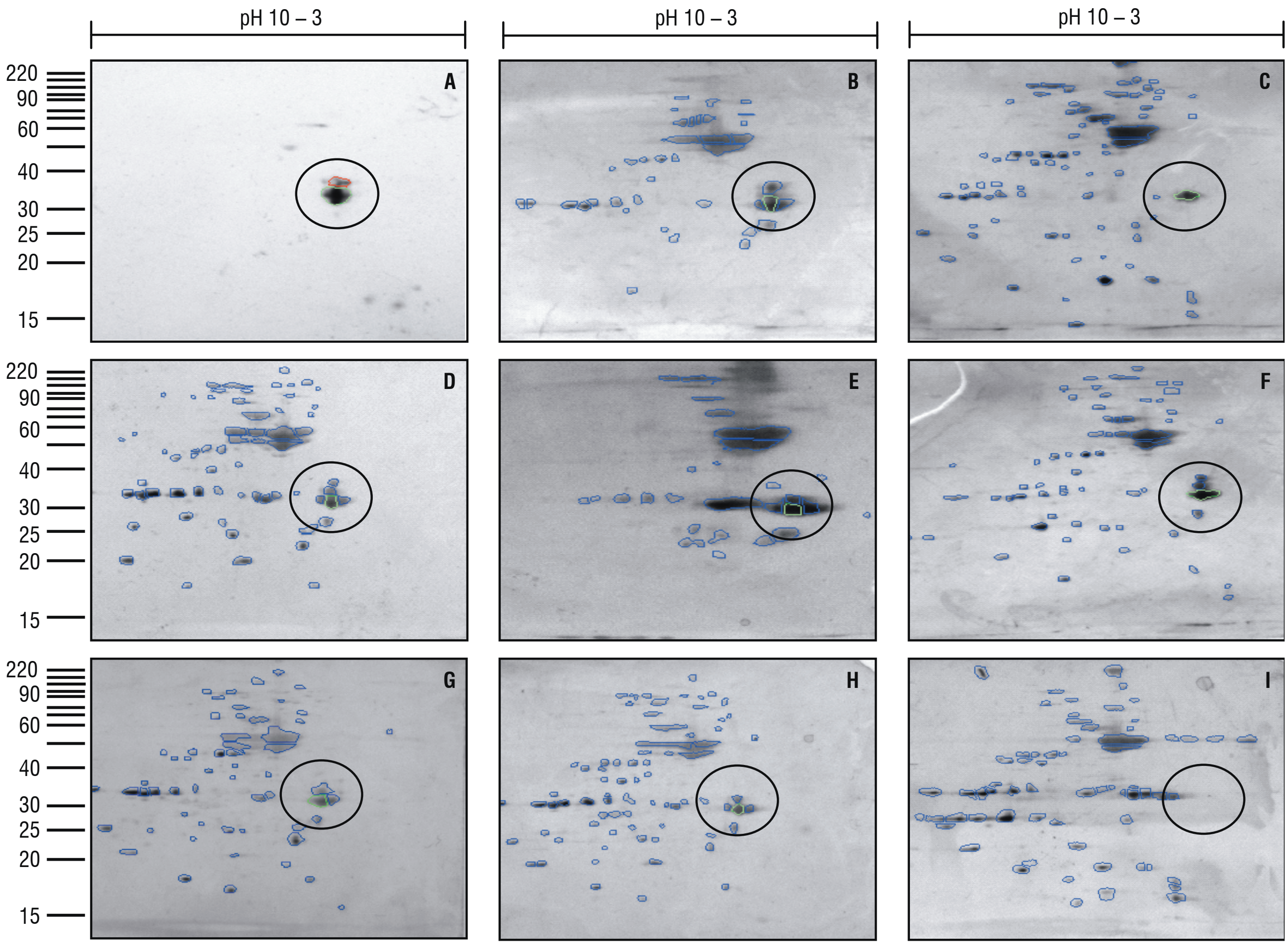

FIGURE 2. (A) 2-DE immunoblot of extracted proteins from a commercial variety of *P. vulgaris*. (B-I) 2-DE of extracted proteins from eight different accessions. Immuno-detection of the arcelin region with a specific antibody (A). Circles highlight the Two-DE region corresponding to arcelins in the different proteomes according to the immunoblot and the Phoretix 2D profile analysis.

TABLE 1. Peptide sequences and mass-specs for arcelin identification. Theoretical pI: 6, 16 / Mw (average mass) 29375.87 / Mw (monoisotopic mass): 29357.62.

Mass	Position	Peptide sequence
4564.2340	125 - 165	DYFLGLFNKPDDPEAHIVAVVFDTSSNQIEIDMNSISPVAR
3718.7547	215 - 248	VLNDWVSVGFSATSGLYDPTSETHDVLSWSFSSK
3401.7627	6 - 36	LLSLALFLVLLTHANSASETSFNFTSFDTNK
1369.5875	203 - 214	CNFSTSSVHMEK
1217.6736	37 - 48	LILQGDASVSSK
1205.5215	58 - 69	GNGDPTVDSMGR
1092.5068	249 - 257	FSQHTTSER
1022.5517	193 - 202	ASLVYPSGTK
813.3989	128 - 188	ITYDSSK
772.4927	49 - 55	GQLLLTK
610.2831	173 - 177	YNGEK
608.2886	80 - 85	DSTTGK
516.2889	189 - 192	NNLR
502.2983	178 - 181	VEVR

Parreira *et al*., 2016); however, it does not present a useful tool for the study of protein families of interest, such as lectins. The inmuno-proteomic approach described in this study is a reliable alternative technique to easily localize/identify a subset of proteins from complex bidimensional gels. This is the first immuno-proteomic approach used for the characterization of arcelins from *P. vulgaris*.

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Effect of irrigation suspension on the growth, water state and production of spinach (*Spinacia oleracea* L.) plants

Efecto de la suspensión del riego sobre el crecimiento, estado hídrico y producción de plantas de espinaca (*Spinacia oleracea* L.)

Andrea Reyes[1], Oscar Alvarado[1], and Javier Álvarez-Herrera[1*]

ABSTRACT

Spinach is susceptible to drought conditions, and, because of climate change, it is necessary to optimize water application to crops. Therefore, a test was carried out in a completely randomized design (CRD) with four treatments consisting of the following irrigation suspension durations: T1: 0 d, T2: 4 d, T3: 7 d and T4: 10, with six replicates for a total of 24 experiment units (EU). Each EU consisted of 6 plants, for a total of 120 plants that were transplanted to 2 L pots with a mixture of peat and soil (2:1). The upper part of the substrate was maintained with a higher moisture content in the treatment without irrigation suspension and in the one with irrigation suspended for 4 d. An inversely proportional relationship was observed between the moisture content and the leaf water potential. The treatment without irrigation suspension recorded the highest leaf water potential value during the measurement period. There were no significant differences between the chlorophyll content in the SPAD units or for the leaf area, stomatal conductance and dry mass. The fresh mass presented significant differences and had the highest value in the treatment without suspension of irrigation.

Key words: stomatal conductance, relative water content, water potential, SPAD, leaf area.

RESUMEN

La espinaca es una planta sensible a las condiciones de sequía y debido al cambio climático se hace necesario optimizar la aplicación de agua a los cultivos. Por lo anterior, se llevó a cabo un ensayo en un diseño completamente al azar (DCA) con cuatro tratamientos correspondientes a la duración de la suspensión del riego T1: 0 d, T2: 4 d, T3: 7 d y T4: 10, con seis repeticiones para un total de 24 unidades experimentales (UE). Cada UE estuvo conformada por 6 plantas, lo que significó emplear un total de 120 plantas, las cuales fueron trasplantadas en materas de 2 L con una mezcla de turba y suelo (2:1). La parte superior del sustrato se mantuvo con mayor contenido de humedad tanto en el tratamiento sin suspensión de riego como en el que se suspendió por 4 d. Se observó una relación inversamente proporcional entre el contenido de humedad y el potencial hídrico de la hoja. El tratamiento sin suspensión de riego registró los valores más altos de potencial hídrico foliar durante el tiempo de medición. No se presentaron diferencias significativas entre el contenido de clorofila en unidades SPAD, así como en el área foliar, conductancia estomática y masa seca. La masa fresca presentó diferencias significativas y obtuvo los mayores valores en el tratamiento sin suspensión de riego.

Palabras clave: conductancia estomática, contenido relativo de agua, potencial hídrico, SPAD, área foliar.

Introduction

In recent years, the consumption of spinach (*Spinacia oleracea* L.) has increased worldwide. This vegetable provides a balanced supply of vitamins and minerals to diets, preventing many diseases (Jiménez *et al*., 2010). In Colombia, the cultivated area of spinach is close to 500 ha (MADR, 2017), and it is considered a crop with great export potential for markets in the United States and other consuming countries.

Water scarcity is one of the main consequences of climate change, which is one of the most important constraints for crop development (Farooq *et al*., 2009) since it generates economic losses and threatens global food security (Earl and Davis, 2003). Spinach is susceptible to drought since this condition reduces photosynthesis as a result of stomatal closure, which prevents the entry and assimilation of CO_2. This effect may be reversible (acclimatization) or irreversible (damage to photosynthetic metabolism) (Lipiec *et al*., 2013).

The damage caused to plants by a water deficit depends on the duration and intensity of the water stress. Severe stress can reduce growth and absorption of nutrients such as phosphorus and nitrogen by up to 30%, while mild stress

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does not significantly affect these variables. A plant's capacity of recovery depends on both the intensity of the stress condition and the genotype (Subramanian *et al*., 2006).

Plants exposed to a water deficit present slow growth and a low photosynthetic rate, which is affected mainly by a decrease in the cellular turgidity and in the assimilation of CO_2, which in turn causes lower accumulation of dry mass and poor plant development (Lipiec *et al*., 2013; Batra *et al*., 2014). In addition to these processes, variables related to water status in plants such as water potential, relative water content and osmotic potential have been reported as being sensitive to water deficits. Therefore, these variables are generally used as water deficit indicators (Lipiec *et al*., 2013).

Although water deficit is one of the more studied abiotic stresses, there are few studies on spinach, and there is no information on the recovery capacity of this plant and the main variables that are affected by water deficits. Since the importance of spinach is growing and its demand for water can be considerable, the study of this abiotic stress should be addressed. Therefore, the objective of this research was to evaluate the effect of water deficit on spinach growth, water status and plant production.

Materials and methods

This project was carried out in the mesh house of the Universidad Pedagógica y Tecnológica de Colombia (UPTC) in Tunja, located at an altitude of 2,782 m a.s.l. with coordinates 5º32' N and 73º23' W. The mesh house had a plastic cover, and the average temperature during the study was 16ºC with 70% relative humidity (RH).

Hybrid Select 4-24 spinach seedlings, 20 d after germination, were transplanted to 2 L pots with a mixture of peat and soil (2:1). A completely randomized design (CRD) was used with four treatments (Fig. 1) corresponding to the duration of the irrigation suspension: T1: 0 d, T2: 4 d, T3: 7 d and T4: 10 d, with six replicates for a total of 24 experiment units, each one consisting of 6 plants for a total of 120 evaluated plants in the test. The irrigation was suspended according to the established treatments, after which the water supply was resumed. The sampling was done at 25, 29, 32, 35 and 50 d after transplanting (dat), and the growth variables were determined as detailed below.

The accumulation of fresh leaf mass was determined using a VIBRA AJ220E (Shinko Denshi Co, Tokyo, Japan) 0.001 g semi-analytical balance; for dry mass accumulation, the leaves were dried in a Memmert UNB500 drying

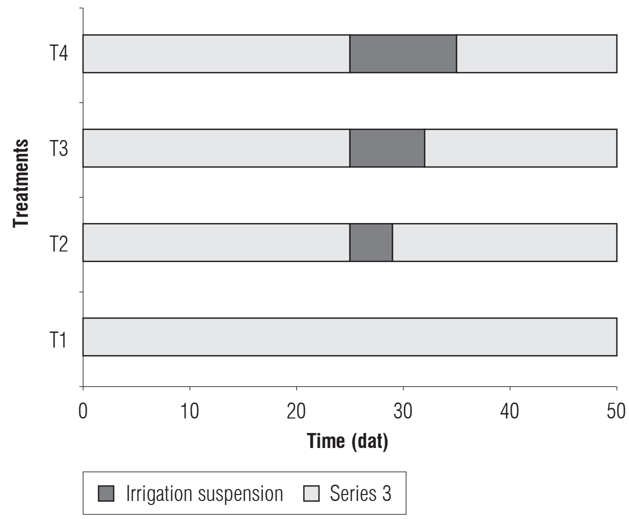

FIGURE 1. Irrigation treatments applied to the spinach plants.

oven (Memmert GmbH + Co. KG, Schwabach, Germany) at 80°C for 48 h. The leaf area and canopy leaf area were determined by taking photographs of the complete leaves and the canopy of the plant, respectively. These photos were analyzed with ImageJ (University of Wisconsin, USA).

The relative water content (RWC) was determined using 8 discs per leaf (1 cm in diameter) and the following equation (Smart and Bingham, 1974):

$$RWC(\%) = \frac{(FW-DW)}{(TW-DW)} \times 100 \qquad (1)$$

where *FW* is the simple fresh weight, *DW* is the leaf dry weight and *TW* is the turgid weight. *FW* corresponded to the mass of the discs immediately after cutting them from the leaf, *TW* corresponded to the mass of the discs after subjecting them to a relative humidity close to 100% for 24 h, and *DW* corresponded to the mass of the discs after drying at 80°C for 48 h.

The stomatal conductance was determined using a SC-1 meter (Decagon Devices Inc., Pullman, WA, USA). The relative content of chlorophyll was determined with a SPAD-502 Plus meter (Decagon Devices Inc, Pullman, WA, USA). The volumetric water content in the substrate was measured with a portable FieldScout TDR 100 meter (Spectrum Technologies, Aurora, IL, USA), and the water potential was determined at 12 m with a Scholander PMS 600 (PMS Instrument Co, Albany, OR, USA) on leaves that had been previously enfolded. A statistical analysis of variance and Tukey's comparison tests ($P \leq 0.05$) were carried out using the SAS v9.2e statistical program (SAS Institute Inc., Cary, NC, USA).

Results and discussion

Volumetric water content (VWC) in the substrate

There were significant differences between the treatments (Tab. 1). The control treatment (without irrigation suspension) and the treatment with 4 d of irrigation suspension reached the maximum values of VWC in the substrate (65.4%) at a depth of 4 cm at 32 and 35 dat, respectively. When analyzing the moisture behavior over time, it was observed that the VWC decreased as the suspension of irrigation progressed until 35 dat (Fig. 2), when the water supply was resumed, after which the soil moisture increased and remained almost constant until the day of harvest.

As for the variation of the VWC in the substrate with respect to depth, it was observed that the upper part of the substrate maintained a higher moisture content, especially in the treatment without irrigation suspension and in the treatment with 4 d of irrigation suspension, probably because the water stress time was the lowest (Fig. 3). Durand *et al*. (2016) stated that roots extract water from the zone of contact with the medium; if there is a certain level of water deficit, the VWC of the substrate decreases, which can slow down or even stop the water absorption rate in plants because of the energy used for water absorption.

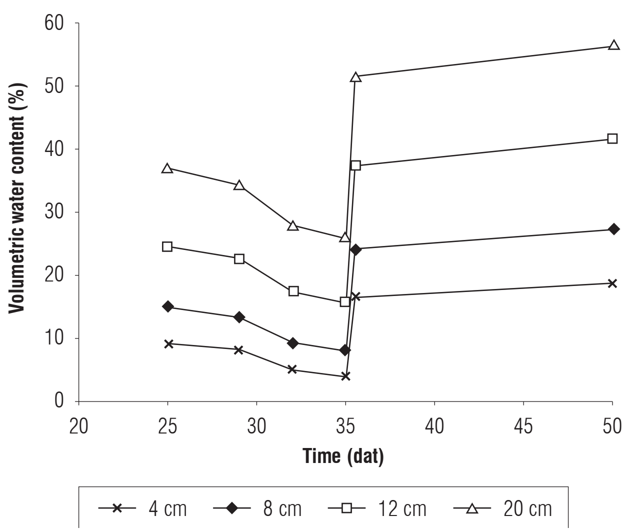

FIGURE 2. Behavior of the volumetric water content during the irrigation suspension in the substrate of the spinach plants.

Water stress causes a decrease in the synthesis of auxins and, in turn, an increase in ethylene synthesis. This increase in endogenous ethylene is slight, but sufficient to initiate the process of leaf abscission. In particular, the expression of genes encoding polygalacturonase, cellulase and aminocyclopropane-1-carboxylic acid synthase (ACS), the latter being the catalyzer of ethylene synthesis, has been found in the organ abscission zone (Estornell *et al*., 2013). Furthermore, to avoid water deficit problems, the recommended VWC value for a medium varies between 24% and 40% (Abad *et al*., 2004); this value depends on the physical characteristics (Bougoul and Boulard, 2006).

Chlorophyll content (SPAD)

There were no significant differences in the chlorophyll content between the plants submitted to different irrigation suspensions. The values showed a downward trend over time for the SPAD units, which ranged from 43.4 for the treatment with 10 d of irrigation suspension on day 25 (dat) to 28.4 SPAD units for the treatment with 4 d of irrigation suspension on day 50 (dat) (Tab. 1). The water deficit to which the plants were subjected was not intense and could not have affected the chlorophyll content. La Rosa *et al*. (2011) stated that, in spite of subjecting plants to certain levels of water stress, they did not present alterations in the synthesis of chlorophyll. In adittion, Ors and Suarez (2017) found values that ranged from 47 to 49 SPAD units for spinach plants subjected to different levels of water stress (soil water potentials -44.7 kPa, -231 kPa, and -446 kPa) and an electrical conductivity of 0.85 dS m^{-1}.

Stomatal conductance

There were no significant differences between the treatments except for day 29 (dat), where the treatment with 10 d of irrigation suspension showed an average value of

TABLE 1. Anova F values of the variables evaluated in the spinach plants submitted to different levels of water deficit.

Time	VWC				SPAD	CE	pH	FLM	DLM	RWC	LA	CLA
(dat)	4 cm	8 cm	12 cm	20 cm								
25	0.8786	0.9176	0.9082	0.7631	0.1721	0.1114	0.0055*	0.0104*	0.5730	0.6138	0.4950	0.0511
29	0.0094*	0.0048*	0.0053*	0.0015*	0.7691	0.0200*	0.2809	0.4243	0.7009	0.2685	0.2003	0.5940
32	0.0013*	0.0008*	0.0015*	0.0018*	0.1174	0.1678	0.0350*	0.0295*	0.0982	0.5880	0.5495	0.2406
35	0.0001*	0.0001*	0.0001*	0.0001*	0.2544	0.4173	0.0142*	0.2699	0.2782	0.4090	0.5028	0.3525
50	0.7485	0.7891	0.7634	0.7811	0.0561	0.1613	0.1229	0.5925	0.2948	0.7359	0.2924	0.3639

dat: days after transplating; VWC: Volumetric water content; SC: Stomatal conductance; FLM: Fresh leaf mass; DLM: Dry leaf mass; RWC: Relative water content; LA: leaf area; CLA: canopy leaf area. * Indicates significant differences at $P \leq 0.05$.

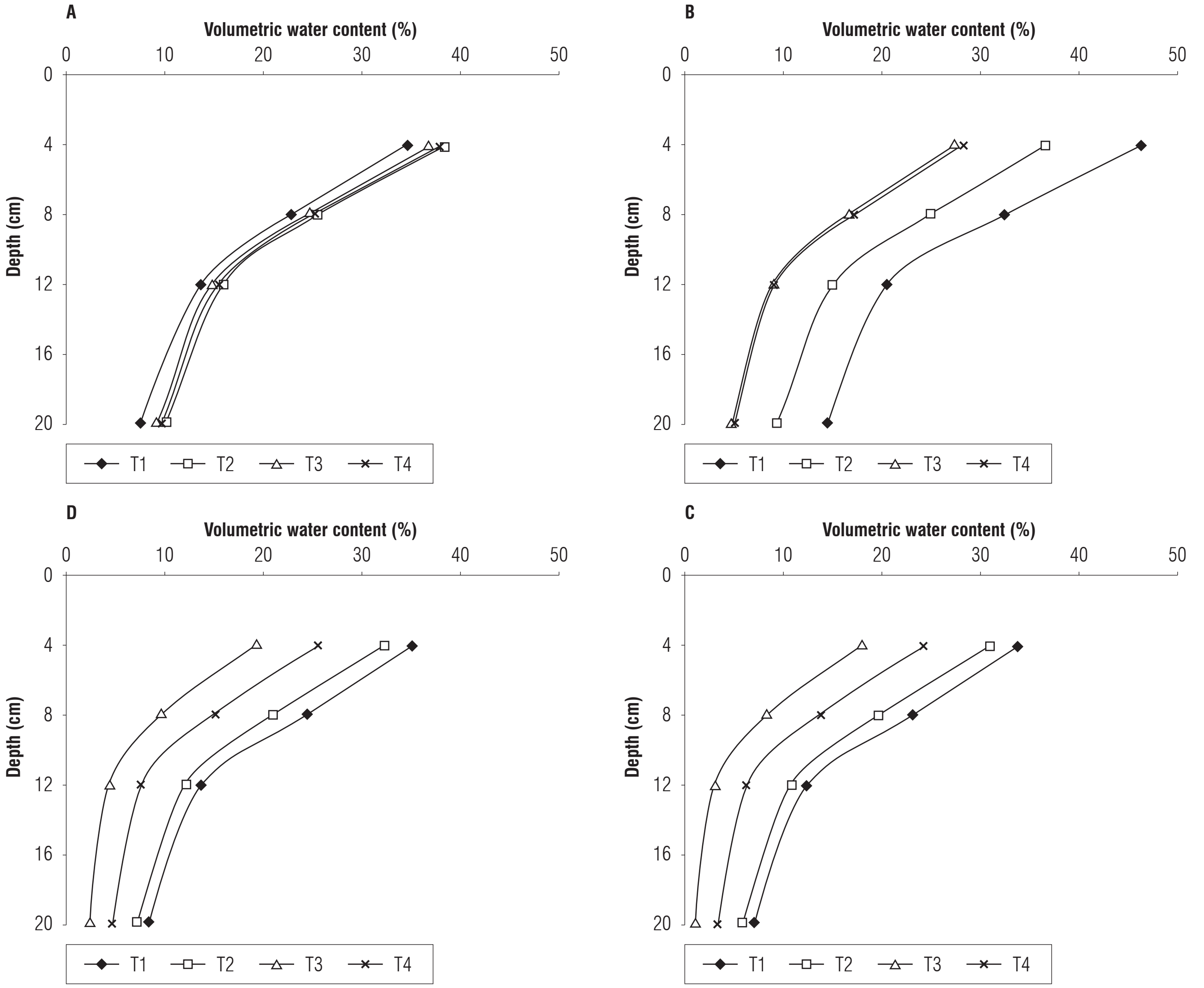

FIGURE 3. Volumetric water content in the substrate of the spinach plants with respect to the substrate depth, A: 25 dat; B: 29 dat; C: 32 dat; D: 35 dat.

333.8 mmol m^{-2} s^{-1}, which then decreased markedly during the evaluation period (Tab. 1). It should be emphasized that the reduction of stomatal conductance has protective effects, allowing water savings in plants and improving water use efficiency (Chaves *et al*., 2009). Plants have also generated responses to water stress with adaptations at the morphological, anatomical and cellular levels, which allow them to live in conditions of constant water stress. In this regard, the stomatal closure process acts as a resistance mechanism at the physiological level. It occurs when the mesophyll begins to undergo dehydration, which is regulated by abscisic acid (ABA), increasing its content in the leaves. This happens as a result of the decompartmentalization and redistribution of chloroplasts in mesophyll cells and its synthesis and transport from the roots, where it is released to the apoplast, reaching the guard cells through the transpiration stream (Moreno, 2009).

Water potential

This variable presented significant differences in the Anova test. An inversely proportional relationship was observed between the moisture content and the water potential of the leaves. The treatment without irrigation suspension registered the highest leaf water potential value during the measurement period, obtaining a value of -0.59 MPa for day 25 (dat) and a value of -0.91 MPa for day 34 (dat), whereas the treatment with the least water (T4: 10 d of irrigation suspension) recorded the lowest water potential values, -1.02 MPa for day 25 (dat) and -1.32 MPa on day 34 (dat) (Tab. 1).

On the other hand, these results are consistent with those reported by Godoy *et al*. (2005) and Ismail (2010), who indicated that, the lower the availability of water in a soil, the lower the water potential. In this regard, the substrate retained an amount of homogeneous water, and the evapotranspiration, infiltration and absorption by the plants resulted in water loss, which manifested a greater decrease in the moisture content in the treatments where the water stress lasted longer. It should be noted that the water potential values were lower than those reported for other crops, such as the faba bean (Kajerti *et al*., 2011), which ranged from 0.15 to 0.5 MPa because the leaf water potential in this research was measured at noon since the objective was to determine the maximum stress to which the plants were subjected in the different treatments.

Leaf area (LA)

No significant differences were found for LA; however, for the first LA measurement at 25 dat, irrigation restriction for 4, 7 and 10 d decreased the leaf area production by 6%, 10% and 26%, respectively. This indicates that increasing the water restriction of spinach is detrimental to leaf area development and decreases the moisture content in a substrate because, as the irrigation suspension increased, the substrate presented a lower moisture content at the all of the measured depths (Fig. 3). In this regard, Quintal *et al*. (2012) stated that a water deficit restricts cell growth, which leads to a smaller leaf expansion. Balaguera *et al*. (2008) indicated that, with a lower turgidity pressure resulting from a water deficit, the leaf area is smaller, and there is greater stomatal closure.

Dry mass and fresh mass of leaves

No significant differences were found between treatments in the dry mass, but there were significant differences for the fresh mass. The plants that were not submitted to irrigation suspension presented a greater recovery of biomass at 32 dat (Tab. 1). Quintal *et al*. (2012) indicated that, when there is a water deficit, leaves are less developed, and there is a smaller leaf area. This is why crop production correlates directly with the availability of water in the substrate. Reyes-Matamoros *et al*. (2014) concluded that water stress induces metabolic irregularities such as a decreased leaf growth rate and the consequent decrease in dry mass. These stress conditions have a strong impact on the morphology and physiology of plants, which depends on the degree of tolerance of the tissues to dehydration, mainly in leaves which are the photosynthetic surface. Furthermore, water stress affects the ability to accumulate solutes, which are required to maintain an adequate water content in plants to prevent diminished growth. A greater reduction in the production of fresh mass in spinach leaves and water use efficiency was also reported by Ors and Suarez (2017) when plants were subjected to a combined hydric and saline stress instead of receiving a separate effect from these two types of stress.

Relative water content (RWC)

For the relative water content, there were no significant differences between the treatments. The values ranged from 85.5% for the treatment that did not have irrigation suspension at 28 dat to 73.9% for the treatment with 10 d of irrigation suspension at 34 dat (Tab. 1). Bartlett *et al*. (2012) pointed out that a total relative water content in cells of less than 75% can drastically inhibit the production of adenosine triphosphate (ATP), Ribulose 1,5-bisphosphate (RuBP) and proteins; however, this depends on the resistance to water movement outside the cells and the water potential in the vascular bundles. Because of water deficits, the cell membrane undergoes changes, such as permeability and decreased turgidity (Blokhina *et al*., 2003). In addition, microscopic investigations of dehydrated cells revealed damage, including membrane rupture and sedimentation of cytoplasm content, which can reduce the ability of osmotic adjustment (Ganji Arjenaki *et al*., 2012).

Conclusions

The irrigation suspension for 10 d decreased the moisture content of the substrates in which the spinach crop was planted. The volumetric water content was higher in the first centimeters of the substrate and decreased with depth. The water potential of the spinach plants was affected by the irrigation suspension because, with the longer period without water, there was higher water stress, and the plants presented a lower leaf area and fresh mass. The leaf chlorophyll content of the spinach plants was not affected by the different irrigation suspension treatments.

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Potential yield and efficiency of N and K uptake in tubers of cvs. Capiro and Suprema (*Solanum tuberosum* Group Andigenum)

Potencial de rendimiento y eficiencia en la demanda de N y K en tubérculos de cvs. Capiro y Suprema (*Solanum tuberosum* Group Andigenum)

Manuel Iván Gómez[1], Stanislav Magnitskiy[1]*, and Luis Ernesto Rodríguez[1]

ABSTRACT

Potato yield depends on the genotype-environment interaction, edaphic nutrient supply, and fertilization rates. The total tuber yield (FWt), dry weight of tubers (DWt), harvest index (HI) and nutrient use efficiency in tubers (NUEt) were evaluated in the Andean region in Colombia at 75, 100, 125, and 150 d after sowing using two cultivars (Capiro, Suprema), three locations with contrasting soils (Subachoque, Facatativa and Choconta) and two levels of fertilization: F0 (unfertilized) and F1 (fertilized). The Humic Dystrudept soils with fertilization (Choconta) presented late tuber filling with increases of 48 and 64% for the DWt in the cvs. Suprema and Capiro, respectively. In Suprema, the highest production potentials were obtained in fertilized soils with low fertility, with increases of 60.9% for the DWt and 75% for the HI. On the other hand, Capiro was better adapted to soils with medium to high fertility, with increases of up to 86.7% for the FWt, as compared to the unfertilized soils. This increase may be related to higher rates of nutrient recovery efficiency (RFt), higher accumulated nutrients per tuber yield (EPt) and a better NUEt because N. Suprema presented a negative EPt and RFt with HI<45% and the lowest NUE of N and K in high fertility soils, which represents a null response to fertilization and possible mechanisms of luxury consumption for the evaluated elements.

Key words: nutrient use efficiency, productive potential, luxury consumption, macronutrients.

RESUMEN

El rendimiento de papa depende de la interacción genotipo-ambiente, el suministro edáfico de nutrientes y las dosis de fertilización. Se evaluó el rendimiento total de tubérculos (PFt), el peso seco de tubérculos (PSt), el índice de cosecha (IC) y la eficiencia de uso de nutrientes en tubérculos (UENt) en la región Andina de Colombia a los 75, 100, 125, y 150 d después de la siembra utilizando dos cultivares (Capiro, Suprema), tres localidades con suelos contrastantes (Subachoque, Facatativá y Chocontá) y dos niveles de fertilización variable por tipo de suelo, F0 (no fertilizado) y F1 (fertilizado). Los suelos Humic Dystrudepts con fertilización (Chocontá) presentaron un llenado tardío con un incremento PSt de 48 y 64% en los cvs. Suprema y Capiro, respectivamente. En Suprema se obtuvieron los mayores potenciales de producción en suelos de baja fertilidad con incrementos en rendimiento del 60,9% y con IC de 75%, mientras que Capiro se adaptó mejor a suelos de media a alta fertilidad con incrementos hasta 86,7% en rendimiento (PFt) con respecto a suelos no fertilizados. Este incremento se encuentra relacionado con la mayor recuperación de nutrientes del fertilizante por el tubérculo (RFt) y la mayor eficiencia de nutrientes para la producción de tubérculos (EPt). Suprema presentó EPt y RFt negativo con IC<45% y la menor UENt de N y de K en suelos de alta fertilidad, lo que representa una respuesta nula a la fertilización y posibles mecanismos de consumo de lujo de los elementos evaluados.

Palabras clave: uso eficiente de nutrientes, potencial productivo, consumo de lujo, macronutrientes.

Introduction

In 2016 in Colombia, an estimated production of 2,623,700 t ha^{-1} was achieved on 126,100 ha, with Cundinamarca and Boyaca provinces generating 76% of the potato production (Riascos, 2016). Diacol Capiro and Pastusa Suprema are considered cultivars with high economic importance and represent 80% of the area cultivated for fresh consumption and industrial processing in the country (Ñústez, 2011).

In the Andean region of Colombia, yields of 50-60 t ha^{-1} have been reported for Capiro (Gómez and Torres, 2012) and 40-50 t ha^{-1} for Suprema (Pérez, 2015). However, these yields vary considerably by location or soil type, with an increase in the potential yield in the last decade resulting from technological improvements in certified seed management, irrigation, mechanization, and balanced fertilization.

In potato cultivars, a high application of nitrogen and potassium fertilizers facilitates significant yields (Quinchoa

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et al., 2010; Saravia *et al.*, 2016), but there is low absorption efficiency of mineral nutrients by the plants, probably because of the shallow root systems (Poljak *et al.*, 2011). This implies a low nutrient recovery of up to 50% for N (Vos, 2009) and 70% for K (Gómez and Torres, 2012) as a result of the low influx of potassium used by the roots (Rengel and Damon, 2008). On the other hand, other factors include the availability of water and nutrients in the soil (Saravia *et al.*, 2016) as well as the plant genetics in terms of source-sink efficiency, which is variable for cultivars (Trehan and Singh, 2013) and directly affects the efficiency of nutrient transport to tubers (Poljak *et al.*, 2011; Giletto and Echeverria, 2015; Fernandes and Soratto, 2016). Nutrient Use Efficency (NUE) in potato tubers has recently been researched, mainly for N by Poljak (2011), Saravia *et al.* (2016), and Marouani and Harbeoui (2016), and for K by Trehan and Singh (2013), and Wang and Wu (2015) in Group Chilotanum, while studies on the efficient use of nutrients in Group Andigenum are less common (Zebarth *et al.*, 2012).

Potatoes of the Group Andigenum cultivated in the Andean region respond favorably to fertilization, mainly in soils with a low nutrient supply (Quinchoa *et al.*, 2010). Nutrient uptake rates of about 0.2-0.3 kg m^2 are seen (Quinchoa *et al.*, 2010; Gómez and Torres, 2012), which can reduce the efficient use of fertilizers and negatively impact the profitability and environmental sustainability of potato production in the country. The efficiency of nutrient use for Capiro and Suprema cultivars has not been quantified. The present research evaluated the production potential, accumulation of dry matter, harvest index (HI) and NUEt for N and K in tubers in response to balanced fertilization in three soils with contrasting fertility; this study aimed to establish an optimal management of these mineral nutrients according to the cultivar and soil type.

Materials and methods

Location and soils

The experiments were conducted between 2013 and 2014 in three potato producing locations with contrasting soils on the Cundiboyacense plains in the Andean region of Colombia as shown in Table 1.

In each location, a randomized complete block design was established using a factorial arrangement in a divided subplots-mixed model with three replicates (one plant per replicate), where the main plot corresponded to the cultivars (Capiro and Suprema) and the subplots corresponded

TABLE 1. Environmental and soil fertility characteristics at the study sites (from Gómez *et al.* (2017), with permission from Agronomía Colombiana).

Environment conditions**/Locality	Subachoque	Choconta	Facatativa
Altitude, m a.s.l.	2680	2780	2520
North latitude	4°57'50.1"	5°5'30.37"	4°49'26.9"
West longitude	74°09'28.1"	73°43'2.04"	74°22'29.7"
Annual precipitation, mm	870	1295	951
Max air temperature, ºC	18.7	16.9	18.1
Min air temperature, ºC	6.3	4.4	7.0
Average air temperature, ºC	12.5	10.6	12.6
Soil properties/classification*	Typic Hapludand	Humic Dystrudept	Andic Eutrudept
Soil fertility***	Low	Medium	High
pH	4.95	5.5	6.4
Al, $cmol_c$ kg^{-1}	0.59	0.1	0.0
Soil organic matter, g kg^{-1}	171	67.7	166.7
CIC, $cmol_c$ kg^{-1}	8.3	9.5	31.9
Texture	Loam	Clay loam	Loam
N, g kg^{-1}	8.5	3.3	8.3
P, mg kg^{-1}	24.8	28.2	39.6
K, $cmol_c$ kg^{-1}	0.1	0.7	3.1
Saturation K, g 100 g^{-1}	1.2	7.1	9.8

*Physical-chemical characterization of soils in arable layer (0-30 cm) according to IGAC (2006). Soils classified according to the USDA classification system (Soil Staff, 2010). **Environmental data obtained from IDEAM (2013-2014). ***Potential chemical fertility evaluated in the arable layer 0-30 cm (Castro and Gómez, 2013).

to two levels of fertilization (F0 and F1). The fertilizer doses F1 were not the same in the three locations (Tab. 2) due to the differences in initial soil fertility among the study sites (Tab. 1); therefore, the design was of incomplete nature due to the fertilizer dose factor. The amount of mineral nutrient applied, the sources of fertilizers, and the dose fractionation for the F1 level are shown in Table 2.

TABLE 2. Doses of mineral nutrients applied with fertilizers in the study sites (from Gómez *et al.* (2017), with permission from Agronomía Colombiana).

*Nutrient, kg ha^{-1}	Subachoque (F1s)	Facatativa (F1f)	Choconta (F1ch)
N	198	171	192
P_2O_5	374	261	340
K_2O	380	180	348
CaO	40	110	45
Mg	55	60	56
S	37	74	120
B	2.8	3.4	1.2
Zn	5.6	5.6	2.4
Mn	7.0	7.0	3.0
Cu	1.4	1.4	0.6
Fe	2.8	2.8	1.2

*Recommended fertilization rates derived from the soil-plant balance method (Castro and Gómez, 2013) and fertilizer dose fractionation according to historical references in the areas, where high yields have been obtained (>50 t ha^{-1}): N, 60% at sowing and 40% at 45-50 days after sowing (das); P, 70% at sowing and 30% at 45-50 das; K, 30% at sowing and 70% at 45-50 das. Granulated fertilizer sources were: N-P, DAP; K, KCl (0-0-60), Potassium sulfate (0-0-50); Ca, Calcium Nitrate (25% CaO); Mg, Kieserite; Nutricomplet, complex source of micronutrients B, Zn, Cu, Mn and Fe based on sulfates.

Twelve combinations of factors were evaluated using three factors: two cultivars (Capiro, Suprema), three locations (Subachoque, Facatativa, and Choconta) and two levels of fertilization, F0 (unfertilized plots, initial soil fertility conditions) and F1 (fertilized plots). The factorial design with an intra-subject factor over time was associated with four phenological stages of tuber growth, and was adapted from Valbuena *et al.* (2010): Stage II, 70-75 days after sowing (das) (start of tuberization); Stage III, 90-100 das (flowering, maximum tuberization and start of tuber filling); Stage IV, 120-125 das (end of flowering, filling of tuber); Stage V, 150-160 das (maximum tuber filling and ripening). The sowing was done in experimental units of 50 m^2 (135 plants/plot), with distances of 1 m between rows and 0.37 m between the plants and a density of 27,000 plants ha^{-1}. The agronomic practices of irrigation, weed and phytosanitary management were carried out according to the needs of the locations, as a result the effects of external factors were minimized. For Subachoque and Choconta, dolomite type pre-sowing amendments were incorporated in the soils at rates of 1.5 and 1.0 t ha^{-1}, respectively.

Plant sampling and analysis

At the four stages of tuber growth, four plants per experimental unit were evaluated. Destructive analysis of the leaves, stems and tubers was carried out. All parts of the plants were rinsed with deionized water. For each sampling, fresh leaves, aerial stems and tubers were weighed separately; a sample of 200 g (fresh weight) of each organ was placed in a paper bag and dried in the oven at 70°C for 72 h. The dry matter (DW) of each sample was then weighed, and the DW accumulation in each organ was evaluated per plant and per stage. All of the organs of the dried plants were ground using a 40 stainless steel mesh for subsequent chemical analysis. The harvest index (HI) was evaluated at phenological stage V: HI = (DWt/DWs)*100 according to Giletto and Echeverria (2015), where DWt is the tuber dry weight and DWs is the shoot dry weight. With dry samples, the nutrient concentrations in the tubers were determined according to the methodology of IGAC (2006). The amounts of N and K extracted by the tubers were calculated by multiplying the concentration of the nutrients by the DW accumulated by the tubers at each growth stage.

Efficiency use and recovery of mineral nutrients by tubers

The NUE indices were estimated by considering the treatments without fertilizer application (F0) with respect to the balanced fertilization in each location (F1). The N and K recovery efficiency by the tubers from fertilization, or acquisition efficiency (RFt), was calculated using the equation RFt = (Et_1-Et_0/amount of mineral nutrient supplied in the fertilizer)*100 (Tab. 2), where Et_1 is the nutrient extraction by tubers in fertilized soils (kg ha^{-1}) and Et_0 is the nutrient extraction by tubers in unfertilized soils (kg ha^{-1}). This equation was adapted from Fernandes and Soratto (2016). The efficiency use of N and K by the tubers (NUEt) was estimated as the accumulated dry matter in the tubers/nutrient accumulation in the tubers, as reported for potatoes by Poljak *et al.* (2011) and Rengel and Damon (2008) for N and K. In addition, we evaluated the efficiency of tuber production obtained per unit of accumulated nutrient (EPt) with the equation adapted from Prochnow *et al.* (2009): EPt = (FWT_1-FWT_0) / (Et_1-Et_0), where FWT_1 is the tuber yield in the fertilized treatment and FWT_0 is the tuber yield in the control treatment.

Statistical analysis

Multivariate analysis of variance was used, assessing differences in factor interaction with a confidence level of P<0.01. The Pearson correlation matrix for the qualitative variables was analyzed and the efficiency indices were adjusted. The statistical program SAS version 2014 was used.

Results and discussion

Yield, harvest index, and dry weight of tubers

The DWt and FWt presented significant differences ($P\leq0.01$) in response to the phenology*fertilization interaction, with a higher yield obtained for Suprema in fertilized treatments (F1) in Subachoque (70.5 t ha^{-1}) and Choconta (73.7 t ha^{-1}), in soils with medium to low fertility (Fig. 1) and a higher DWt accumulation, between 17.1 and 19.98 t ha^{-1}, respectively (Fig. 2). For Capiro, the highest yield was registered in fertilized soils with the highest K availability (Tab. 1): Humic Dystrudepts and Andic Eutrudepts in Choconta (67.3 t ha^{-1}) and Facatativa (73.1 t ha^{-1}), respectively (Fig. 1). which was directly related to the accumulation of DWt.

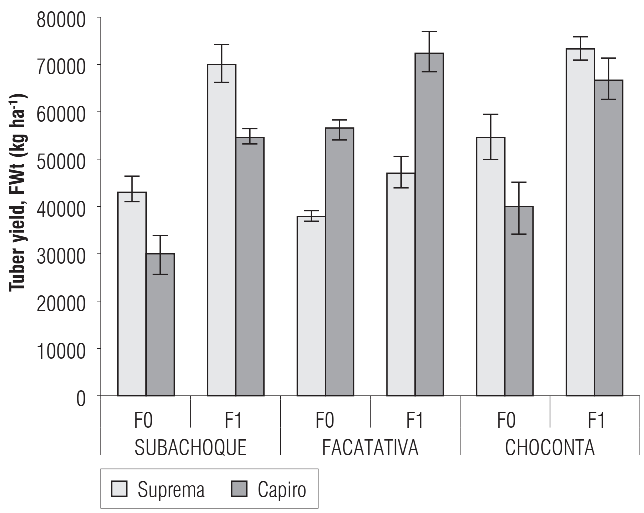


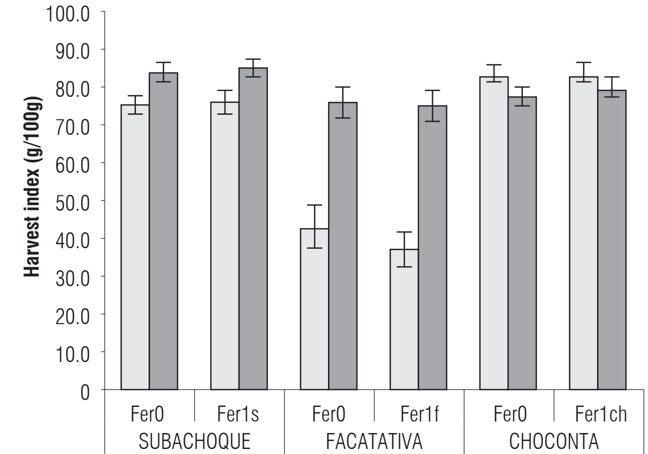
FIGURE 1. Yield (FWt) of the cvs. Capiro and Suprema at phenological stage V (maximum tuber filling and maturation) in the absence of fertilization F0, with respect to balanced fertilization by location: F1s, Typic Hapludands (Subachoque); F1ch, Humic Dystrudepts (Choconta), and F1f, Andic Eutrudepts (Facatativa). $P<0.01$ for fertilization (location*cultivar). Error bars indicate standard errors.

The yields of 70 t ha^{-1} obtained in both cultivars proved the high genetic potential of these Andean potatoes under optimal environmental and fertilization conditions; these yields were dependent on the soil-plant conditions, where a greater number of tubers and a better translocation of assimilates were promoted, exceeding the yields of 45 t ha^{-1} observed by Quinchoa *et al.* (2010), Ñústez (2011), and Pérez (2015) in the same cultivars.

For the fertilized Humic Dystrupets soils (Choconta) (Fer1ch), an increase in yield of 36.4% and 69.9% was observed for Suprema and Capiro, respectively, with respect to the initial soil conditions. These values were lower than the ones found in Typic Hapludands in Subachoque, but with higher increases in FWt, 60.9% (Suprema) and 87.1% (Capiro). This shows the importance of fertilization for both cultivars in low fertility soils (Tab. 1) and agrees with the report by Quinchoa *et al.* (2010), who found positive responses to fertilization of up to 95% YFt in Capiro in low fertility andisoles in the Antioquia province (Colombia). The lowest response to fertilization was found in Andic Eutrudepts (Facatativa) with 20.1% for Suprema and a marginal and non-significant increase with respect to the control treatment (Fig. 1); in addition, there was a 30.3% yield increase in Capiro, which corroborates a higher nutrient availability in this soil, as compared to the other evaluated soils.

For the two cultivars in the three locations, positive and significant responses in yield were observed, with a better response to fertilization in Capiro than in Suprema since Capiro is better adapted to soils with higher fertility. Capiro had the best response to the higher edaphic K supply, a better base ratio (Ca/K, Ca+Mg/K) (Tab. 1) and a balanced fertilization (Fig. 1). The differential response of the two cultivars, which was greater in Humic Dystrudepts (Choconta), could be explained by the better balance in the edaphic supply of K (0.68 cmol kg^{-1}), with lower P fixation, higher average contents of soil organic matter and absence of interchangeable Al^{+3} (Tab. 1). This was observed in the Typic Hapludands (Subachoque), which had P fixing soils associated with low mineralization and Al^{+3}, where lower levels of available N might be present. Additionally, deficient K levels were observed below the critical levels (0.1 cmol kg^{-1}) as reported by Castro and Gómez (2013) for similar soils.

The cvs. Suprema and Capiro showed a greater conversion of assimilates in the fertilized Humic Dystrudept soils (Choconta), with 27.03% and 28.2% DWt at harvest, respectively, a higher value than the one reported by Ñústez (2011) in the same cultivars (24% DWt). This greater contribution of DWt% favors their industrial use. In contrast, in the high fertility Andic Eutrudepts soils (Facatativa), lower yields and a lower accumulation of assimilates were obtained in the tubers, with 22.01% DWt and 19.8% DWt in Capiro and Suprema, respectively. The best DWt in Suprema was related to the fertilized soils with lower fertility in Subachoque and Choconta (Figs. 2 and 3), with a total DWt of 634 g $plant^{-1}$ and 740 g $plant^{-1}$, respectively. The DWt was higher than those reported by Ñústez *et al.* (2009), 450 g $plant^{-1}$, for this cultivar in low fertility soils in the Colombian potato producing zone of Zipaquira (2,580 m a.s.l.).



	Fer0 SUBACHOQUE	Fer1s SUBACHOQUE	Fer0 FACATATIVA	Fer1f FACATATIVA	Fer0 CHOCONTA	Fer1ch CHOCONTA
Suprema	75.0	75.9	42.5	36.7	83.2	83.1
Capiro	84.0	85.0	75.6	75.1	77.5	79.5

FIGURE 2. Harvest index (HI) in the cvs. Capiro and Suprema in the absence of fertilization Fer0, with respect to balanced fertilization, by location: F1s, Typic Hapludands (Subachoque); F1ch, Humic Dystrudepts (Choconta), and F1f, Andic Eutrudepts (Facatativa). $P<0.01$ for location*cultivar. Error bars indicate standard errors.

Capiro, with low to high fertility soils, presented a HI between 75% and 85%, as compared to Suprema, which presented a HI between 75 and 83% in low fertility soils in the locations of Subachoque and Choconta, respectively. Suprema limited its assimilate partition in the high fertility soils of Facatativa with a lower than 45% HI because of its more indeterminate growth habit and a later growth cycle with less accumulation of DWt (21.2%); similar results were obtained by Giletto and Echeverria (2015) for late-cycle and indeterminate-type cultivars, such as Markies Russet.

The lower HI in Suprema was related to the lower adaptation of this genotype because it was cultivated in Facatativa at marginal altitudes close 2,500 m a.s.l. and with average air temperatures higher than 13ºC, as compared to the environmental parameters defined by Ñústez (2011). Additionally, there was an availability of soil nutrients (Tab. 1), which may have generated excess N that could have inhibited tuberization and tuber growth, confirming the report by Ruza *et al.* (2013). On the other hand, Capiro was more adapted to high levels of N and K because of its better ability to partition assimilates to the tuber, as characterized by its genotype, with a determinate habit and better aerial shoots/tubers ratio. This makes it a more efficient crop; coinciding with research conducted by Kleinkopf *et al.* (1981) and Trehan and Singh (2013) for Group Chilotanum.

The HI in Capiro and Suprema did not show significant differences in response to the fertilization, but did present differences in the cultivar x location interaction (Fig. 2). The non-significance of HI in response to fertilization is consistent with results obtained by Zelalem *et al.* (2009) and Burga *et al.* (2014), who reported that increasing doses of N and K, respectively, had no influence on this parameter. This can be explained by adaptation mechanisms and characteristics of the genotype and also coincides with reports by Rengel and Damon (2008) and Gileto and Echevarria (2015).

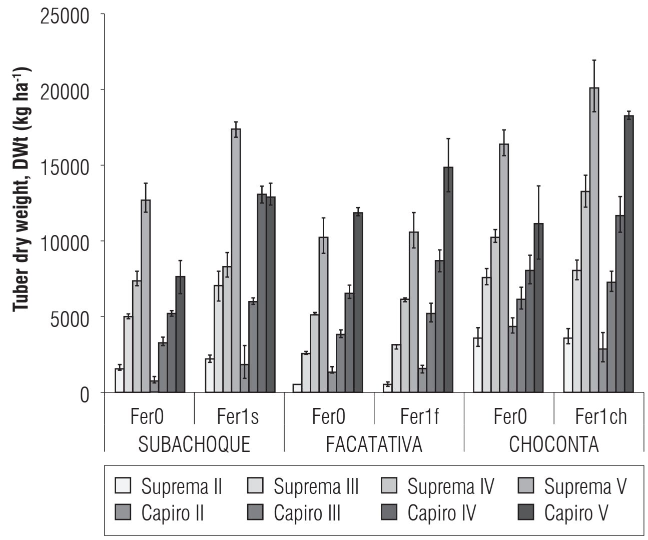


FIGURE 3. Tuber dry weight (DWt) in the cvs. Capiro and Suprema at four phenological stages (II, start of tuberization, III, maximum tuberization-start of filling, IV, filling of tuber, V, maximum filling and maturation) in the absence of fertilization F0, with respect to balanced fertilization by location for the soils: Typic Hapludands (Subachoque), F1s; Humic Dystrudepts (Choconta), F1ch and Andic Eutrudepts (Facatativa), Fer1f; $P<0.01$ for fertilization (location*cultivar). Error bars indicate standard errors.

As for the accumulation of DWt in Suprema at 125 das (Stage IV) and in Capiro at 100 das (Stage III), an earlier filling of tubers was observed with the Typic hapludands (Subachoque) soil as compared to the other locations. This was probably because these cultivars were grown in soils with lower fertility and a low contribution of K (0.1 cmolc kg^{-1}), as compared to the Choconta and Facatativa soils (Fig. 3, Tab. 1), where tuber filling in both cultivars was concentrated towards the end of the cycle. This could possibly be due to the better availability of nutrients, mainly K, during all of the phenological stages, which presented a gradual and linear extraction of these elements (Fig. 3). The greater DWt accumulation at stages IV and V in the evaluated cultivars could be associated with phenological stages of higher photosynthetic demands, and coincided with stages of high translocation and assimilation partition, verifying the report by Valbuena *et al.* (2010) for these cultivars.

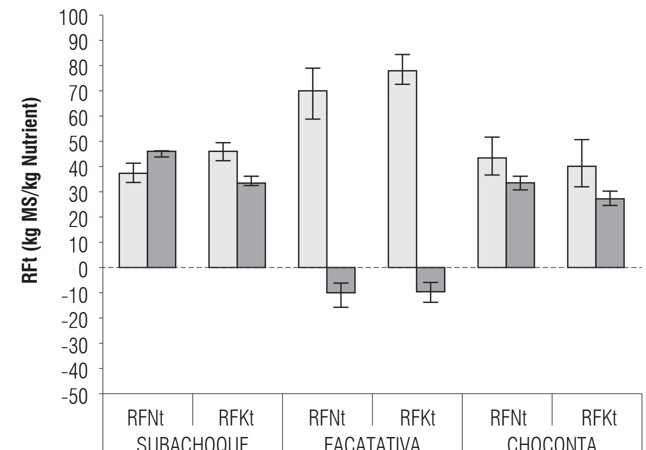
Efficiency in the nutrient use by tubers

Suprema in the Andic Eutrudepts with high fertility (Facatativa) presented a negative response in the recovery of N (-10.8 RFNt) and K (-9.6 RFKt) for the balanced fertilizer application (Fig. 4) and a negative physiological response of N (-267.9 EPt N) and K (-250 EPt K) for tuber production when compared to positive NUE indices for Capiro (Fig. 5), indicating significant differences between location and cultivar. The highest RFt of N and K for the applied fertilizer was observed for Capiro in Facatativa in high fertility soils in the basin zone, with an RFNt of 70.2% and an RFKt of 79.8%; these indexes were higher than those found in Choconta, with an RFNt of 44.8% and an RFKt of 42.3%, and in Subachoque (38.1% RFNt and 46.9% RFKt) probably because these soils present greater losses of these nutrients as a result of runoff since they are located in mountainous terrain. Choconta and Subachoque also presented a high yield potential for Capiro and a lower than 60% RFNt, as reported by Vos (2009) for nitrogen applications between 150-200 kg ha^{-1}, and had similar indices for K acquisition, as reported by Gómez and Torres (2012) in the cv. Capiro.

Suprema presented a low recovery efficiency (<45%) of N (RFNt) and K (RFKt), with lower rates than Capiro for K in the low fertility soils (Fig. 4). This is a characteristic of high yield and indeterminate cultivars, as reported by Kleinkopf *et al.* (1981) for the cvs. Russet Burbank and Centennial Russet. In addition, the low K efficiency was probably due to a low supply of non-exchangeable K to the roots, with low K extraction in these soils associated with a low root/shoot ratio. Similar results were reported by Trehan and Singh (2013) for the cultivars Group Chilotanum Kufri Jyoti and Kufri Badshah. Therefore, it is necessary for soils with low K availability to increase the diffusion and mass flow of this element by means of an adequate K contribution and fractionation in the mineral and/or organic fertilizers starting at sowing. This helps counteract the effect of antagonistic elements, such as Al^{+3}, maintaining a balance in the Ca^{+2} and Mg^{+2} ratio, and, thus, improving the rhizosphere environment and root growth.

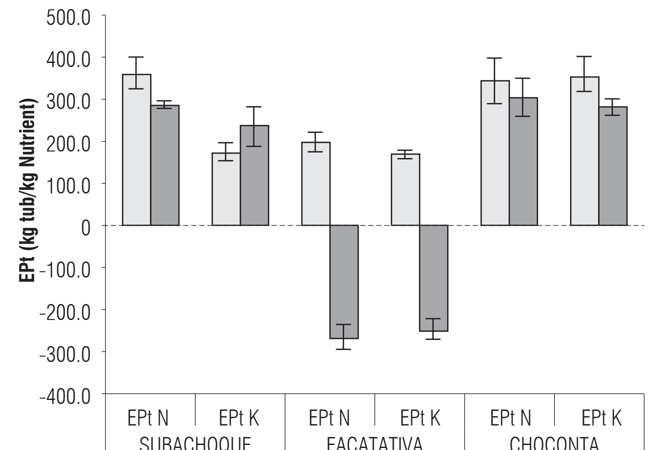
In the Humic Dystrudepts soils with lower fertility in Choconta, where the best yields were obtained, a better physiological efficiency in the production of tubers by nutrient extraction (EPt) was observed, with significant differences in the interaction between the location and the cultivar (Fig. 5). Under these conditions, Capiro and Suprema produced an EPtN of 347 and 308 kg FWt kg^{-1} N, respectively, and an EPtK of 361 and 283 kg FWt kg^{-1} K. These results were higher for N and similar for K when compared to those reported by Trehan and Singh (2013), with an EPtN between 250 and 318 kg FWt kg^{-1} N and an EPtK between 256-360 kg FWt kg^{-1} K for the efficient Group Chilotanum cultivar Kufri and the hybrid JX 576.

This is associated with the higher response to fertilization in FWt and DWt in Capiro and Suprema in this location. It also coincides with the greater use efficiency of N and K in the tubers (Fig. 6), which can be explained by the better partitioning and conversion of N, K and assimilates to



	RFNt SUBACHOQUE	RFKt SUBACHOQUE	RFNt FACATATIVA	RFKt FACATATIVA	RFNt CHOCONTA	RFKt CHOCONTA
Capiro	38.1	46.9	70.2	79.8	44.8	42.3
Suprema	45.78	35.0	-10.8	-9.6	34.2	28.1

FIGURE 4. Efficiency of recovery of N and K in tubers (RFt), kg nutrient extracted per 100 kg of nutrient applied in balanced fertilization, for the cv. Capiro and cv. Suprema in contrasting soils in the Andean region of Colombia. $P<0.01$ in N and K for location*cultivar. Error bars indicate standard errors.



	EPt N SUBACHOQUE	EPt K SUBACHOQUE	EPt N FACATATIVA	EPt K FACATATIVA	EPt N CHOCONTA	EPt K CHOCONTA
Capiro	365.6	174.4	200.2	172.8	347.1	361.2
Suprema	291.1	238.3	-267.9	-250.0	307.9	283.9

FIGURE 5. EPt of N and K (kg of tuber harvested per kg of nutrient extracted) in Capiro and Suprema in contrasting soils in the Andean region of Colombia. $P<0.01$ in N and K for location*cultivar. Error bars indicate standard errors.

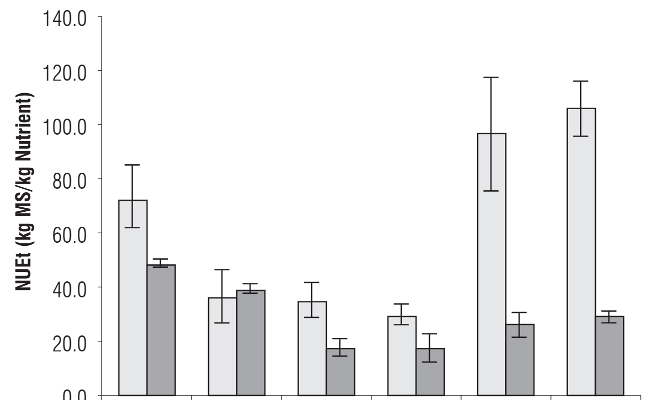
the tubers with a better sink strength of the tubers during filling for both cultivars under the edaphic-environmental conditions of this location. In addition, the greater efficiency in the cv. Capiro in contrasting soils coincides with the better adaptation of this cultivar to a wider range of altitudes (1,800-3,200 m) and soils, similar to the optimal environmental conditions reported by Ñústez (2011).

Capiro responded better to the fertile soils, with HI>75% (Fig. 2). Lower soil loss factors were observed as a result of the location in lacustrine basin areas and the high native K and N contents (Tab. 1). Again, this confirms that the best adaptation for Capiro is in flat areas and in the high fertility soils of the Bogota plateau. This is seen in a better efficiency in the translocation of nutrients, which are assimilated to the tuber, and a high removal of N and K from the plant (Fig. 4), which needs to be replenished with fertilization plans. The better adaptation of this cultivar can be explained by possible differential absorption mechanisms with a greater flow of K and N to the root and tuber. In addition, the presence of specialized channels that also favor assimilate translocation, together with a lower aerial shoots/tuber ratio, represent mechanisms that have been explained for K by Trehan and Singh (2013) and Wang and Wu (2015), and for N by Vos (2009), who verified the differences between the genotypes.

Under the conditions of high K availability (3.14 $cmol_c\ kg^{-1}$) in the Andic Eutrudepts (Facatativa), Suprema responded negatively to the fertilization of this nutrient with an EPtK of -250 and presented the lowest EptK, 172.8 for Capiro (Fig. 5). Additionally, the tuber production response of Suprema was marginal for the high potassium saturation soils (>9%) in the Andic Eutrudepts (Facatativa), as shown in Figure 1.

These data suggest a possible luxury K uptake for Suprema and a marginal uptake for Capiro, phenomena that have been explained for the Group Chilotanum by Kang *et al.* (2014). On the other hand, Karam *et al.* (2009) found less efficiency in the use of K with K_2O levels higher than 289 kg ha^{-1} for the cvs. Derby and Umatilla Russet. In addition, Burga *et al.* (2014) verified that, in tetraploid cultivars, high levels of K affected the development of tubers, to the detriment of yield, where excesses of this element might limit the transport of other assimilates or hormones.

On the other hand, the Facatativa soils had the lowest physiological efficiency of N (NUEt of 18 kg kg^{-1} for Capiro and 36 kg kg^{-1} for Suprema) and K (NUEt of 36 kg kg^{-1} Suprema and 30 kg kg^{-1} Capiro) with contributions of 171 kg ha^{-1} of N and 180 kg ha^{-1} of K, respectively, with high N and K availability (Tab. 2). This agrees with the findings of Zebarth *et al.* (2012) and Saravia *et al.* (2016), who found low NUE values in potatoes, 40 and 10 kg kg^{-1}, respectively; increasing the availability of N in crops with doses between 200 and 300 kg ha^{-1} of N can help with positive plant responses.



	NUEt N	NUEt K	NUEt N	NUEt K	NUEt N	NUEt K
	SUBACHOQUE		FACATATIVA		CHOCONTA	
Capiro	73.2	37.2	36.0	30.5	97.3	106.7
Suprema	49.3	40.1	18.2	18.2	26.8	29.8

FIGURE 6. Efficient use of N and K in tubers with balanced fertilization, NUEt (kg of dry matter of the tuber per kg of nutrient extracted) in the cvs. Capiro and Suprema in Typic Hapludands, Subachoque, Andic Eutrudepts, Facatativa, and Humic Dystrudepts, Choconta in the Andean region of Colombia. Error bars indicate standard errors.

The highest physiological efficiency in the use of nutrients in the tubers was obtained in Capiro, with an NUEt of 97.3 kg kg^{-1} for N and 106.7 kg kg^{-1} for K in the fertilized soils with lower fertility in Choconta. This corroborates the better efficiency of Capiro, a characteristic of this genotype that has already been mentioned, and verifies that the best NUE was significantly different from that in Suprema. Capiro appears to be a genotype of a determinate type, in agreement with results of Kleinkopf *et al.* (1981) for cultivars of a similar type. These indices, found in the soils with lower fertility, were higher than those reported by Poljak *et al.* (2011), with a NUEt of 71 to 76 kg kg^{-1} for N in the Group Chilotanum with an N fertilizer contribution between 150-200 kg ha^{-1}. This was probably due to the positive interaction of the nutrients in the balanced fertilization of macro and micronutrients in unsaturated soils, with an improvement in the efficient use of nutrients in tropical soils, which confirms the observations of Prochow *et al.* (2009).

The low physiological efficiency of N and K in the tubers for the cv. Suprema in the soils with excesses of N might suggest a luxury consumption of these elements that could be due to a lower growth of tubers resulting from the low transport of assimilates to organs associated with low HI (Fig. 2). With a higher average temperature than in the other locations, this could result in a high aerial shoots/tubers ratio, which limits the flow of carbon and nutrients to tubers. These results are similar to those reported by Fandika *et al.* (2012) and Saravia *et al.* (2016) for N and Wang and Wu (2015) for K. These authors suggested agronomic and genetic strategies to decrease the air shoot/tuber ratio and improve the translocation of assimilates to the tubers. According to Roumeliotis (2012), excess N and high temperatures limit tuber formation and growth. Fandika *et al.* (2012) also reported a less efficient use of N with lower partitioning of assimilates to the tubers and a low number of physiological sources at the start of tuberization under high N doses.

An imbalance resulting from excess available N in a soil during tuber filling at the start of tuberization could also cause a reversal of tubers into stolons, affecting the productive potential, a phenomenon that should be evaluated in these cultivars in future research. Similar results were proposed by Güller (2009) for the potato Group Chilotanum cv. Ana, where it was found that, at doses higher than 200 kg ha^{-1}, a smaller number of tubers were present at the tuberization stage, with a higher generation of source structures. In addition, Zelalem *et al.* (2009) reported that doses higher than 140 kg N ha^{-1} increased the number of stems and delayed flowering, which, according to Roumeliotis (2012), affected the synthesis and transport of the FT-like protein tuberigen, which favors tuberization in this species.

The lower physiological indices found in the use of K obtained for Suprema in high fertility soils (Figs. 4-6) coincided with a rapid vegetative growth, later tuberization and less growth of the source organ. This agrees with the lower accumulation of K and dry matter and the presence of smaller tubers despite the presence of the same number of tubers as in Capiro. This can be explained by the excess K, which can generate an imbalance by allowing nitrate accumulation with a lower assimilation in the aerial part and a decrease in the transport of carbohydrates and proteins towards the tubers. Similar effects from excess K were found by Kang *et al.* (2014).

Conclusions

Capiro is more efficient in the use of N and K than Suprema regardless of the soil type and location, although Suprema presented the best responses and physiological indices in the soils with lower fertility at the higher altitude and with a lower ambient temperature in response to the balanced fertilization. This suggests the use of integrated fertilization management aimed at improving the N and K availability in the rhizosphere (acidity and nutrient balance) and a specific fertilization in both cultivars, considering the environmental supply per site and the soil supply given by the soil pedogenesis.

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Growth response surface for optimizing fertilization in Andean blackberry (*Rubus glaucus* Benth.) nurseries

Superficie de respuesta en crecimiento para optimizar fertilización en mora de los Andes (*Rubus glaucus* Benth.) en viveros

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ABSTRACT

Fertilization in soils cultivated with the Andean blackberry has been carried out empirically because there is no complete knowledge on its nutrient requirements. Therefore, the aim of this study was to estimate the effect of variable doses of N, P, K and Ca on the growth of thornless Andean blackberry in nurseries. This research was carried out in the nursery of AGROSAVIA C. I. Tibaitatá (Mosquera, Colombia) using vitroplantlets sown in peat moss, sand and rice husks (2:1:1). A completely randomized block design with 25 treatments and 15 plants per experiment unit was used. The nutritive solution application frequency was established as every four d for three months with a volume of 44 mL/plant; the concentration was increased each month. Destructive sampling was carried out 30, 60 and 90 d after transplanting, registering plant height, leaf area, root length and volume, number of leaflets, and leaf, stem and root dry matter. Regression models were used establishing significance (P<0.05 and <0.01) between N, P, K and Ca using SAS 9.3. Doses of 36 N, 43 P_2O_5, 18 K_2O, and 9 CaO g/plant improved the root development in terms of dry matter and length and increased the number of leaflets, aerial length and leaf dry matter.

Key words: vitroplantlets, dry matter, optimum dose, application rate.

RESUMEN

La fertilización en suelos cultivados con mora se ha realizado empíricamente, puesto que no se tiene pleno conocimiento de sus requerimientos nutricionales. Tomando como base lo anterior, el objetivo de esta investigación fue estimar el efecto de dosis variables de N, P, K y Ca sobre el crecimiento de la mora sin espinas en vivero. La investigación se realizó en los invernaderos de AGROSAVIA C. I. Tibaitatá (Mosquera, Colombia), utilizando vitroplántulas sembradas en turba, arena y cascarilla de arroz (2:1:1). Se estableció un diseño en bloques completos al azar con 25 tratamientos y 15 plantas por unidad experimental. La frecuencia de aplicación fue cada cuatro días durante tres meses, con un volumen de solución nutritiva de 44 mL/planta, incrementando la concentración cada mes. Se realizaron muestreos destructivos a los 30, 60 y 90 días después del trasplante, registrando altura de planta, área foliar, longitud y volumen radical, número de foliolos y masa seca en hoja, tallo y raíz. Se trabajaron modelos de regresión determinando significancia (P<0,05 y <0,01) entre variables utilizando el software SAS 9.3. La dosis 36 N – 43 P_2O_5 – 18 K_2O – 9 CaO g/planta permitió mejor desarrollo radical en masa y longitud, mayor número de foliolos, longitud aérea y masa seca foliar.

Palabras clave: vitroplántulas, masa seca, dosis óptima, tasa de aplicación.

Introduction

Scientific and technical literature on the Andean blackberry (*Rubus glaucus* Benth.) yield regarding nutrient requirements and extraction levels is still in its initial stages, and there are still big differences in fertilizer applications (sources and amounts). This information is of utmost importance for achieving higher production and yield (Santos *et al.*, 2014). Currently, the Andean blackberry is produced with a mixture of diverse materials and agronomic management practices, urgently requiring a different type of management. One of these materials is the thornless Andean blackberry, which was obtained from the Colombian coffee-growing region, possibly through spontaneous generation (Morales and Villegas, 2012). It has a higher number of productive branches (female) and a lower number of non-productive branches (whips). Additionally, it shows higher tillering than the Andean blackberry with thorns (15 to 20% superior) (Bernal and Díaz, 2006), inferring a consumptive use that is different from that of the genotype with thorns. In this sense, various researchers have evaluated nutrient consumption in the thornless Andean blackberry, comparing it with the one with thorns. Bolaños-Benavides *et al.* (2014) carried out applications of 36 g, 43 g, 18 g and 9 g of N, P_2O_5, K_2O

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and CaO per plant, respectively, finding that thornless plants had a higher height with significant differences ($P \leq 0.05$) (9.93 cm and 20.83 cm, 30 and 90 d after transplanting (dat), respectively), as compared to plants with thorns, with height values of 8.11 and 20.55 cm at 30 and 100 d of accumulating nutrients, respectively (Cardona *et al.*, 2016). However, the experience acquired by producers and technicians has led to fertilizing 60 d after planting, and this practice continues every 90 d until one year of establishment is reached (Betancur-Cardona *et al.*, 2014). Adequate and accurate nutrient application for plants starting in the initial growth stages (Marschner, 2011) favors the rooting process and gives seedlings higher vigor (Razaq *et al.*, 2017).

Based on this empirical practice and in order to have plants with adequate nutrition when transplanted to uncontrolled conditions, the aim of this study was to estimate optimum doses of N, P, K and Ca in thornless Andean blackberry plants under nursery conditions, developing their genetic potential once they have been established as a commercial crop.

Materials and methods

Study site location

This study was carried out in the Tibaitatá Research Center of the Corporación colombiana de investigación agropecuaria - AGROSAVIA located at 4º41'43.1349" N and 74º12'18.7666" W, with a mean temperature of 13.1°C, a relative humidity of 80% and an altitude of 2,600 m a.s.l.

Plant material

Thornless Andean blackberry vitroplantlets were propagated in the micropropagation laboratory of AGROSAVIA. Then, the plant material was placed in transparent trays with inert peat moss, adding 1 g of the fungus *Glomus proliferum* Dalpé et Declerck strain GB02 (60 to 70 spores g^{-1}) for the hardening stage (30 d). Finally, each plantlet was sown in recipients with 500 g of peat moss: sand: rice husk at a volume: volume ratio of 2:1:1.

Experimental design and statistical analyses

The treatments were analyzed using a composite central design, defining 25 treatments and applying the following equation: $2^k + 2{*}k + 1$ (where k is the number of factors: N, P, K and Ca).

This experiment was established in a greenhouse (the climatic conditions during the evaluations had a maximum temperature of 21°C, with an average of 19°C and a minimum of 17°C; in the case of relative humidity, a maximum humidity of 71%, an average of 57% and a minimum of 35% were registered) in complete randomized blocks with 25 treatments, three (3) blocks and 15 plants per experimental unit. The application frequency was every four d for three months, i.e. 24 applications, administering a volume of 44 mL of nutrient solution per plant. The concentration of the nutrient solution applied increased every month according to plantlet development (Tab. 1). The basis for this nutrient solution was the Hoagland and Amon solution, N, P, K and Ca concentrations according to the fertilization treatment and maintaining the original Mg, S and micronutrient concentrations. Destructive sampling was carried out 30, 60 and 90 d after transplanting (dat). The following variables were registered: plant height (PH) using a ruler with millimeters and measuring from the base of the stem to the growth point, leaf area (LA) using a CI - 202 portable measuring device (CID Bio-Science, USA), and root volume (RV). In this method, the entire root was placed inside the test tube with a known water volume, with the root system volume corresponding to the displaced water. Other variables included the number of leaflets (NL), leaf dry matter (LDM), stem dry matter (SDM), root dry matter (RDM), and total dry matter or plant dry mater (TDM). In each destructive sampling, the plants were processed as follows: a) petiole and lamina, b) stem, and c) roots. To measure the dry matter, the material was placed in paper bags and placed

TABLE 1. Nutrient level specifications for constructing the treatment matrix for 30, 60 and 90 dat according to the Central Composite Design.

Code level		N (g/plant)				P_2O_5 (g/plant)				K_2O (g/plant)				CaO (g/plant)			
		Days after transplanting (dat)															
		30	60	90	Total	30	60	90	Total	30	60	90	Total	30	60	90	Total
Lower axial (LA)	-1.41	1.6	2.4	3.0	7.0	0.5	0.7	0.9	2.1	2.0	2.4	6.2	10.6	1.6	1.9	3.0	6.5
Low dose (LD)	-1	2.3	4.0	5.7	12.0	2.1	3.1	3.8	9.0	4.7	5.7	7.5	17.9	2.0	3.0	4.0	9.0
Average dose (AD)	0	4.9	6.3	12.8	24.0	6.0	9.0	11.0	26.0	9.6	11.4	15.0	36.0	4.2	4.9	6.0	15.1
High dose (HD)	1	4.8	13.4	17.8	36.0	12.0	14.3	16.7	43.0	12.4	17.0	24.6	54.0	6.0	7.0	8.0	21.0
Upper axial (UA)	1.41	7.3	14.2	19.5	41.0	14.0	16.8	19.2	50.0	15.7	21.4	24.4	61.5	6.7	7.8	9.0	23.5

in an oven at 70°C for 24 to 48 h until constant weight; then, an analytical balance (Mettler Toledo Model AB204) was used to measure the dry matter.

The statistical analysis included regression models selecting significant terms (P<0.05) of a saturated model with lineal and quadratic effects for doses of N, P, K and Ca with their double interactions, simple effect and interaction over time. The data processing was carried out with SAS 9.3 (SAS Institute, Cary, NC, USA).

Results and discussion

At 60 d after transplanting, the Andean blackberry plants presented differential values for the recorded allometric variables, showing higher averages for the leaf area, plant height and root length, as compared to 90 d after transplanting (Tab. 2). In contrast, the variables related to accumulation of dry matter in each organ and number of leaflets presented incremental averages over time.

Plant weight accumulation

According to the ANOVA (not included), the dry matter accumulation in the leaves, stems, roots and in the entire plant (total) was significantly influenced (P<0.05) by the doses of N, K, P and Ca, with some interactions described below. In all of the plant organs, the biomass varied because the Ca dose effect followed quadratic tendencies, but the response rate was variable over time, indicating that the Ca requirement increased with plant growth, but an excess Ca dose can be detrimental.

The P doses had a significant quadratic effect (P<0.05) on RDM accumulation, at a rate that varied according to plant growth; hence, the temporal role in initial root system growth was found; likewise, K can modify P response through an interaction. These results contrast with those found by Ascencio and Lazo (2001), who argued that P absorption by roots is better explained by total root length and not by root system dry weight, which were taken as a reference point. On the contrary, Bayuelo-Jiménez *et al.* (2011, 2012) stated that root formation is not related to plant size, but to the efficiency in phosphorus absorption. On the other hand, Benavides-Mendoza (2011) stated that root system dry matter can increase its length. "increasing root dry weight might reflect an increase in root length"

However, when developing biomass in any of the plant organs, the Ca dose effect interacted with the N dose effect consistently over time. This means that the excess Ca dose inhibited or reduced the effect of the N dose. Castaño *et al.* (2008) evaluated nutritient deficiencies in the Andean blackberry, finding that a lower RDM was registered in the Ca treatment, stimulating cellular division in the meristematic points and stabilizing cells during division. Riveras *et al.* (2015) mentioned the role that Ca plays in the regulation of nitrogen absorption. A P x Ca effect was observed in the leaves (P<0.05), indicating that the excess Ca also affected the plant response to the P dose in the LDM.

The response rate of the N dose was affected by plant growth only in the LDM (P<0.01). Likewise, Yang *et al.* (2014) stated that absorption in leaves changes over time and has a narrow relationship with the growth rate. Moreover, Castaño *et al.* (2008), in a treatment without nitrogen, found the lowest SDM resulted from the role of this element in growth, tissue formation and dry matter accumulation.

TABLE 2. Descriptive statistics of the analyzed variables.

Variable	30 dat				60 dat				90 dat			
	Mean	Min	Max	SD	Mean	Min	Max	SD	Mean	Min	Max	SD
LA (cm^2)	21.735	0.050	70.120	10.824	98.673	4.080	272.380	77.187	48.980	0.720	279.210	48.872
PH (cm)	9.981	4.000	16.500	2.416	13.007	2.000	29.100	4.345	12.212	2.500	29.500	5.268
RL (cm)	11.071	2.500	24.000	4.054	14.780	1.500	28.000	5.007	10.802	1.000	28.000	5.115
NL (n)	6.824	1.000	17.000	2.060	8.726	3.000	21.000	3.553	13.043	3.000	31.000	5.498
LDM (g)	0.067	0.010	0.226	0.034	0.180	0.005	0.924	0.125	0.265	0.018	1.340	0.252
RDM (g)	0.022	0.003	0.068	0.012	0.054	0.003	0.551	0.047	0.064	0.001	0.325	0.061
SDM (g)	0.022	0.001	0.096	0.013	0.054	0.003	0.272	0.042	0.092	0.005	0.741	0.105
TDM (g)	0.100	0.003	0.324	0.052	0.261	0.001	1.339	0.190	0.383	0.001	2.081	0.393
RV (cm^3)	0.420	0.050	5.000	0.431	0.698	0.030	15.000	0.974	0.774	0.050	4.000	0.803

Min: Minimum; Max: Maximum; SD: Standard deviation; LA: Leaf area; PH: Plant height; RL: Root length; NL: Number of leaflets; LDM: leaf dry matter; RDM: root dry matter; SDM: stem dry matter; TDM: total dry matter; RV: Root volume.

The RDM was affected by every evaluated nutrient; however, according to the analysis of variance, N and K had a stable response over time, indicating that the root absorbs these elements in the amount required by growth. Castaño *et al.* (2008) found that the highest RDM average was obtained in the treatment without nitrogen, as seen by Cánovas *et al.* (2016), who stated that a decrease in the nitrogen concentration promotes an increase in root system length during initial growth stages.

The SDM responded to the K and Ca doses at a different rate according to the plant growth; however, there was no interaction between the K and Ca doses, indicating independent functions in this organ. One of the main functions of Ca is the formation of the structural part of the protopectin, keeping cells together as they are located in the medium layer and the primary cell wall (Al-Shemmar *et al.*, 2013).

The surface analysis for the RDM response (Tab. 3) showed a positive response to the N dose increase; however, this effect was reduced with the higher doses of Ca. Likewise, there was a quadratic effect towards the maximum RDM, with an increase in the P doses (P^2), but it was reduced when high doses of K were applied (Fig. 1). Therefore, doses of Ca and K must be low ($CaO \leq 9$ and $K_2O \leq 18$) in order to favor positive N and P effects. Kim and Li (2016) found that high doses of P favored biomass accumulation in the root system when analyzing the effects of reduced phosphorus on shoot and root growth, partitioning, and phosphorus utilization efficiency in *Lantana camara*. The most favorable treatment with these conditions was the one that had the doses of 36 g of N, 43 g of P_2O_5, 18 g of K_2O and 9 g of CaO per plant, which match the highest average observed between the treatments. Another proposed dose is 36 g of N, 50 g of P_2O_5, 18 g of K_2O and 9 g of CaO per plant; this could increase RDM by 12% (0.15 units of weight) according to the established model.

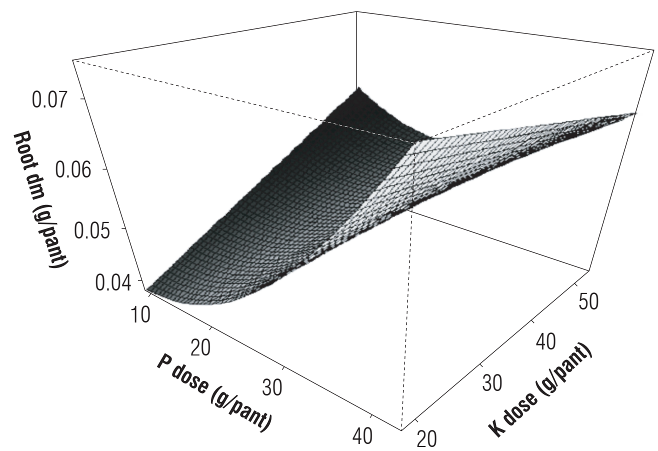

FIGURE 1. The surface analysis for the root dry matter (RDM) response to the P x K interaction.

In the case of the LDM (Tab. 3), there was a positive effect, with an increase in the N dose that was reduced with high amounts of Ca ($CaO \geq 15$ g/plant) (Fig. 2). Accordingly, the Ca effect had a quadratic tendency, indicating that the LDM followed a curve as a function of the Ca dose, with a maximum response at 15 g of CaO/plant according to the derivative calculations. Moreover, P had a positive effect on

TABLE 3. Regression coefficient estimation and associated error per regression model in terms of RDM, LDM and SDM.

Terms	RDM		LDM		SDM	
	Estimation	Error	Estimation	Error	Estimation	Error
Intercept	-0.0241	0.0126	-0.9188	0.1684	-0.3721	0.0673
N	0.0050	0.0006	0.0168	0.0036		
K					0.0050	0.0022
Ca			0.1295	0.0217	0.0536	0.0087
N x P					0.0001	1.6E-05
N x Ca	-0.0002	3.3E-05	-0.0004	0.0002		
P x K	-2.6E-05	1.0E-05				
P x Ca			-0.0003	0.0001		
K x Ca	0.0001	1.9E-05				
P^2	3.0E-05	8.0E-06	0.0001	4.1E-05		
K^2					-0.0001	2.9E-05
Ca^2			-0.0041	0.0007	-0.0018	0.0003
r^2	0.52		0.54		0.5	

All of the coefficients in the table are significantly different from zero (0) according to the *t*-test at 5% significance. Empty cells indicate terms that were not included in the model because they did not contribute significantly. r^2: coefficient of determination; RDM: root dry matter; LDM: leaf dry matter; SDM: stem dry matter.

the LDM, although it may have been unfavorably affected by the high Ca doses. Finally, the model discarded K, with lower doses chosen because of economic costs ($K_2O \leq 18$ g/plant). According to these conditions, the dose of 36 g of N, 43 g of P_2O_5, 18 g of K_2O and 9 g of CaO per plant was favorable for the LDM. However, with this model, it is possible to predict that, with a dose of 15 g of CaO per plant (optimum value by derivation), a 4% higher point is reached (0.52 units of weight). For the observed averages, the dose of 41 g of N, 26 g of P_2O_5, 36 g of K_2O and 15 g of CaO per plant also showed a good LDM result. This treatment had a Ca level that matched the level recommended by the model, and the effect of reducing the P dose was compensated by the higher N dose; nevertheless, the K dose can be reduced to 18 g/plant according to the model without changing the result in the LDM, which would reduce costs for potassium fertilizers. In other words, adjusted proposal is 41 g of N, 26 g of P_2O_5, 18 g of K_2O and 15 g of CaO per plant; however, this result should be validated. Parra-Terraza *et al.* (2010) found that a higher LDM may be related to higher leaf area values, decreasing the stress caused by *Solanum lycopersicum* plantlet transplantation, facilitating establishment.

For the SDM (Tab. 3), the model had a quadratic tendency in response to the K dose and a maximum weight with 40 g of K_2O per plant. Parra-Terraza *et al.* (2010) assessed the nitrate/ammonium/urea ratio and potassium concentrations in *S. lycopersicum* plantlet production, finding that K concentrations did not present significant differences in leaf, stem and root dry weight.

As in the results for the LDM, there was a positive Ca effect that followed a quadratic tendency with a maximum LDM with 15 g of CaO per plant (Fig. 3). Finally, a positive N x P interaction was found, indicating an advantage in increasing their doses for higher SDM effects (N>36 g, P_2O_5>43 g/plant). The closest treatment for the conditions suggested a dose of 41 g of N, 26 g of P_2O_5, 36 g of K_2O and 15 g of CaO per plant. This treatment had the best response although, when using this model, a maximum SDM with a dose of 43 g of P_2O_5 per plant is expected, generating an increase of 24% in the SDM (0.25 units of weight); however, this result should be validated. The adjustment of 36 g to 40 g of K_2O per plant did not have a strong effect on the SDM according to the model (<1%).

Furthermore, the application of N and P was more important for the leaf and root development, whereas the high application of K and Ca was unfavorable. In this plant development stage, when root system development must be strengthened, the treatments with good levels of N and P must be selected even if they are not the most favorable to increasing stem biomass.

Allometric variables

The allometric variables were significantly influenced ($P<0.05$) by the N, P, K, Ca doses and their interaction, as described below. On one side, the K and P negative interaction had a significant effect on the root volume (RV) (Tab. 4). The effect of Ca was positive and followed the curve with a peak when the Ca dose was 12.5 g per plant. Additionally, the effect of N was positive and lineal, and, according to the model, this treatment achieved high RV values with a dose of 36 g of N, 9 g of P_2O_5, 54 g of K_2O and 9 g of CaO per plant, with an average of 1.24 cm^3. However, the Ca dose could be increased to 12.5 g of CaO per plant in order to achieve a 9% higher volume (1.35 cm^3). Then, in this proposal, a dose of 9 g of P_2O_5 per plant is low, but produces a higher response in RV when 54 g of K_2O per

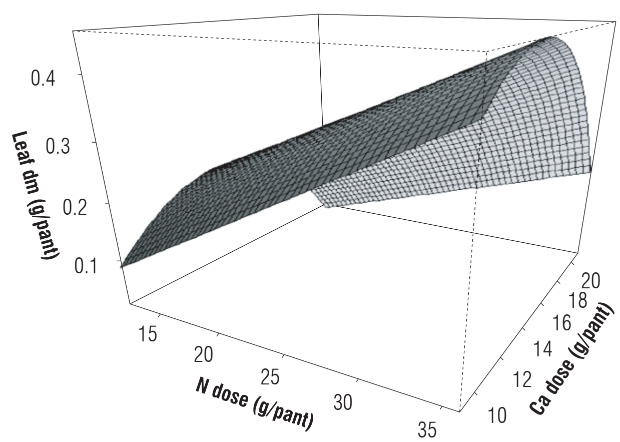

FIGURE 2. The surface analysis for the leaf dry matter (LDM) response to the N x Ca interaction.

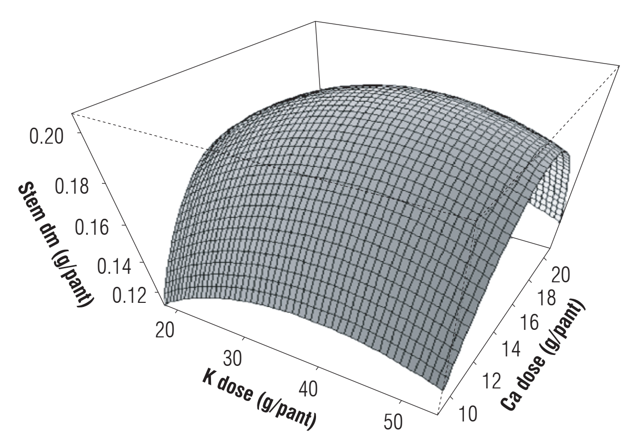

FIGURE 3. The surface analysis for the stem dry matter (SDM) response to the combined effect of K and Ca.

plant is applied. RV results would be better if P dose were increased and K dose were decreased, according to P x K interaction. So, in the data observed, the dose of 24 g of N, 50 g of P_2O_5, 36 g of K_2O and 15 g of CaO per plant had a higher average (1.46 cm^3), 30% higher than what was expected by the model (1.01 cm^3). Another treatment that can be proposed is 36 g of N, 43 g of P_2O_5, 18 g of K_2O and 12 CaO per plant, which had an expected average of 1.30 cm^3 and produced the best RDM development.

These results contrast with those found by Koning *et al.* (2015), who evaluated the effects of nitrogen and phosphate fertilization on the leaf nutrient content, photosynthesis, and growth of *Fallopia sachalinensis* cv. 'Igniscum Candy'; they observed a significant increase when the nitrogen concentration was increased in the growth media. Nitrogen had a significant effect ($P<0.05$) on root system growth (root length and volume) over time; these results agree with the findings of Rivera-Espejel *et al.* (2014), who evaluated the fertilization of *S. lycopersicum* with nitrate and ammonium, finding significant differences when compared to the absolute control (root that only grew with water). According to Frias-Moreno *et al.* (2014), high N doses induce a higher development of the stem and leaves in *S. lycopersicum,* but decrease root system growth.

For leaf area (LA) (Tab. 4), the Ca effect was very strong according to the model and showed a response curve with a peak when the CaO dose was 14.6 g per plant (by model derivation). This result is very close to the mean model dose (i.e. 15 g of CaO per plant). In this respect, Vargas-Bolívar *et al.* (2009) evaluated nutrient deficiencies in *S. quitoense* in nurseries, finding that the absence of calcium had a significant positive effect on LA as compared to plants irrigated only with water and calcium.

Calcium interacts negatively with P; however, the dose effect of P was positive, such as N and K. Therefore, high doses of N, P and K increase LA (≥ 36 g of N, ≥ 43 g of P_2O_5, and ≥ 54 g of K_2O per plant). Xiang *et al.* (2012) found a decrease in height, leaf area and stem diameter in *Glycine max* plants cultivated without phosphorus fertilization because this nutrient is essential as a tissue constituent that acts directly on plant height (PH). Because of the Ca x P interaction, a P dose should not be too high, with a Ca dose near the medium level. The results established that the dose of 41 g of N, 26 g of P_2O_5, 36 g of K_2O and 15 g of CaO per plant was most similar to the assessed conditions, obtaining the highest result, 26% higher than what was expected by the model.

Luo *et al.* (2017) stated that a lack of nitrogen inhibits elongation and cellular division. Likewise, White *et al.* (2015) argued that nitrogen is present in many essential compounds, and because of that a plant grows slowly when there is low availability of this nutrient. These results agree with results found by different authors for plant height (PH) when nitrogen is applied together with phosphorus and potassium (Dhillon *et al.*, 2011; Xiang *et al.*, 2012).

For the leaflet number (LN) (Tab. 4), the model indicated the importance of Ca dose management, showing a maximum response level with 11.1 g of CaO per plant according to the derivation model. Moreover, there was a negative Ca interaction with N and P. For the N dose, there was a positive effect, indicating that high doses are recommended for increasing LN (≥ 36 g of N per plant). Nevertheless, including P, the model had a quadratic tendency, where an increase in dose produced a maximum LN response. P doses associated with the maximum were out of the range of the experimental design, so the LN response to 50 g of P_2O_5 per plant is not known. Finally, K did not influence the LN; and therefore, low doses are recommended for decreasing costs (≤ 18 g of K_2O per plant). Nonetheless, the treatment with the best response was 36 g of N, 43 g of P_2O_5, 18 g of K_2O and 9 g CaO per plant, but, according to the model, a slightly superior response was obtained with 11 g of CaO (19.3 leaflets as compared to the 19 leaflets obtained with 9 g of CaO per plant).

Additionally, Preciado-Rangel *et al.* (2002) mentioned that the number of leaves is related to photosynthesis and, therefore, to a higher production of carbonate skeletons that are used or stored in the stem. On the contrary, Magdaleno-Villar *et al.* (2006) stated that the number of leaves is not a reliable indicator of plantlet production because it mostly depends on the age of the plant.

In addition, the PH (Tab. 4) was defined primarily by the Ca dose, with a quadratic tendency and a peak when the CaO dose was 6 g per plant. This element interacts negatively with N and P. Since the N and P effects were positive in the aerial length, high doses are better (≥36 g of N, and ≥43 g of P_2O_5 per plant). Li *et al.* (2016) reported that phosphorus contributes to the growth and elongation of internodes, stimulating cellular division.

The effect of K was not significant, so the application of low doses is recommended because of economic costs (≤18 g of K_2O per plant); this effect is similar to the one found by Sá *et al.* (2014), who did not register an effect of potassium applications on the growth of *Corymbia citriodora* plants,

TABLE 4. Regression coefficients and their associated error for regression model terms per plant growth variable.

Terms	Number of leaflets		Leaf area		Plant height		Root length		Root volume	
	Estimation	Error	Estimation	Error	Estimation	Error	Estimation	Error	Estimation	Error
Intercept	-9.4654	3.5528	-179.1964	29.5621			27.8844	3.3535	-1.4252	0.5423
N	0.3894	0.0752	1.9954	0.2313			-0.5362	0.1657	0.0174	0.0043
P			1.4561	0.4649					0.0178	0.0074
K			0.3789	0.1493					0.015	0.0057
Ca	2.2712	0.455	23.0843	3.8844	1.1567	0.0966	-1.7964	0.4433	0.2197	0.0702
N x Ca	-0.0117	0.005			-0.0098	0.004				
P x K									-0.0004	0.0002
P x Ca	-0.0071	0.003	-0.059	0.0307	-0.0106	0.0026				
N^2					0.008	0.0012	0.0124	0.0034		
P^2	0.0033	0.0009			0.0043	0.0007	0.0007	0.0004		
Ca^2	-0.0693	0.0146	-0.793	0.1287	-0.0297	0.0066	0.0526	0.015	-0.0088	0.0024
r^2	0.6		0.53		0.96		0.41		0.51	

All of the coefficients in the table are significantly different from zero (0) according to the *t*-test at 5% significance. Empty cells indicate terms that were not included in the model because they did not contribute significantly. r^2: Determination coefficient.

even when they were cultivated in a soil with a low content of this nutrient. Therefore, the most favorable treatment according to these conditions was 36 g of N, 43 g of P_2O_5, 18 g of K_2O and 9 g of CaO per plant; moreover, it had the highest average (20.8 cm). A slight improvement with a dose of 6 g of CaO per plant is expected, but only 2%.

Conclusions

The dose of 36 g of N (high), 43 g of P_2O_5 (high), 18 g of K_2O (low) and 9 g of CaO (low) per plant allowed good development of the root system under nursery conditions, both in dry matter and in length, as compared to the other evaluated doses. Likewise, it favored a higher number of leaflets, aerial length and leaf dry matter, which are decisive variables in plant establishment under field conditions. Therefore, this dose is adequate for an integrated fertilization program because it will result in plants with adequate growth, which can later be transplanted to uncontrolled conditions under standard cultivation systems.

Furthermore, high doses of K (above the doses proposed) can favor a greater leaf area, but are unfavorable for root system development and other allometric variables.

There was a negative interaction between the nitrogen and phosphorus fertilization and the calcium fertilization. The high doses of Ca did not favor the application of elevated levels of N and P, and, under that condition, it was, in general, detrimental for plant growth.

These conclusions apply only for the first three months of Andean blackberry plant growth. According to the analysis of the results during the growth period, the plants tended to be saturated quickly, so it would be difficult to extrapolate plant behavior beyond three months.

Acknowledgments

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Life plan for the Yaquivá indigenous reservation in the municipality of Inza, Cauca Colombia, from the perspective of agroecology

Plan de vida del resguardo indígena de Yaquivá del municipio de Inzá, Cauca, Colombia, desde la perspectiva de la agroecología

Marco Heli Franco-Valencia[1*] and Marina Sánchez de Prager[2]

ABSTRACT

The Nasa indigenous community on the Yaquivá reservation, located in the municipality of Inza (Cauca-Colombia), has designed a "Life Plan", in which education plays a fundamental role. This is reflected in the Community Education Project carried out at the *Jiisa Fxiw* agroecological school. However, within the Colombian ethnographic literature, there are no records that systematize these life plans from the agroecological approach. In order to help fill this gap, the objective of this study was to analyze the life plan for the Yaquivá reservation from the agroecology perspective. Participatory action research was used for the research methodology. The results identified the legal and constitutional frameworks that support this plan and action document as the main strength. In addition, the document facilitates the development of institutionalism with autonomy and identity. It was concluded that the life plan for the Yaquivá reservation, in itself, constitutes a force that surpasses the technological and productive (distributive), socioeconomic (structural), and sociopolitical (dialectical) dimensions. Additionally, as part of the Nasa indigenous community, the entire life plan is influenced and determined by its own worldview, i.e. its spiritual perspective.

Key words: dimensions of agroecological research, distributive dimension, structural dimension, dialectical dimension, spiritual dimension.

RESUMEN

El trabajo se realizó en la región de Tierradentro, en el Resguardo de Yaquivá, municipio de Inzá (Cauca-Colombia), comunidad indígena perteneciente al pueblo Nasa, la cual dentro de las estrategias para recuperar su cultura de producción agraria han diseñado un "Plan de Vida" en el cual la educación juega un papel fundamental. Este reconocimiento lo plasman en el Proyecto Educativo Comunitario desarrollado en el colegio agroecológico *Jiisa Fxiw*. La comunidad Yaquiveña ha orientado su quehacer con un plan de vida que le ha permitido existir como comunidad importante para la nación. Sin embargo, dentro de la literatura etnográfica colombiana, se carece de registros que sistematicen estos importantes documentos desde diferentes perspectivas, incluyendo la agroecología. Con el fin de contribuir a llenar este vacío, se planteó como objetivo analizar desde la agroecología el plan de vida del resguardo de Yaquivá. La metodología de investigación utilizada se basó en investigación acción participativa. La interacción de sus resultados permitió identificar como principal fortaleza de este documento de planificación y acción, el marco jurídico y constitucional que lo respalda y le confiere jurisdicción especial propia, que facilita el desarrollo de la institucionalidad con autonomía e identidad. Se concluye que el plan de vida de la comunidad yaquiveña, en sí mismo, constituye fortaleza que supera lo tecnológico y productivo (perspectiva distributiva), lo socioeconómico (perspectiva estructural), y lo sociopolítico (perspectiva dialéctica). Además, por ser parte de la comunidad indígena Nasa, todo el plan de vida está impregnado y atravesado por su propia cosmovisión, es decir, por la perspectiva espiritual.

Palabras clave: dimensiones de investigación agroecológica, perspectiva distributiva, perspectiva estructural, perspectiva dialéctica, perspectiva espiritual.

Introduction

In the Tierradentro region, the Yaquivá reservation has compiled its life guidelines, proposals and budgets into a single document called the "Life Plan". This plan can be defined as a way to organize the future of a community, taking into account advances in terms of territory, economy, rights, education and culture, while exercising autonomy (Huila, 2017). An integral life plan starts with the history of a town, values the awareness and socialization of

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each community's knowledge, analyzes its problems and needs, and enables the creation of pedagogical and methodological proposals that dynamize life projects (ONIC, 1999). These documents generally include a diagnosis of the fundamental problems and needs of communities, as well as a set of possible solutions and programs to guide the development of public policies in accordance with the indigenous people's worldview and history. Therefore, each town designs its own life plan according to its ethnic and cultural characteristics (Resguardo Indígena de Yaquivá, 1999).

The expression "Life Plan" can be interpreted with the way each ethnic group puts it into sociocultural and historical context and the level of "acculturation" the ethnic group has. Therefore, the ethnic and cultural diversity of Colombia is reflected in the multiplicity of ways to view a plan. For this reason, it is not possible to have a unified concept of what a life plan is, how it should be done, and who should do it. However, life plans have been defined in two complementary ways: as a tool for cultural, social, political and economic affirmation of indigenous peoples and, on the other hand, as a strategy of negotiation and agreement with society in the construction of a multiethnic and pluricultural nation (ONIC, 1999). Consequently, a life plan is also a possibility for a community to recreate its culture through a process of permanent self-diagnosis, which must be set in its own reality: yesterday, today and tomorrow (Franco, 2010).

A life plan is a cultural expression and a negotiation strategy to establish relationship principles with factors outside indigenous communities. The real reasons for a life plan are fundamentally linked to the way in which non-indigenous communities wish to relate. Moreover, they are determined by historical moments that, in almost all indigenous peoples and nations, make a big difference with the remaining population of Colombia (Monje, 2014). However, all these efforts to incorporate indigenous people into Colombian society, especially in the economic and political systems, have had negative consequences for indigenous cultures and organizations.

In spite of this, one of the subjects that indigenous communities have insisted on the most has been education, especially the teaching of native languages as an ancestral and cultural heritage. According to the Regional Indigenous Council of Cauca (CRIC for its acronym in Spanish), memory teaches and shows us the way; we all resist aggression that mistreats us, but everyone respects diversity and differences, so the future depends on a set of collective consciences and autonomies that are in balance and harmony with all beings (CRIC, 2004).

On the other hand, a life plan is a response to the sustainability of an indigenous process, which is based on a permanent construction of social and natural spaces. It should also take place in fair environments for the parties immersed in it, with respect to their social and cultural constructions. This respect should not only come from non-indigenous or government entities, but also from themselves to ensure that what they build day by day is reflected in their reality. Additionally, the basis for this respect should be their own history, with an autonomous presence in their territories, in order to survive, sustain, recover and highlight their culture, retrieve their ancestral work and resist modernization (Monje, 2014). Therefore, these communities try to function without industry and capitalism, which paralyze them, using models of self-organization, restoration of relationships, revaluation of food and agricultural functions, readjustment to natural cycles and de-commodification of processes and values (Naredo, 2006).

Although indigenous communities, and especially the Yaquivá reservation, have had a life plan throughout history that has allowed them to exist as important communities for the nation, within the Colombian ethnographic literature, there are no records that account for the existence of systematized life plans from the agroecological approach. Therefore, the objective of this study was to analyze the life plan for the Yaquivá reservation from the four dimensions of agroecological research: distributive, structural, dialectic and spiritual.

Materials and methods

Location

The Yaquivá reservation is located to the east of the central mountain range, in the municipality of Inza, province of Cauca, Colombia (Fig. 1). It has an approximate population of 3,900 inhabitants and a total area of 16,184 ha, at an altitude between 1,600 and 4,000 m a.s.l. in a paramo area. The precipitation and average annual temperature are between 1,000 and 2,000 mm and 18°C, respectively. In order to bring institutions and academia closer to rural and indigenous communities, 2008 participatory research has been carried out between the Universidad Nacional de Colombia and the indigenous community of the Yaquivá reservation. In this study, Participatory Action Research (PAR) was used as the methodology, and participant observation, discussion groups, participatory diagnoses, surveys and interviews, dialogical dialogues (dialogue of knowledge and dialogue of doing), literature review and secondary sources were used as the methodological tools.

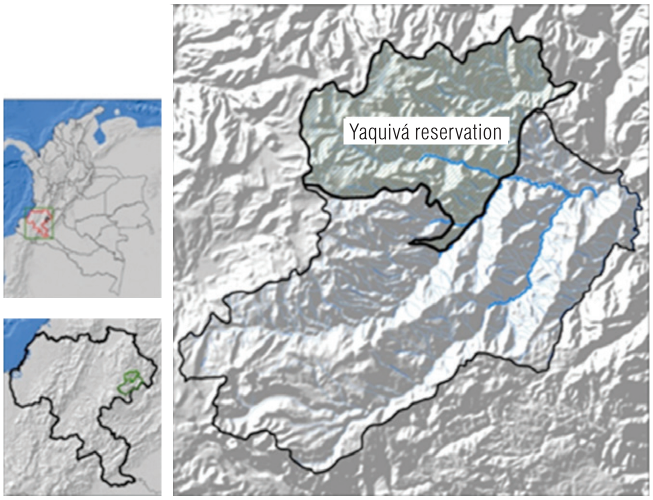

FIGURE 1. Map of the Yaquivá indigenous reservation.

Other techniques used to collect the information included meetings, visits, pedagogical *tulpas*, labor activities and mingas, of both work and thought. The term *Tulpa* refers to a word derived from the *Tul* (ancestral Nasa orchard) production system, where training events are held. Minga corresponds to collective efforts to fix roads or aqueducts and build homes, health centers, or schools, in which all the members of the community participate.

Dimensions of agroecological research

According to Ottmann (2005) and Sevilla (2006, 2009), agroecology is based on three dimensions that develop the study of existing strategies for social and ecological reproductions of agroecosystems. These dimensions propose the rescue of dynamics that, at another time, were based on the recognition, use and creation of diversity. These dimensions act in accordance with natural laws and do not contravene them (Toledo and Barrera-Bassols, 2008). The three dimensions of agroecological research proposed by Ibañez (1996) are: the ecological and technical productive (distributive), the socioeconomic and cultural (structural) and the political (dialectic) dimensions. In addition, this research proposed the spiritual dimension (Fig. 2).

The distributive dimension of agroecological exploration-action moves in a productive space (Sevilla and Ottmann, 2000). In the ecological and technical productive dimension, agroecology aims to learn and promote modes of appropriation of nature that: a) respect mechanisms of self-maintenance, self-regulation and self-repair that

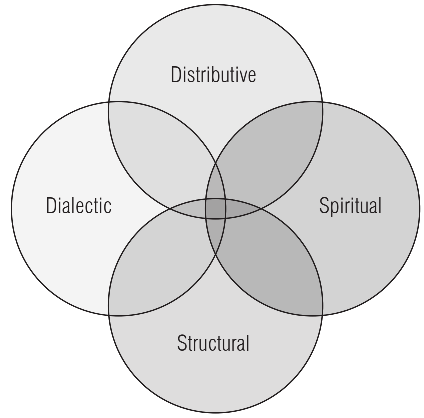

FIGURE 2. Agroecological research perspective.

every ecosystem possesses independent of societies and human intervention (Toledo, 1985) and b) come from adaptation and integrated coevolution between culture and the environment (Norgaard, 1985; Norgaard and Sikor, 1999). On the other hand, the structural dimension is made up of agroecology as rural development and refers to it as a socioeconomic process for obtaining the optimum level of life allowed by existing resources; it is a productive and participative strategy for obtaining sustainability through forms of collective social action (Sevilla and Ottmann, 2000).

The dialectical dimension, in which participatory action research breaks the subject-object power structure of the scientific methodology (known as "laboratory rebellion"), generates the possibility of change in social actions within performance events, such as "historical analyzers" (Delgado and Gutiérrez, 1995). The socio-political dimension of agroecology or the cultural and political management of natural resources (Cuéllar, 2008) complements the socioeconomic concept of the structural dimension from the perspective of breaking with the conventional scientific and epistemological paradigm. Without this, it is not possible, from the agroecological perspective, to achieve fair environmental, social and economic sustainability in accordance with existing cultural diversity (Sevilla, 2009).

As a consequence of the multiple forms of cultural resistance, certain values were forged, which are incorporated into social memories and which are rescued along with local rural and indigenous knowledge with agroecology. Therefore, the sociopolitical dimension states that, without intervention in the established power structures, it will not be possible to propose alternative integral development strategies. These strategies should truly satisfy this focus and be placed in a new "ecological paradigm" that is built on the basis of contributions from both criticism of the dominant mechanistic model and the emergence of new scientific disciplines (Garrido, 2007).

The last level is the spiritual one; according to Boff (2000), modern culture tends to occupy humans with a giant avalanche of messages and requests. Our being has become contaminated with materialism and dehumanized, with a loss of all transcendence that feeds the spirit. The spirit, in its original sense, from which the word spirituality derives, is in every being that breathes. Therefore, it is in every being that lives, such as human beings, animals and plants. However, Earth and the universe live as bearers of spirit because life comes from them, and they provide all the elements for life and maintain the creative movement.

In this sense, human beings represent a unitary compound of body and spirit. Spirituality is one of the dimensions of humans, that of the spirit. In addition, spirituality motivates inner transformation. It can happen at any age or state or time and in both sexes. Spirituality is that attitude that places life at the center, promoting it against all mechanisms of death. The opposite of spirit is not body, but death and everything that is attached to the system of death, taken in its broad sense of biological death, social death and existential death (failure, humiliation, and oppression) (Mejía, 2011).

Feeding spirituality means cultivating the inner space, from which things are connected and re-connected. This means overcoming stagnant behaviors and living the realities beyond their opaque and sometimes brutal existence, as values, inspirations, and symbols of deeper meanings. A spiritual man/woman always perceives the other side of reality and is capable of capturing the depth that is hidden and the reference of everything with the ultimate reality, which religions call God (Boff, 2000).

Results and discussion

The analysis of the life plan is presented from perspective of the four dimensions: distributive, structural, dialectical (Ibáñez, 1996) and spiritual (Chate, pers. comm.; Mejía, 1999; Boff, 2000). Taking into account these dimensions, the different constitutive elements of the life plan are grouped.

Distributive dimension

The income of most of the population of the Yaquivá reservation comes from the agricultural sector, mainly from the production and commercialization of coffee (*Coffea arabica*). Two types of production are clearly identified: the first one belongs to the rural economy, characterized by permanent crops such as coffee, sugar cane (*Saccharum officinarum*) and banana (*Musa paradisiaca*), with the application of some techniques from the green revolution technological package (for cash crops). In contrast, the second type of production, of indigenous family economy, is carried out for self-consumption, with subsistence crops of produce such as: corn (*Zea mays*), beans (*Phaseolus vulgaris*), potatoes (*Solanum tuberosum*), broad beans (*Vicia faba*), ulluco (*Ullucus tuberosus*), onions (*Allium cepa*), cabbage (*Brassica oleracea*), and coriander (*Coriandrum sativum*), among others. This type of production has scarce commercial surpluses, is developed in systems associated with traditional techniques such as slash, burn and rotation of land, and does not use pesticides or fertilizers made with petrochemical synthesis. Additionally, the contribution of labor from family members (mainly female and child labor) and neighbors (Franco, 2010) stands out.

However, in Tierradentro, vegetable crops have decreased in diversity compared to the crops that the Spanish colonizers found (Patiño, 1969). Apparently, this is one of the reasons for the conditions of impoverishment, but at the same time, one of the possible explanations for the persistence of traditional crops. The traditional use and management of Tierradentro's useful plants is the result of the persistence of a traditional agricultural system and the cultural resistance of the Nasa ethnic group in its territory. In addition, under the notion of *Kiwe* (Nasa term), which means "see, sow and harvest", agricultural activities are central to the conservation and defense of territories: it is the means of life (work) for its inhabitants, which in part assures the survival of the ethnic group (Sanabria, 2001).

The persistence of vegetables under the traditional agricultural system is based on a subsistence economy in the ecological and socio-cultural context of the Nasa territory (Sanabria, 2001). However, the persistence of different Andean edible crops and the ones adapted from other regions is due to the political process of indigenous autonomy and the resistance and socioeconomic marginalization of the Nasa territory (Sanabria, 2001). On the other hand, the traditional production practices carried out on the reservation guarantee the continuity of the productive initiatives developed in the *Jiisa Fxiw* (Nasa term meaning seed of knowledge) agroecological school over time. Acevedo (2015) found that the systems of traditional agriculture showed the highest values of multifunctionality and the best performance in terms of environmental and productive sustainability, while the farmers and semi-annual monocultures showed a lower degree of multifunctionality and sustainability. The ability of farmers to perform multiple tasks in their farm systems is a strategy of resistance for overcoming adversities that put the productivity of the systems at risk. Finally, the ecological and productive aspects are grouped as: the land and the territory, size of the land and reserve lands, agricultural sector, agricultural calendar, livestock sector, environment, and ecological life zones (Fig. 3).

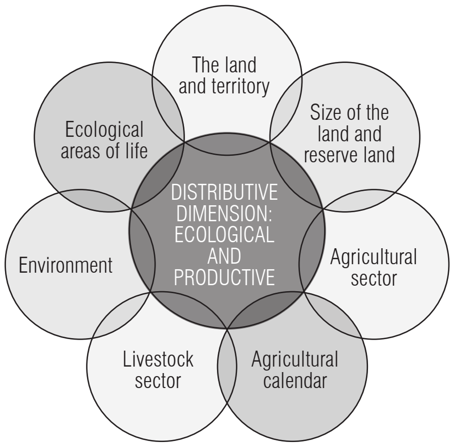

FIGURE 3. Illustration of distributive dimension.

Structural dimension

For the Nasa people and the Yaquivá community, the conservation of their culture and cosmogony is of great importance, which is reflected not only in the life plan that was created in 1999, but also in the interest shown in developing productive projects that improve socioeconomic conditions and the level and quality of life for its commoners. Therefore, the Yaquivá reservation is integrating the Nasa culture into an ethno-education and production strategy in the Community Education Project (PEC for its acronym in Spanish) of the *Jiisa Fxiw* agroecological school, which seeks to strengthen sovereignty, dignity and food and nutritional autonomy. Education for ethnic groups is a public service and is based on a commitment to collective elaboration where different members of the community exchange knowledge and experiences in order to maintain and recreate processes that development their cultures. This is achieved through real policies of bilingual and intercultural education in accordance with their language, traditions and indigenous privileges (Mineducation, 2009; Franco and Chate, 2015).

Therefore, since its inception, the CRIC has created strategies for education that take indigenous culture into account, which is how the alternatives of bilingual education and processes of ethno-education were born (CRIC, 2004), as stated in article 68 of the National Political Constitution of 1991. In addition, with the firm purpose of advancing the guidelines of the life plan and dynamizing its policies through the PEC, teachers meet with students, parents and traditional authorities in order to assume the organization of new technologies to improve the educational quality and strengthen agroecological production for the educational institution *Jiisa Fxiw* and the reservation (Franco, 2010).

However, the construction processes of the PEC in the *Jiisa Fxiw* school, as well as the life plan, have faced problems such as: a lack of infrastructure and resources, difficulties of appropriation of the territory, natural disasters, and problems of exclusion and violence in the region. This project has reached the productive and political areas of the community and is part of all the dynamics of participatory governance of the reservation (Franco, 2010).

Finally, the aspects related to the socioeconomic part were grouped as: population, health and education sectors (education, organization and culture, the Institutional Educational Project (PEI for its acronym in Spanish), the PEC, educational autonomy, arts, sports and non-formal education (Fig. 4)).

Dialectical dimension

The political organization is based on Cabildo, a legal figure implanted by the Spanish crown, supported by the laws of the liberator Simon Bolivar and ratified in Law 89 of 1890. The organizational dynamics of indigenous communities is a major factor in long-term, proactive and constructive tasks; they have a great capacity for action, reaction and adaptation, which have survived millennia, even after suffering attacks throughout history, an indication that they will continue towards the achievement of many objectives and accomplishments.

As for the organization, there have been substantial advances that have led to improvements in living conditions, such as belonging to an association of Cabildos, setting up their own working committees, establishing some parameters of action that guarantee peaceful coexistence with autonomy and freedom and achieving progress in the solution of unsatisfied basic needs for the community. On the other hand, from the point of view of identity, specifying the origin of the inhabitants of the reservation is not difficult since the cultural heritage is mostly preserved, such as the ancestral language (Nasa Yuwe), governance, spirituality, food, economy, folklore and territory.

If there is self-governance, it is because the territory is constituted in a reservation with autonomy, where different

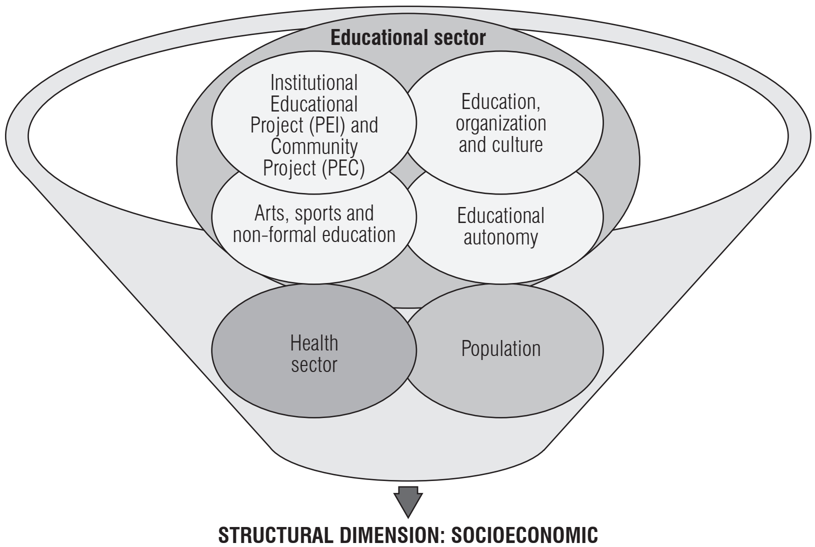

FIGURE 4. Illustration of structural dimension.

activities of an indigenous council are performed; the commoners, with the minimum amount of land, have autochthonous technology learned through oral tradition from elders. Hence, its history is based on ancestry (Resguardo Indígena de Yaquivá, 1999). Finally, the aspects related to the sociopolitical part were grouped as follows: political organization (the governor, mayors, marshals, commissaries, treasurer, secretary, and councils), internal authority and special jurisdiction and institutional development (Fig. 5).

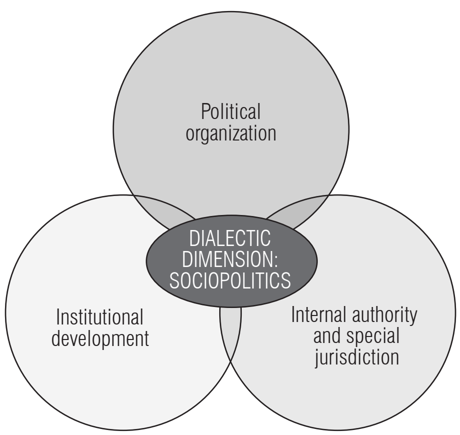

FIGURE 5. Illustration of dialectical dimension.

Spiritual dimension from the Nasa cosmovision

When talking about the origin of the Nasa world, Chate (pers. comm.) stated that, at the beginning, everything was dark and lonely; there were only two superior beings that moved from one place to another through the universe. One of them was called *Uma* and the other was named *Tay*; male and female cosmic entities, these two spiritual beings were very sad because they felt alone, so they agreed to procreate and their children were scattered throughout the universe. Among their children, *Kiwe* (Earth) was born and became a very beautiful young woman. *Sek* (sun), a handsome young man, started to court her, but he had difficulty approaching her since she was very hurt by his high temperature. *Uma* and *Tay* agreed that the young *Sek* could only see their daughter and get close to her from a certain distance. This way, life was possible on *Kiwe* (Earth), and *Kiwe* and *Sek* shared their lives in the universe. This union produced an innumerable diversity of offspring, to the point that everything became chaotic as *Kiwe*'s children started to fight and caused many difficulties. *Uma* and *Tay* were forced to intervene and establish rules of coexistence, but, since the universe was so big, and they could not be vigilant, they established some delegates who were in charge of ensuring the balance and harmony of everyone in the *Kiwe* family.

These delegates were located at strategic places, such as rivers, lagoons, swamps, hills, springs, mountains and stubbles; they penalized *Kiwe's* children, especially humans, when they broke the rules of balance and harmony. These delegates are the spiritual beings who are in charge of maintaining the equilibrium with everyone. The person in charge of establishing this communication between the beings of the cosmos and the Nasa or humans is the *The Wala* (a wise member of the Nasa community who has been selected by the beings of the cosmos to do this task). Thus, the *The Wala* has specific functions, such as being the conciliator between the Nasa and the sacred cosmic beings. There is a language that has been established between the *The Wala* and the spiritual beings that only they know; this communication is done mainly through vibrations in

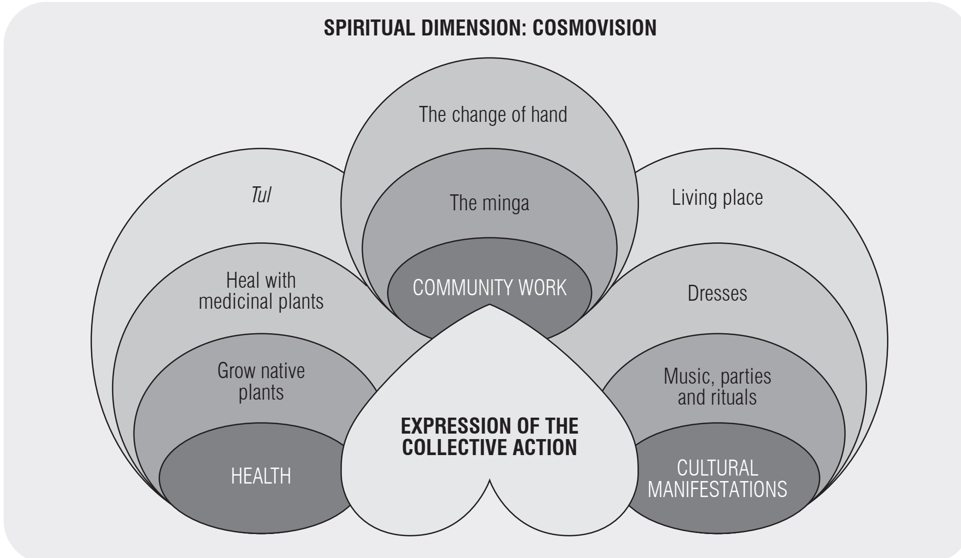

FIGURE 6. Illustration of spiritual dimension.

the body, visions, and signs of nature that the wise man interprets while being very concentrated.

Additionally, from the point of view of Nasa spirituality and feasts and rituals, every 1st of November, the *Tafxi* (ritual of the souls) is performed at the family and community levels, which consists of making an offering to people who have passed away and embarked on the long journey. Currently, other rituals, such as *Cxapuc* (appreciation for Mother Earth) and *Sakhelu* (ritual of seeds) are being recovered. On the other hand, from the organizational and political point of view, the ritual of territorial renewal of the command insignia of the council directors has taken place. From the socio-economic point of view, renewal or harmonization of diversified autochthonous crops, the Nasa *tul*, the seeding of the navel, the dwelling and the body are carried out. The cleaning of the body is done when women are menstruating, at the time of delivering a baby and after a funeral.

In view of multiple religions existing on the reservation, it is necessary to look for strategies of agreement and understanding towards Nasa spirituality.

On the other hand, the organization based on PEC and *Tul* converge in an ethnoecological proposal that was developed through participatory talks with professionals, technicians, teachers, students, the community in general, the *The Wala* and elders, who are fundamental to rescuing ancestral knowledge (Franco, 2010), which is very necessary to overcoming difficulties. From the local perspective, and from the impulse of the *Tul*, which is defined as those tissues of the soil with food plants, bits of mountain that are already planted to eat, an environmental and cultural tissue is reconstructed, in which the *The Wala* (traditional doctors) and elders direct what to sow, how to sow and how much to sow, as exercises of autonomy, territoriality, spirituality, culture and unity.

The *Tul* is considered the expression of the capacity of domestication and technological adaptation under the diverse Andean agro-ecological conditions. In the *Tul*, the steps or meeting places of basins and sub-basins constituted points of Andean economic dynamism for a living local territory. These points of dynamization, which are more than 500 years old and have withstood the attack of production models of the green revolution, continue to offer their benefits to the communities; at these points, systems of reciprocity and exchange of products and food continue to be dynamic and intense. This testimony of historical permanence constitutes a reliable indicator of its sustainability (Vásquez, 2004).

Despite western sociocultural attacks, the Nasa have skillfully preserved much of the cultural heritage, such as: thought through language, spirituality manifested through rituals, traditional medicine, minga, a collective form of work, and the *Tul* as part of autonomy, dignity and food sovereignty. Therefore, the technology transfers and agroecological practices are designed to make the sovereignty, autonomy and food dignity of the Yaquivá community possible. For food autonomy, the residents of the reservation state that "he who knows what he sows decides what he eats"; in addition, they say that "food is produced to eat first and, if there is something left over, it can be used to barter or to sell". Food dignity is understood as "mutual respect between all beings to produce food in harmony", with a more spiritual concept (Chate, pers. comm.).

The following activities are grouped in Figure 6: health (grow native plants, heal with medicinal plants and *Tul*), cultural manifestations (living place, dresses, music, parties and rituals), and community work, i.e. minga and change of hand. Change of hand refers to the work dynamics in which members of the community work from farm to farm in order to accomplish certain goals that could not be achieved by working individually.

Conclusions

The life plan for the indigenous reservation of Yaquivá has a high level of appropriation and represents a novel advance in both the theoretical and practical aspects. By its nature, it is understandable that the formulation of the life plan is an exercise in decision-making for collective action. It is based on the orality of their culture and the revitalization of traditions; it is a useful tool for promoting reflection processes within communities for their political, social, cultural, economic and spiritual dynamics in order to reach an acceptable level of formalization and implementation of these dynamics.

Finally, in the analysis of the life plan for the Yaquivá reservation from the agroecology perspective, agreements are gathered on the most appropriate forms of behavior in social relationships and with nature. In this analysis of the life plan of the community, emphasis was placed on the fourth dimension, the "spiritual" one, given the need to resume its contributions within the process of change for purposes that transcend the economic level and that are inserted in welfare. This emphasis intends to collaborate the construction of conceptual and methodological references at the level of other reservations, citizens and the scientific community on apparently evident subjects that are invisible to a culture that believes that the goods of the Earth are infinite.

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Organic substrate and organic foliar fertilizer effect on chlorophyll index and growth in Umbuzeiro seedlings

Efectos de substratos orgánicos y fertilizantes orgánicos foliares en el crecimiento e índices de clorofila en plántulas de Umbuzeiro

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ABSTRACT

The umbuzeiro (*Spondias tuberosa* Arr. Cam.) is a fruit tree originating from the Caatinga biome that enjoys high acceptance in the market for *in natura* consumption or industrialized products; as a result, its cultivation has become of great importance for the region of Northeast Brazil. The objective was to evaluate growth, substrate temperature and chlorophyll indices in umbuzeiro seedlings at organic substrate levels and with organic foliar fertilizer (EcoLife®) applications. The experimental design was a randomized block with a factorial arrangement of 5×2, with four replicates and three plants per experiment unit. The factors corresponded to the proportions of the organic compost to the substrates (0, 25, 50, 75 and 100%), with and without foliar applications of 1% organic foliar fertilizer. The growth, leaf area and chlorophyll indices of the umbuzeiro seedlings were higher in the treatments with organic compost and foliar applications of organic foliar fertilizer.

Key words: *Spondias tuberosa*, organic inputs, native fruit.

RESUMEN

El umbuzeiro (*Spondias tuberosa* Arr. Cam.) es un árbol frutal originario del bioma Caatinga con gran aceptación en el mercado para consumo *in natura* o productos industrializados. Debido a esto, su cultivo ha adquirido gran importancia para la región del Nordeste brasileño. El objetivo fue evaluar el crecimiento, temperatura del sustrato e índices de clorofila en plántulas de umbuzeiro en niveles de compuestos orgánicos en el sustrato y aplicación foliar de fertilizante foliar orgánico (EcoLife®). El delineamiento experimental utilizado fue en bloques al azar, con arreglo factorial de 5×2, con cuatro repeticiones y tres plantas por unidad experimental. Los factores corresponden a las proporciones de compuesto orgánico a los sustratos (0, 25, 50, 75 y 100%) en el sustrato con y sin aplicación foliar de fertilizante foliar orgánico a 1%. El crecimiento, área foliar e índices de clorofila de plántulas de umbuzeiro fue mayor en los tratamientos con compuesto orgánico y aplicación foliar de fertilizante orgánico.

Palabras clave: *Spondias tuberosa*, insumos orgánicos, frutal nativo.

Introduction

In the present study, some responses by umbuzeiro (*Spondias tuberosa* Arr. Cam.) to organic inputs were evaluated. The umbuzeiro is an endemic plant of the Caatinga biome characterized by a seasonally dry tropical forest (Mertens *et al*., 2017).

Among the tropical and subtropical fruits grown in the northeast region of Brazil, fruits of the genus *Spondias,* such as umbu (*Spondias tuberosa*) and umbu-cajá (*Spondias* sp.), are generally consumed *in natura*, as juice and in the form of ice cream (Galvão *et al*., 2011; Medeiros *et al*., 2015). Umbu fruit has great prominence and acceptance by consumers in the north and northeast of Brazil, mainly because of its pleasant, refreshing, acidic taste with high antioxidant activity (Zeraik *et al*., 2016).

The umbuzeiro is a species highly adapted to semi-arid regions, with long periods of drought in the dry season. This fruit represents an additional income for farmers in the dry season because it is a nutritious food for humans (Lins Neto *et al*., 2010). Despite the high demand in the northeastern region for umbu fruits, there are no commercial plantations and production is exclusively extractive (Costa *et al*., 2015).

This species is dicotyledonous and can be propagated by cutting, grafting or, predominantly, seeds. The plants originating from seeds have pivotal roots, a structure that

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cannot be observed in the plants originating from cuttings. Seed-derived plants become more resistant to tipping caused by strong winds (Reis *et al*., 2010).

In order to evaluate adaptation of *Spondia tuberosa* in different conditions, the response of its root system to soil changes should be evaluated. Additionally, seedling growth and development with the use of organic substrates and fertilizers that improve soil and consequently the plant's nutrition should be monitored for the development of this species in natural conditions (Cruz *et al*., 2016; Mertens *et al*., 2017).

The use of organic fertilization in agriculture with crop residues and animal manure has been highlighted as one of the main strategies for agriculture with sustainable use and management, especially in regions with low fertility soils (Chiti *et al*., 2012; Ansari and Mahmood, 2017). When properly managed, organic residues from agricultural activities such as crop residues and animal manure, can also be used as substrates for seedling production (Cavalcante *et al*., 2016; Mota *et al*., 2018). Organic fertilizers stimulate the production of extracellular enzymes (Medeiros *et al*., 2014) that contribute to the gradual release of nutrients and substances that stimulate plant growth (Silva *et al*., 2013).

Because of the scarcity of information on the development of umbuzeiro seedlings, the objective was to evaluate the growth, substrate temperature and chlorophyll indexes in umbu seedlings at organic compound levels in the substrate and with organic foliar fertilizer applications.

Materials and methods

This experiment was conducted from July to October, 2015 in a protected environment belonging to the Center for Human, Social and Agricultural Sciences (CCHSA) at the Federal University of Paraíba (UFPB), Bananeiras-Paraíba, Brazil.

The experiment design used randomized blocks in a 5×2 factorial arrangement, with four replicates and three plants per experiment unit. The factors corresponded to the substrates for sowing (0, 25, 50, 75 and 100% of organic compound) in the substrate, with and without foliar applications of 1% organic foliar fertilizer (EcoLife®, Palm Harbour, Florida, USA). The composition of the organic foliar fertilizer is described in Supplementary material 1.

For the preparation of the organic compound, rabbit manure was used and rest of cultures (brachiaria, common bean and jackfruit). The soil used was collected at a depth of 0 to 20 cm in a Dystrophic Yellow Latosol classified according to the criteria of the Brazilian Soil Classification System - SiBCS (EMBRAPA, 2013). The foliar application of the organic fertilizer was carried out at 20, 40 and 60 d after transplanting the seedlings to the bag, at a concentration of 1% of the organic input.

The seeds were collected in the Riachão municipal district, Paraíba, Brazil. Then, the seeds were taken to the soil laboratory, where the endocarp was removed with sandpaper (number 120) in the distal part of the seeds to avoid damaging the embryo (Melo *et al*., 2012).

The seeds were allowed to germinate on polyethylene trays containing vermiculite as the substrate, with emergence starting 25 d after sowing, with a duration of 10 d. After 40 d of emergence, the seedlings were transplanted to 18×30 cm polyethylene bags. After 90 d, when the seedlings were fit to be taken to the field, the following biometric variables were analyzed: stem diameter, which was measured with a digital caliper, plant height, with a ruler graduated in centimeters, number of leaves, leaf area, a, b and total chlorophyll indexes, which were measured with a portable chlorophyll meter (ClorofilLOG® CFL 1030, Falker, Brazil) and substrate temperature at 12 pm with the help of a Digital Infrared Thermometer (AK35new, Akso, Brazil).

The data were compiled to perform an analysis of variance (F test, $P \leq 0.05$). The mean values for the organic foliar fertilizer application were compared by the F test, which in this case was conclusive; the means of the substrate composition were compared by regression. A statistical analysis was carried out using SISVAR version 5.3 (Ferreira, 2011).

TABLE 1. Data of chemical analysis of the soil and the organic compound used in the substrate composition.

Sources	**pH H_2O	P mg dm^{-3}	K^+	Na^+	H^+Al^{3+}	Al^{3+}	Ca^+	Mg^{2+}	BS	CTC	V	M	OM g kg^{-1}
			cmol$_c$ dm^{-3}								%		
Soil	4.57	16.17	0.26	0.09	12.46	0.55	2.40	1.45	4.21	16.67	25.25	11.55	9.48
OC	6.84	233.5	9.53	2.67	4.54	0.00	6.30	6.70	25.18	29.72	84.72	0.00	164.0

* pH in water; OC: organic compost; BS: base sum (Ca^{2+} + Mg^{2+} + K^+); CTC: cation exchange capacity [BS + (H+ + Al^{3+}); V: saturation by exchangeable bases (BS/CTC)*100; OM: Organic matter; M: Al^{+3} saturation.

Results and discussion

The analysis of variance (Tab. 2) verified that the organic compound × foliar fertilization interaction exerted a significant influence on the plant height, stem diameter, number of leaves, leaf area, chlorophyll b index, and total chlorophyll index and an isolated effect on the substrate temperature and chlorophyll a index.

The height of the umbuzeiro seedlings was higher in the treatments with organic compost with up to 50% of the organic compound in the substrate and foliar fertilization with organic foliar fertilizer at 90 d after transplanting the seedlings in polyethylene bags (Fig. 1A). According to Cruz *et al*. (2016), the use of 40 to 50% organic matter with bovine manure in the substrate provided better growth of the umbuzeiro seedlings because of nutritional balance in the substrate, increasing the availability of nutrients to the seedlings.

Organic fertilizers cause the production of extracellular enzymes (Medeiros *et al*., 2014) as a result of maintenance and stimulation of enzymatic activity in the presence of the organic material (Li *et al*., 2018a). This contributes to the gradual release of nutrients and substances that stimulate plant growth (Silva *et al*., 2013). The foliar application of organic fertilizer may have contributed simultaneously to the growth of the umbuzeiro seedlings.

The treatments with organic compost and applications of organic foliar fertilizer at the estimated dose of 46.9% of the organic compound in the substrate provided the best seed diameters of the seedlings (Fig. 1B). These results may have resulted from improvement in nutrient balance of the substrate, as well as the improvement in water availability and nutrient retention near the root, which is a limiting factor for growth in umbuzeiro plants (Mertens *et al*., 2017). The addition of organic matter increases the contents of fulvic acid and humic acid in the organic

TABLE 2. Summary of analysis of variance, including mean values of the variables plant height (PH), stem diameter (SD), number of leaves (NL), leaf area (LA), substrate temperature (ST), chlorophyll a index (ICa), chlorophyll b index (ICb) and total chlorophyll index (ICt).

SV	DF	PH	SD	NL	LA	ST	ICa	ICb	ICt
Block	3	17.95*	0.02ns	8.73ns	1.75	0.29	3.00	0.49ns	4.42
Proportion (P)	4	126.72**	2.78**	492.96**	47.97**	67.85**	31.47**	2.68ns	51.02**
Foliar Fertilizer (FF)	1	1010.02**	0.22ns	90.00**	11.02ns	1.22ns	0.40ns	0.62ns	0.02ns
P x FF	4	594.90**	5.78**	60.32**	18.90*	1.97ns	19.52ns	3.43*	51.40**
Residue	27	4.34	0.08	4.91	1.75	1.12	6.92	1.21	4.42
Total	39	-	-	-		-	-		-
CV (%)	-	5.66	7.23	10.36	16.11	3.78	9.86	14.94	8.78

SV: source of variation; DF: degrees of freedom; CV: coefficient of variation; ** and * significant at 1% and 5% probability, respectively; ns: not significant at 1% and 5% probability.

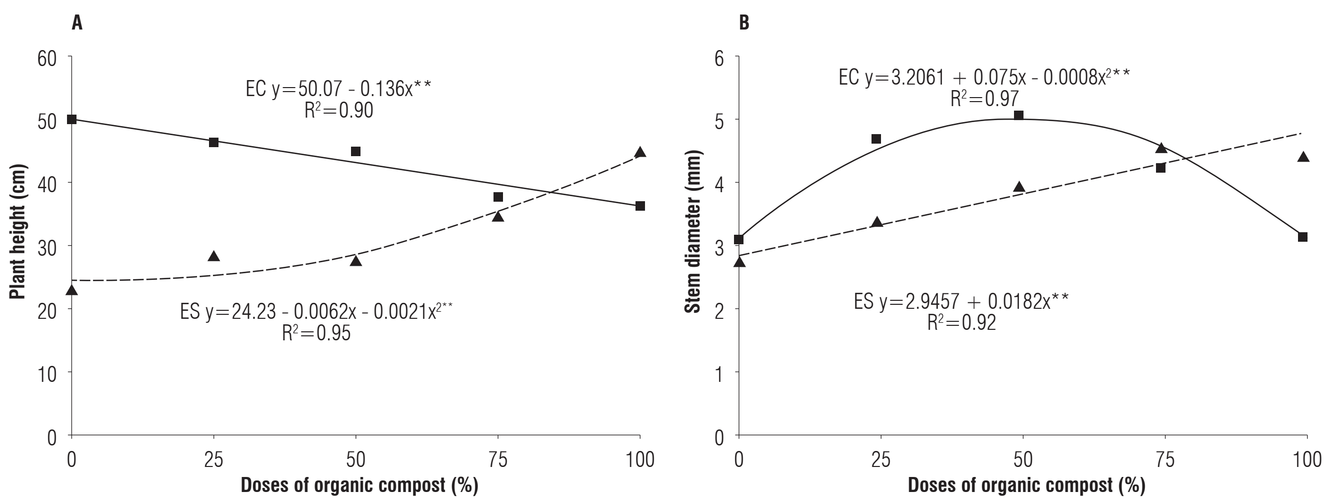

FIGURE 1. Plant height (A) and stem diameter (B) of the umbuzeiro seedlings in organic substrate with EC (—) and without ES (---) organic foliar fertilizer applications. *significant at 1% and ** significant at 5% probability.

matter, which can favor the growth and development of seedlings (Bacilio *et al*., 2016).

For the number of leaves per umbuzeiro plant, the treatments with organic compounds at the estimated dose of 54.4% with organic foliar fertilizer applications and 84% organic compound in the substrate without organic foliar fertilizer provided the best results (Fig. 2A). The addition of leaf fertilization with an organic foliar fertilizer increases the availability of nitrogen to plants, as well as a plant's resistance to pathogens (Nascimento *et al*., 2008), which may have favored an increase in the number of leaves and leaf area in the umbuzeiro seedlings in this study (Fig. 2B). The organic foliar fertilizer EcoLife® is a commercial product composed of bioflavonoids; in addition to improving plant resistance to stress, this product has a synergistic action between its components and can regulate vegetative vigor, causing better growth in plants (Cavalcanti *et al*., 2006; Furtado *et al*., 2010).

The temperature was higher in the substrates with more than 75% organic compound, which may have increased the accumulation of heat and, consequently, the temperature (Fig. 2C). According to Li *et al*. (2017), soil temperature may directly influence seedling growth. The increase of organic matter in a substrate can raise its temperature and consequently influence the growth of seedlings (Hartley and Ineson, 2008; Conant *et al*., 2011; Moinet *et al*., 2018).

The chlorophyll a and b rates increased in the organic foliar fertilizer treatments. For the total chlorophyll index, the best results were obtained in the treatments without applications of the organic foliar fertilizer at an estimated dose of 58% organic compound in the substrate (Fig. 3). In order to obtain a better balance of organic residues in the substrate, it is possible to provide better conditions and relative chlorophyll indexes in the seedlings, which may favor light uptake by chlorophyll and consequently improve the photosynthetic regulation of the plants (Braga *et al*., 2017; Li *et al*., 2018b).

According to Mota *et al*. (2018), the evaluation of chlorophyll in seedlings growing in substrates with organic residues is a quick and simple way to increase the quality of seedlings.

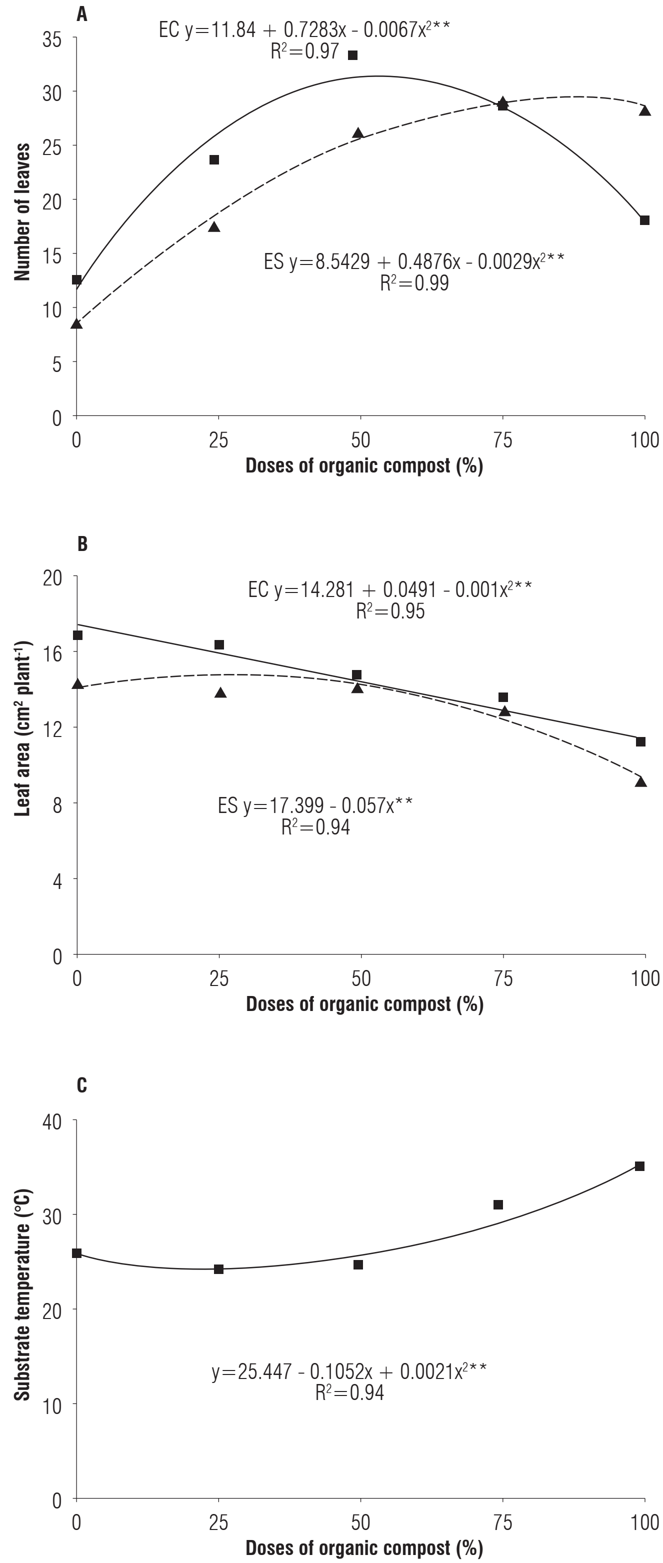

FIGURE 2. Number of leaves (A), leaf area (B) and substrate temperature (C) in the umbuzeiro seedlings in the organic substrate with EC (—) and without ES (---) organic foliar fertilizer applications. * significant at 1% and ** significant at 5% probability.

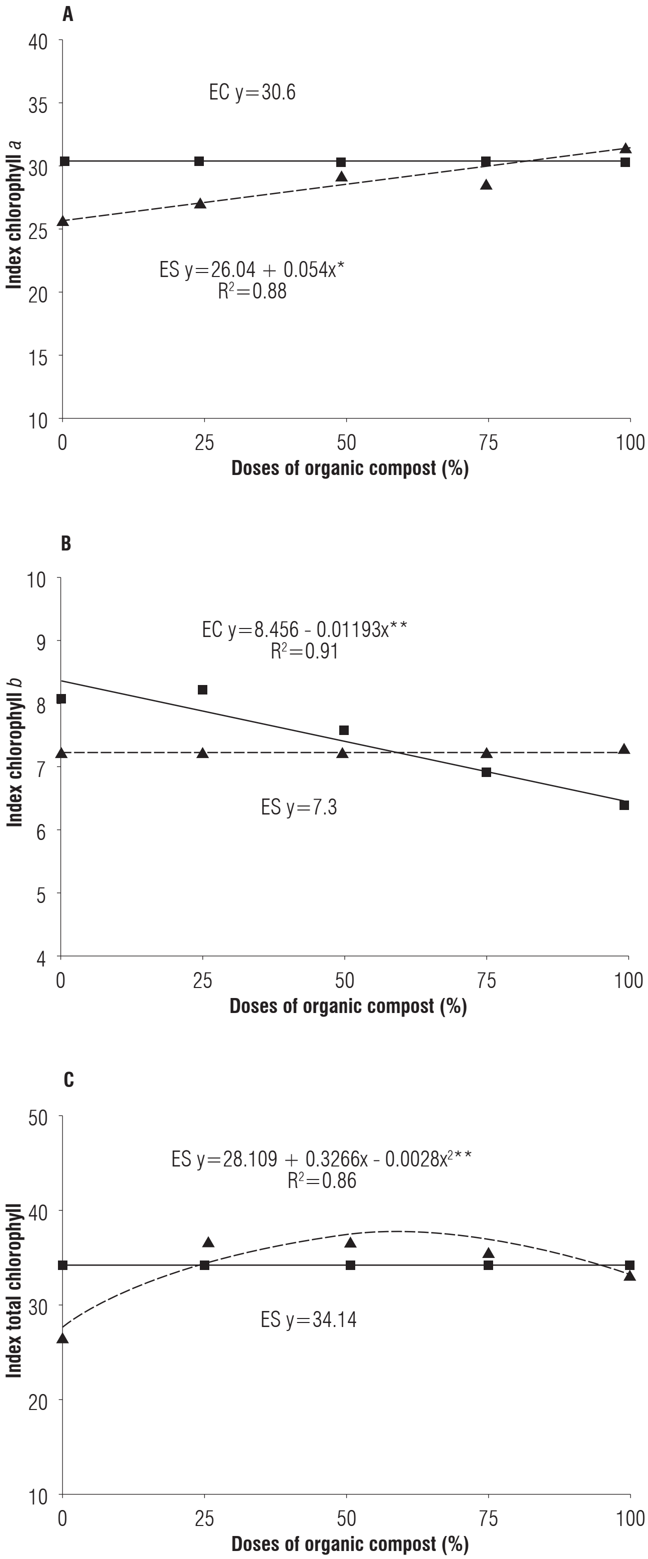

FIGURE 3. Chlorophyll a index (A), chlorophyll b index (B) and total chlorophyll index (C) in the umbuzeiro seedlings in the organic substrate with EC (—) and without ES (---) foliar applications of the organic foliar fertilizer. * significant at 1% and ** significant at 5% probability.

Conclusions

The substrate with up to 58% of the estimated dose of organic compound with organic foliar fertilizer applications provided better growth in terms of height, stem diameter and number of leaves.

The temperature of the substrate increased regardless of the proportion of the substrate or the application of the organic foliar fertilizer.

The indexes of chlorophyll a and b were higher in treatments with the organic foliar fertilizer up to the dose of 50% organic compound in the substrate, and the total chlorophyll was higher in the treatments without the organic fertilizer at the estimated dose of 58% organic compound in the substrate.

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SUPPLEMENTARY MATERIAL 1. Components of the organic foliar fertilizer EcoLife®.

Nitrogen (N)	1%
Total organic carbon (C.O.)	9.50%
Density	1.06 g L^{-1}
Additives - citric acid, polyflavonoids and polyols	-

Empowerment and associative process of rural women: a case study of rural areas in Bogotá and Cundinamarca, Colombia

Empoderamiento y asociatividad de las mujeres campesinas: un caso de estudio en áreas rurales en Bogotá y Cundinamarca, Colombia

Eliana Suárez[1], Teresa Mosquera[2], and Sara Del Castillo[1]*

ABSTRACT

There are no *formal female food production organizations* in the municipalities of Sibate, Sopo or Sumapaz (Bogotá rural area). Additionally, in the organizations in which women participate, they usually do not hold a decision-making position, which inhibits economic and social development despite the fact that women play an important role in the entire food system (production, preparation, consumption and food utilization). The aim of this research was to characterize the empowerment of rural women as a consequence of associative processes that contribute to food and nutrition security using the "descriptive case" qualitative research methodology. The main results indicated that, even though the participation of women in associative processes promotes decision-making, access to agricultural inputs, technical assistance and economic autonomy, there are still inequalities and inequities between men and women. For these reasons, it is necessary to strengthen associative processes with a cross-cutting gender approach and provide space for participation where both women and men analyze and question traditional roles in the household in order to transform inequity gaps and, therefore, contribute to food and nutrition security.

Key words: farmers organizations, household food security, rural development, woman.

RESUMEN

En los municipios de Sibaté, Sopó y Sumapaz (Bogotá rural), no existen organizaciones de mujeres productoras de alimentos legalmente constituidas, y en las organizaciones en las que ellas participan, usualmente no ocupan cargos directivos. Esta situación va en contra del desarrollo económico y social, puesto que las mujeres ejercen un rol relevante en todo el sistema alimentario (producción, preparación, consumo y aprovechamiento biológico de alimentos). Por ello, la presente investigación tuvo como objetivo caracterizar los procesos de empoderamiento desde la asociatividad de las mujeres campesinas como una contribución al logro de la seguridad alimentaria y nutricional, mediante una metodología de investigación cualitativa de "casos descriptivos". Los principales resultados indican que, si bien la participación de las mujeres en los procesos asociativos ha promovido en ellas la toma de decisiones, el acceso a insumos agrícolas, asistencia técnica y autonomía económica, aún persisten desigualdades e inequidades entre hombres y mujeres. Por esta razón se hace necesario fortalecer los procesos asociativos mediante un enfoque de género transversal, donde se brinden espacios de participación para que juntos, mujeres y hombres analicen y cuestionen los roles tradicionales en el hogar con el fin de transformar las brechas de inequidad y así contribuir a la seguridad alimentaria y nutricional.

Palabras clave: organizaciones campesinas, seguridad alimentaria del hogar, desarrollo rural, mujer.

Introduction

An evaluation of the Millennium Development Goals for Colombia (PNUD, 2015) indicates that, although women participate more at the decision-making levels and have achieved a reduction of inequity gaps in the labor market in the last 20 years, inequity still exists. In 2014, men occupied 77% of the congressional seats in the Republic of Colombia. In the realm of labor, there were salary differences of about 20% between the genders. This situation is possibly associated with the fact that the informal work rate for women is 52%, seven percent higher than for men (PNUD, 2015). Therefore, inequity and inequality are still present domestically.

Rural women have traditionally been in charge of household tasks, which include feeding, cleaning, taking care of and educating children, and taking care of the elderly and sick relatives. Additionally, they have done other activities related to the entire agri-food system, which range from caring for natural resources and animals for household-consumption to seed protection, planting, harvesting,

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commercialization, preparation, consumption and biological use of food (OSAN Colombia, 2015; Suárez and Del Castillo, 2016). Daily, rural women are in charge of activities that require a lot of time and dedication, without any kind of economic remuneration or, in many cases, without any recognition of their contribution to food and nutrition security (FNS) and to the economic and social development of their country and region (Suárez and Del Castillo, 2016).

Rural women suffer double discrimination: first because they are women and second because they are rural (OSAN Colombia, 2015). Estimating the precise number of rural inhabitants in Colombia and conceptualizing that number is very difficult because the term "rural inhabitant" does not appear in the national census; instead the category "the remainder" population is used (PNUD, 2011). Furthermore, in Colombia, the term "rural inhabitant" is not reflected in agricultural policies and does not appear in the current National Agricultural Census of 2014, wherein they are classified as "Producers of the countryside". Nevertheless, this concept is considered a cultural category and a reference of self-recognition by a large part of the Colombian rural population (Universidad del Rosario, 2016).

In spite of the important work that women do contributing to food and nutrition security, especially in the production and processing of food products, there are currently no formal female organizations in this area according to the Agriculture and Economic Development Secretaries of the municipalities of Sibate, Sopo and Location 20 of Bogota (Sumapaz).

In the organizations that women participate in, they usually do not hold decision-making positions, a situation that could lead to less access to technical assistance, agricultural inputs, technology, land, commercialization chains and credits for rural women (Suárez and Del Castillo, 2016). The National Agricultural and Livestock Census of 2014 indicates that, in spite of the fact that Colombian men and women have the same illiteracy level (12%) and that more women attend schools in rural areas, land and services continue to be handled by men at a greater proportion (DANE, 2014).

This situation, along with a lack of knowledge and/or an underestimation of women's role in communities, goes against local development, food production, local value chain strengthening, equity and FNS. As was pointed out at the International Conference on Nutrition held in Rome in 1992, "*women and formally constituted women's food production organizations are often more effective, efficient and essential to improve household food security*" (FAO, 1992). It has been shown that, if the role of women is strengthened, and activities that significantly increase their productivity are prioritized, hunger and malnutrition will be reduced and livelihoods will improve. This benefits not only women, but also the entire population (FAO, 2014).

Colombia has a broad normative compendium in relation to gender equity and protection of women's rights, which fosters non-discrimination and elimination of all forms of gender-based violence. This compendium promotes the strengthening and conformation of women's organizations, wage equity and employment quality, support and financing of women's productive projects and/or initiatives and implementation of municipal policies for rural women, among other things (Alcaldía Municipal de Sibaté, 2016; Alcaldía Municipal de Sopó, 2016). However, despite these initiatives, it is necessary to continue strengthening women in order to make their role and organizational strategies more visible in order to reduce poverty and inequity, as well as to contribute to improved health and nutrition of their families and community.

Accordingly, this research used the following concepts as the starting point:

- The concept of "the empowerment of women", which has been pursued since the Third World Conference on Women held in Nairobi in 1985, is a process of acknowledgment and extension of the control of women over themselves and their environment, in the personal, social, political, economic and cultural spheres (PADEM, 2004).
- The concept of "associative process for work", coined by authors such as (Haeringer *et al*., 1997), refers to the capacity that social actors have to solve individual and collective problems and needs by working with a principle different from that of the market economy. It is based on solidarity, reciprocity and democratic participation for the development of objectives and rules of internal order, where the big difference between the associative process and companies is the production of goods and services. For companies, the good or service generates the social bond, while for the associative process, the social bond generates the good or service (Maldovan and Dzembrowski, 2009).

Finally, the Observatory of Sovereignty and Food and Nutrition Security of the Universidad Nacional de Colombia (OBSSAN UN) states that FNS is "The right of all people to access the food they need in a timely and permanent manner in adequate quantities and quality for their consumption and biological utilization, guaranteeing them a state of nutrition, health and well-being, which contributes to human development and allows them to be fulfilled and happy" (OBSSAN UN, 2010).

The concept from OBSSAN UN points out that the FNS in Colombia not only depends on food and nutritional factors, but also on environmental, social, economic, political and cultural factors. Aspects such as educational level, distribution of tasks within households, economic autonomy and empowerment of rural women play a fundamental role in the progressive achievement of the human right to food. These aspects also contribute to attaining other human rights and, consequently, the economic and social development of the country. In this context, the present research aimed to characterize the processes of empowerment in rural women's associations as a possible contribution to the FNS of their families and their community.

Materials and methods

This research was based on a qualitative research methodology called "descriptive case" and was developed according to the methodology of Yin (2009). This methodology is defined as empirical research that investigates a contemporary phenomenon in depth within its real context, especially when the limits between the phenomenon and the context are not apparent. This same author pointed out that the descriptive case research tries to describe what happens in a particular case, facing a technically distinctive situation in which there will be many more variables of interest than the observational points (Yin, 2009).

Selection of case studies

The selection of the case studies was performed hand in hand with the Ministry of Agriculture of the Municipality of Sibate, the Secretary of Economic Development of the Municipality of Sopo, and the Nazareth Hospital in the Sumapaz location, who helped contact the leaders of 19 rural organizations in territories that produce and/or process food.

Subsequently, the three organizations with the largest number of female members were selected from these 19 organizations for this research:

- The Municipal Association of Rural Users from Sibate (AMUC Sibate)
- The Municipal Association of Rural Users from Sopo (AMUC Sopo)
- The Rural Producer Network of Life and Peace of Sumapaz.

These cases were selected because of their homogeneity since Sibate, Sopo and Sumapaz are located in Cundinamarca, a province in the Colombian Andean region that is very close to the urban area of Bogota. The studied municipalities have a large rural area, a strong agricultural tradition and vocation, and an economy based on agriculture and livestock, where they grow and process cold weather foods such as vegetables and dairy.

Data collection instrument

An operational gender survey from Emory University and the United Nations Entity for gender equality and the empowerment of women (UN women) was used for the data collection. This survey was previously translated into Spanish, validated and adapted for Colombia by the World Food Program (WFP) and the Universidad Nacional de Colombia for the study: "Evaluation of the effect of marketing interventions for women on economic empowerment and risk of intimate partner violence" (PMA and Universidad Nacional de Colombia, 2016). This instrument was adapted to the research objective and the context of the rural population of the municipalities of Sibate, Sopo and the rural area of Bogota. The final survey was composed of 201 questions, grouped into six chapters, which included questions on demographic characterization, economic income and livelihood, associative process, role in decision-making at the associative and family levels, norms and gender attitudes, financial empowerment and coercion.

Field operational scheme

The data were collected during household visits with women from the selected organizations during the months of July, August and September, 2016. The collected data were then systematized and analyzed.

The following table shows the number of women surveyed in each organization (Tab. 1).

TABLE 1. Organizations selected for the study, with the name of the organizations, their acronyms, municipality where they are located, and the number of women interviewed.

Name of rural organizations	Acronym	Municipality	Number of women interviewed
Municipal Association of Rural Users from Sibate	AMUC	Sibate	9
Municipal Association of Rural Users from Sopo	AMUC	Sopo	8
Sumapaz Rural Network Producer of Life and Peace			9
TOTAL			26

Analysis plan

The information obtained in the surveys was classified by food producing and processing organization in order to organize the three case studies. Then, following the methodology of Yin (2009), a consistency matrix between the proposed objective and the obtained information was created, which determined the categories of interest for the analysis and discussion presented in this document.

Results and discussion

First, each of the selected cases and the common socio-economic characteristics in the three cases were generally described. Then, the processes of empowerment from the associative perspective and the empowerment of the rural women and their relationship with food and nutrition security were analyzed.

Case of the Municipal Association of Rural Users from Sibate - AMUC Sibate

AMUC Sibate is a non-profit, autonomous and independent association with more than 17 years of experience and with a legal organizational structure since 1997. AMUC Sibate is a democratic association; it has a board of directors that is responsible for representing the opinions of its members and making decisions on their behalf (Cámara de Comercio de Bogota, 2015a).

It currently has 16 active members (ten women, six men), whose main activity is the production and commercialization of food such as peas, potatoes, strawberries, carrots, vegetables, blackberries, honey, eggs, curds, and minor species, among others. They also process items such as cheese, curds, desserts, arepas and envueltos, among others. For the last two years, the association has been able to promote and make these products visible in the monthly farmer's markets that are held in the municipality.

Women from the association have been registered for more than a year, and they joined after being summoned by relatives, friends and the municipal government. Motivated by meeting new friends, these women leave their homes in order to get some time for themselves. Currently, two of them are part of the association's Board of Directors. The majority pointed out that they participate by attending meetings and selling their products in the farmer's markets, a situation that makes them feel satisfied with the way in which decisions are made within the association.

Case of the Municipal Association of Rural Users from Sopo - AMUC Sopo

AMUC Sopo is a formally constituted association, registered with the Chamber of Commerce in 2015; however, it has been operating since 1972. It is composed of rural farmers from the municipality in a collective and voluntary manner, under a democratic and non-profit model that promotes cooperation and commitment for their enrollment in productive and agro-industrial projects (Cámara de Comercio de Bogotá, 2015b).

It currently has 21 active members, of which ten are women and eleven are men. Thanks to their resources and support from the Departmental Association of Rural Users (ADUC) and the Mayoralty, they receive technical assistance, plots on loan for crops, and resources on loan, such as tents, tables and transport. Altogether, this helps them participate in the farmer's markets of the municipality on a fortnightly basis, a space where the rural economy is promoted and made visible, from production to the final consumer, with conditions of quality, safety and fair prices.

This is how rural women began to formally join AMUC Sopo two years ago, with ideas such as leaving home, learning more about the management of home gardens, having greater access to technical assistance, obtaining their own economic resources, being self-employed and supporting the household economy and expanding their social circle. This occurred after they were invited by their relatives and friends, the Municipal Agricultural Technical Assistance Unit (UMATA), ADUC and the municipality of Sopo.

In terms of the experience of this organization, only three out of the eight women interviewed have been chosen to occupy a leadership position in this association. One of them currently has the position of auditor. Moreover, when she was asked about how satisfied they felt about the decision-making role in the association's processes, half of the women interviewed said they felt unsatisfied because they did not make decisions within the association, as opposed to the men in the association AMUC Sopo.

Case of the Rural Network Producer of Life and Peace of Sumapaz

The Rural Network Producer of Life and Peace of Sumapaz is an organization of small producers from Nazareth and Betania, two villages of Sumapaz, called the 20th location of Bogota. It is a 100% rural territory that was created by the community after a process of participation and training at three levels of the "Rural School of Managing Leaders in Sovereignty and Food and Nutrition Security". An initiative that was led by the Observatory of Sovereignty and Food and Nutrition Security of the Universidad Nacional de Colombia (OBSSAN UN), whose main objective was to strengthen the social fabric and community empowerment processes as a contribution to food sovereignty and FNS.

The school provided theoretical and practical components, where both teachers and the community had interdisciplinary spaces to learn, debate and build on topics such as planning, participatory management, project formulation and management, the right to food and food sovereignty among others. These topics made possible to strengthen community participation and organization and, as mentioned above, to create the Rural Network of Producer of Life and Peace of Sumapaz. The members of this network have formulated and are implementing productive projects that make the local economy dynamic and contribute to the improvement of FNS in the territory as a consequence of technical assistance, input delivery and constant support from the Universidad Nacional de Colombia and the Nazareth Hospital. In addition, the institutional support has been crucial in defining the structure and the formal and informal rules that govern the organization's behavior and consolidation.

Unlike the cases of Sibate and Sopo, this association is informal, which means that it does not adhere to government regulations. Beyond attending workshops, meetings and farmer's markets, the nine surveyed women said that they actively participate by giving their ideas and opinions, listening to others, making decisions and fulfilling assigned tasks. In addition, they stated that they have applied the acquired knowledge by making food trade and designing and implementing productive projects mainly related to boilers, swine production, and family gardens.

Socioeconomic characteristics of rural women surveyed in the municipalities of Sibate, Sopo and Sumapaz

In the three case studies, the ages of the interviewed women ranged from 23 to 75. Their educational level was low since more than half of them did not manage to finish high school. Their household composition, on average, included four people, and the great majority of the women were married or living in a common law union. The women were mainly engaged in housework tasks, caring for children and grandchildren, and, in some cases, field work, taking care of and breeding farm animals such as chickens, hens, pigs, and rabbits. They also took care of crops and home gardens and produced and sold cheese, curds, *arepas, envueltos* and desserts, among other products. In fact, these women spent more than 18 h a day on average on these kinds of activities, while their husbands or domestic partners devoted themselves mainly to farm work, jobs and livestock.

For the commercialization of their products, the women must leave their farms and villages and transport their products to the main plaza on the days that the farmer's markets are held. In that space, they sell their food directly to the final consumer in small units of measurement such as pounds, liters and pieces since the quantities of food they can transport themselves is limited. Besides, they often do not have fresh and ready to sell products.

When inquiring about the prices of the products, the women surveyed stated that they are set by the women themselves, the men of the association and, in some cases, by their husbands. However, when the women were asked if they knew the total cost of food production, either fresh or processed, 100% of the women of the three study cases declared that they did not know. Additionally, when asked what elements they would consider when establishing the total cost of production, the women did not consider important expenses such as their time, work, land leasing, transportation and public services. They only mentioned the seeds and inputs they acquired outside the household, a factor that harms the economy of their households.

Empowerment of rural women from the associative process

The participation of women in these three associations is incipient since only seven out of the 26 interviewed women stated that they hold positions within the board of directors of these associations. However, the vast majority of the women from the three studied cases declared that the associations presented an opportunity for them to outsource their products, expand their market, make food trades, and allocate a significant percentage of food for household consumption. They also stated that the associative process has allowed them to participate in areas where traditionally only men had access, such as technical assistance, agricultural inputs, financing of productive projects and access to training. This participation has strengthened food production, enabled them to receive their own income and given them the autonomy to freely decide how to spend it.

This is a situation that favors the FNS, as Quisumbing *et al.* (1995) indicate.

The results of this research also indicate that the associative process has also allowed rural women to strengthen abilities they had failed to develop at an individual level, such as cooperation, solidarity, teamwork, leadership, decision making, and economic autonomy, and provided opportunities to be outside their household, free time, chances to meet women in similar situations and the ability to be independent, among other benefits. In this way, the associative process has become a possible protective factor of the FNS of families and their community.

This is reflected in the following testimony of one of the female members of the Rural Network of Sumapaz:

"Belonging to the Network…has allowed us not only to strengthen personal skills such as public speaking and expressing ideas, but has enabled us to acquire knowledge on food and nutrition. We have learned how to identify territorial problems and how we can create solutions to our problems by working as a team and knowing our skills, rights and duties. Participating in the Network has also allowed us to meet neighbors and friends, and, thanks to farmer's markets, we have gotten the opportunity to visit important places such as the Universidad Nacional".

These results agree with authors such as Zimmerman (1999), who states that empowerment is a construct in which strengths and individual competences and collective action are combined to improve the quality of life in a community. Authors such as Vázquez *et al.* (2002) have stated that "empowerment can contribute to improving the lives of women, especially rural women, since not only their personal development is emphasized, but also the structures and forces that marginalize, oppress women and put them at a disadvantage compared to men are transformed" (Vázquez *et al.*, 2002).

However, it should be noted that, although the majority of women in all three cases acknowledged the fact that the associative process has promoted individual, economic and organizational empowerment, it is currently still very uncommon for them to make major decisions within these three associations since men continue to occupy the highest positions on the boards of directors of each association.

Additionally, for the FSN, this study revealed that the women in these three cases continue to experience conditions of disadvantage and inequality since, in a typical day, they are the ones who participate in the whole agri-food chain, guarding seeds, sowing, harvesting, raising animals, milking animals, and processing and/or transforming food; at home, they are responsible for cleaning and keeping things in order; at the food level, they are responsible for selecting, storing, conserving, preparing, distributing, and serving food, making sure all family members have eaten. Also, they are always monitoring food quality and safety.

On a typical day, they are also in charge of the medical care for the whole family; they supervise school activities and homework for their children and grandchildren and take care of the elderly and sick in their household. The rural female members of AMUC Sibate, AMUC Sopo and the rural network producer of life and peace of Sumapaz carry out these tasks daily without the help of their domestic partners or the other members of the household, except in some case when their daughters provide support.

Hence, this research revealed that the participation of women in these rural associations has encouraged empowerment processes, but, at the same time, has generated an additional workload for them. These women spend more than 18 h per day dedicated to their homes and families, producing and processing food, as well as participating in meetings, training, farmer's markets and other activities programmed by their association.

This situation can be compared with the results of the last National Survey on Time Use (DANE, 2017), which showed that Colombian rural women over ten years old devote on average 7 h and 52 min a day to unpaid tasks in their home and other households, whereas men spend 4 h and 50 min on the same type of activities. In addition, this research led to the conclusion that the increase in the workload of rural women was the result of women not being able to acknowledge, visualize and identify their work. They have not been able to distribute workloads within the household since the vast majority of these women affirmed in the survey that *"It is worse for a woman to leave her children than for a man", that "the most important role of women is to take care of their home and cook for their families", and that "tasks such as changing diapers, and bathing and feeding children are the sole responsibility of women"*.

Although these associations promote individual and collective empowerment, in terms of decision-making, leadership, learning, and mainly economic autonomy, there is still a need to strengthen the associative processes through a cross-cutting gender approach that goes beyond using women as a tool. It is necessary to create spaces where men, women, young people, boys and girls question the traditional roles within the household, to create a shared vision of these roles, and to generate spaces where thoughts on the inequality and inequity between men and women can be transformed. As indicated by organizations such as the International Food Policy Research Institute (IFPRI) and the WFP (PMA, 2015), empowering women is not only a process by which women acquire the ability to choose, access to power, possibilities, control and autonomy in their own lives, but actually achieve it. Women must not only have the same skills, access to resources and opportunities as men, but also the necessary autonomy to experience those rights and opportunities in order to choose and make decisions about their own rights as members of society (PMA, 2015). Empowerment depends not only on women, but also on their environment and the possibilities for transformation in the personal, social, legal, cultural and economic spheres (PADEM, 2014).

Conclusions

The female members of AMUC Sibate, AMUC Sopo and the Rural Network Producer of Life and Peace of Sumapaz recognize that, little by little, their participation in these associations has promoted economic autonomy and the strengthening of their abilities and decision-making skills. These achievements are the result of technical assistance, agricultural inputs, training and productive projects that have been provided to them.

The results indicate that empowerment and the associative process of rural women can contribute to improving the FNS of their families and their communities. Indeed, when household income, technical assistance and food production for household-consumption increase, women are encouraged to make the right decisions regarding food, health and nutrition for all household members.

Nevertheless, these associative processes could also increase the workload of women. In the studied cases, the women not only dedicated several hours of the day to reproductive and domestic work that has historically and culturally been assigned to them, but they also devoted many hours of the day to fulfilling their commitments to the associations, for instance participation in meetings, workshops, and food production, processing and commercialization. Despite the fact that the associative processes in the three organizations have promoted individual and collective empowerment, government entities must take action to strengthen the cross-cutting gender approach. These actions should not only provide rural women with physical and economic resources to work in food production and processing, but they should also offer spaces for men, women, young people and children to question the roles within the household, to recognize the important role of women, to learn to distribute burdens within the household and to transform inequity and inequality.

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Ham salting in plastic bags: A way to reduce salt use?

Salado de jamones en bolsas: ¿Una manera de reducir el uso de sal?

Joel Girón[1]*, Raúl Grau[2], and José Manuel Barat[2]

ABSTRACT

The objective of this work was to study salt reduction in the processing and final product of Iberian dry-cured ham manufacturing. For this purpose, high-barrier plastic bags were used in the salting stage, with salt doses of 2.5 and 3.2% at 10 and 15 d. The following aspects were evaluated: the production and salt content of brine, the analytical determinations of the hams after the salting stage, the changes in the total mass of the hams during processing and the analytical and sensory assessment of the dry-cured product. The experiment results showed that the plastic bag salting minimized the production of brine by 61 and 63% and reduced the salt content in the final product without altering the physicochemical characteristics or the hygienic quality of the dry-cured ham. In the sensory analysis, the samples salted with 2.5% doses were preferred by consumers.

Key words: dry-curing, vacuum, sodium chloride, brine.

RESUMEN

El objetivo de este trabajo fue estudiar la reducción de sal en el procesamiento y el producto final en la fabricación de jamón curado. Para este propósito, se usaron bolsas de plástico de alta barrera en la etapa de salado, con dosis de sal de 2,5 y 3,2% a los 10 y 15 d. Se evaluaron los siguientes aspectos: la producción y el contenido de sal en la salmuera, las determinaciones analíticas de los jamones después de la etapa de salado, los cambios en la masa total de los jamones durante el procesamiento y la evaluación analítica y sensorial del producto curado. Los resultados experimentales obtenidos mostraron que el salado en bolsas de plástico minimizó la producción de salmuera en un 61 y un 63% y redujo el contenido de sal en el producto final sin alterar las características fisicoquímicas o la calidad higiénica del jamón curado. En el análisis sensorial, los consumidores prefirieron muestras saladas al 2,5%.

Palabras clave: curado en seco, vacío, cloruro de sodio, salmuera.

Introduction

The manufacturing of dry-cured ham is a relatively simple process, but it must have specific conditions in order to ensure excellent quality. A critical point in the process is the salting stage, which generates the drying conditions and the sensory characteristics of the product (Andrés *et al.,* 2004; Benedini *et al*., 2012), as well as a large amount of brine waste, whose effluents should be treated to prevent environmental pollution. In the salting stage, Serrano/Iberian hams are covered with salt by stacking them in containers for a period of approximately 1 d kg^{-1} (Arboix, 2014). Excess salt is unnecessary because the salt uptake reaches a saturation point, after which the pieces are not able to incorporate more salt despite increasing the salting time. Moreover, the water released from the meat has partially dissolved NaCl, and nitrifying salts form saturated brine, whose main components are organic in nature (Barat *et al*., 2006). Brine production is one of the more significant environmental issues faced by many industries since brine disposal has a negative impact on the environment, is costly, and requires a high amount of energy (Randall and Lewis, 2011). The potential environmental damage from disposed brine include eutrophication, pH fluctuations, and proliferation of heavy metals in marine environments. There are different management strategies for brine disposal, including brine minimization, direct disposal, and direct reuse (Giwa *et al*., 2017). Currently, most of the available brine treatment options do not produce potable water that can be resold, thereby offsetting treatment costs; instead, they produce a mixed salt (waste) product that needs to be disposed of at additional costs (Randall and Nathoo, 2015). Additionally, meat products are identified as products for sodium reduction (Kloss *et al*., 2015). The salt content can be reduced by modifying the salting procedure according to salt uptake information (Fulladosa *et al*., 2015). In this regard, a fish salting methodology with low brine emission proposed by Fuentes *et al*. (2008) was applied for the ham salting. The methodology consists of using an exact amount of salt that is directly dosed for absorption on the lean ham

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surface. The combination of a curing salt mix and vacuum packaging in this controlled salting process has also been studied to obtain salt-smoked salmon for the purpose of accelerating NaCl absorption and dehydration (Rizo *et al*., 2013; Rizo *et al*., 2015). This procedure has proven to be capable of reducing waste and obtaining homogeneous products. Therefore, the aim of this study was to evaluate a salting procedure that uses high-barrier plastic bags in order to reduce brine waste production and obtain an Iberian dry-cured ham that is good quality and safe.

Materials and methods

Fifty fresh Iberian hams with an average weight of 12.8 ± 0.5 kg were selected and processed under the experiment conditions in an Iberian dry-cured ham manufacturing company. Ten hams that were used as the control were salted following the company's standard conditions. The remaining forty hams were salted employing high-barrier plastic bags (size 1300 x 750 x 0.12 mm, water vapor transmission rate 1.8 g 120 L^{-1} m^{-2} 24 h^{-1} (23°C and 85% RH)) supplied by Productos Pilarica, S.A. (Valencia, Spain) with a vacuum application. For this purpose, the hams were randomly assigned to each treatment, in one of the two salt doses, 2.5 and 3.2% (w/w), and one of the two salting times, either 10 or 15 d (Fig. 1). In the post-salting stage, the hams were stored at between 4-8°C and 73-75% relative humidity for 70 d. In the subsequent drying stage, the hams were kept in a room under controlled conditions for 120 d; the temperature was increased from 8 to 20°C, while the relative humidity was progressively reduced to 64%. Finally, the hams were left to mature for 16 months between 20-25°C with relative humidity 55-65% (cellar stage). At the end of the process, the hams were sent to the Polytechnic University of Valencia for the physicochemical analysis and sensory evaluation.

Sampling

The sampling was carried out once the hams had reached about 30% of the total weight loss and at the end of the process. The physicochemical parameters were evaluated in the whole ham after the salting stage. At the end of the process, the analytical determinations were carried out in two zones at the widest section of the ham: zone A, located between the lean surface and near the femoral artery (*Semimembranosus* muscle) and zone B, near the subcutaneous fat (*Biceps femoris* muscle) (Fig. 1). In addition, at the end of the salting stage, the generated brine was collected, and the volume and concentration of NaCl were measured.

Analytical determinations

The moisture content (x^w) was determined with oven drying to constant weight at 100°C (ISO, 1997). The fat content (x^f) was established using a FOSS Soxtec System 2055 Tecator (ISO, 1973). The water activity (a_w) of each sample was determined at 24°C with an Aqualab® dew point hygrometer (DECAGON Aqualab CX-2, Pullman, WA, USA). The sodium chloride was determined in both the brine and hams with an automatic chloride Analyser (Sherwood Scientific Ltd., Cambridge, UK) with the

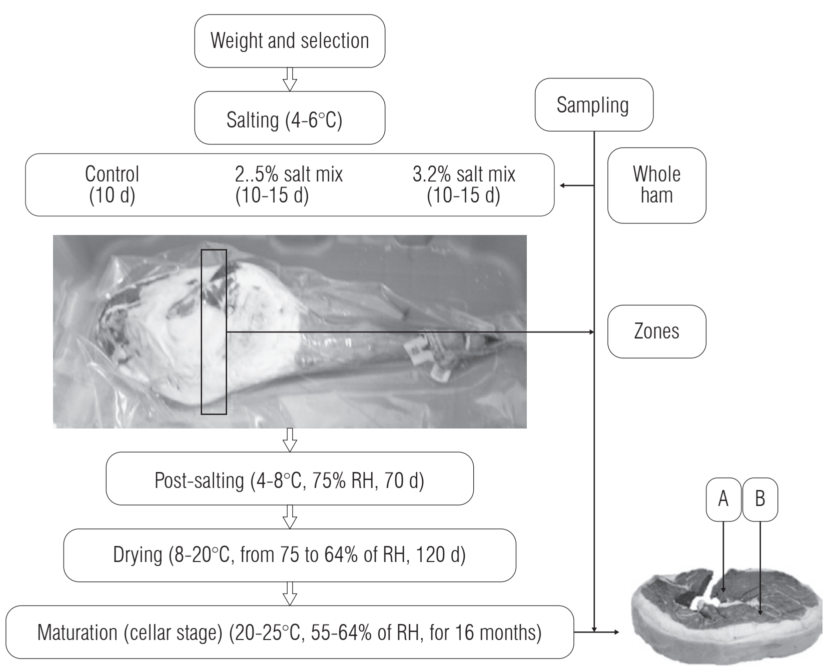

FIGURE 1. Salting process of hams indicating dose, time and sampling zones.

method described by Girón *et al*. (2015). The total sodium chloride concentration in the liquid phase (Z^{NaCl}) was estimated from the weight fractions of water (x^w) and sodium chloride (x^{NaCl}) in accordance with Equation 1. The total weight losses of the post-salted hams were estimated from the weight (M) of the hams (at sampling time t, and 0), with Equation 2.

$$z^{NaCl} = \left(\frac{x^{NaCl}}{x^{NaCl} + x^{w}}\right) \quad (1)$$

$$\Delta Mt = \left(\frac{Mo - Mt}{Mo}\right) \quad (2)$$

Color measurements were taken shortly after cutting the slice in zones A and B using a CR-410 colorimeter (Minolta Chroma Meter, Osaka, Japan) and CIE L*a*b* color system and D-65 illuminant. The texture profile analysis (TPA) was carried out following the procedure described by Aliño *et al*. (2010a) with double compression using a Texture Analyzer TA.XT2 (Stable MicroSystems, UK). The determinations were carried out on cubic samples (15×15×15 mm) from zones A and B; the following parameters were calculated: hardness (g), cohesiveness (dimensionless), springiness (dimensionless), adhesiveness and chewiness (g).

Microbiological analysis

The microbiological analyses were carried out after the salting stage and at the end of the process on the area closest to the bone of the central transversal section of the ham, under sterile conditions and while considering the total mesophilic aerobic flora (Cordero and Zumalacárregui, 2000), Micrococcaceae (Cordero and Zumalacárregui, 2000), salt-tolerant flora (BAM, 1998), lactic acid bacteria (ISO, 1998), Enterobacteriaceae (ISO, 2017), and sulphite-reducing clostridium (BAM, 1998). Microbial counts were obtained in duplicate, and all the results were expressed as a logarithm of colony-forming units per gram (log cfu g^{-1}).

Sensory analysis

A sensory assessment was performed in order to evaluate the sensory acceptance of the obtained dry-cured hams. The sensory analysis was conducted with hams from each treatment. The sensory evaluation test was taken by 106 consumers: 56 women and 50 men aged between 20 and 50, all regular consumers of Iberian dry-cured ham. The tests were done with semi-structured scales (UNE-ISO, 2006), evaluating attributes such as appearance, odor, texture, intensity of saltiness, typical taste and global acceptance. These attributes were selected as being the most important and representative for dry-cured ham. Each assessor answered a questionnaire with 8 cm lines and three anchor points (0 = unpleasant, 4 = acceptable, and 8 = pleasant) for all the attributes. Each assessor evaluated five samples served at room temperature and coded with a three-digit random number.

Statistical analysis

The statistical treatment of the data was performed using Statgraphics Centurion XVI (Manugistics Inc., Rockville, MD, USA). An analysis of variance (One-Way ANOVA) was conducted for each parameter to test if there were significant differences between the samples. All of the physicochemical, microbiological, and sensory parameters were considered as dependent variables in these analyses, and the salt dose and sampling zone were the factors. The LSD procedure (least significant difference) was used to test for differences between the averages at the 5% significance level. A multifactor ANOVA was conducted with the salting parameters to evaluate the effect of the salt dose, sampling zone, and their interactions.

Results and discussion

Evaluation of the salting process

The brine production, sodium chloride content in brine, moisture, lipid content, sodium chloride, a_w, sodium chloride concentration in the liquid phase, and microbial loads for the samples and the control after the salting stage are shown in table 1. There were no significant differences (P>0.05) in the production of brine when increasing the salting time or the salt dose. However, the brine production in the experiment was lower for the samples salted in plastic bags, as compared to the theoretical production of brine obtained in the traditional salting process as reported by Barat *et al*. (2006). Spain produces approximately 26,500 metric t of cured hams and shoulders made with the traditional method, generating a brine volume of approximately 29,818 tons. The salting method using plastic bags at 2.5 or 3.2% for 15 or 10 d would generate approximately 10,910 and 11,823 metric t of brine, respectively, representing a reduction of between 63 and 61%. In addition, when considering the amount of salt present in the brine, the differences would increase to 89% (2.5%, 15 d) and 72% (3.2%, 10 d). Brine from the traditional method is theoretically saturated (0.26 w NaCl / w brine) (Barat *et al*., 2006), which would generate 7,752 metric t of salt. On the other hand, the brine from the plastic bag salting under the conditions of this study was 0.074 ± 0.035 for the 2.5% salting for 15 d (2.5%-15 d) and 0.184 ± 0.050 for 3.2% for 10 d (3.2%-10 d). This represents 809 and 2,181 metric t of salt, respectively.

Physicochemical characterization of the samples after the salting stage

The evaluated parameters in the samples after the salting stage showed significant differences ($P<0.05$) between the control and the treatments for moisture, salt content and water activity. The hams salted at 2.5%-15 d and 3.2%-10 d presented the highest values of moisture. The salt content presented the lower values for treatment 2.5%-10 d (0.060 ± 0.007 w/w) when the salt content was expressed as salt into the liquid phase (Z^{NaCl}); the hams salted at 3.2%-15d had significant differences ($P<0.05$) because of their higher salt concentration (0.038 ± 0.003), as compared to the control (0.031 ± 0.002). The water activity values were inversely related to Z^{NaCl}, with greater or lesser a_w values of the hams related to greater or lesser Z^{NaCl} values. For a_w, the hams salted at 2.5%-10 d presented the higher values (0.982 ± 0.006). The fat content and microbiological counts did not show significant differences ($P<0.05$). The fat values are similar to those reported by other authors in studies on Iberian dry-cured ham (Aliño *et al.*, 2010b). The values obtained in the microbiological determinations for aerobic mesophilic, salt-tolerant flora and micrococcaceae, but not for the isolation of lactic acid bacteria, *Enterobacteriaceae* and sulphite-reducing clostridium, indicated a good level of hygiene in the raw material and the salting process.

Evaluation of the hams' mass variation throughout the processing

There were significant differences ($P<0.05$) for the changes in total mass (Fig. 2). The control exhibited a greater reduction than the hams salted at 2.5 and 3.2% for either 10 or 15 d after the salting stage; the result was consistent with changes in moisture and brine. Nevertheless, the water content was released along the drying stage. Therefore, the differences were reduced during the processing. At the end of the process, no significant differences ($P>0.05$) were observed between the treatments and the control; the accumulated weight losses (ΔM) exhibited an average value of -0.031 ± 0.02 for all of the hams.

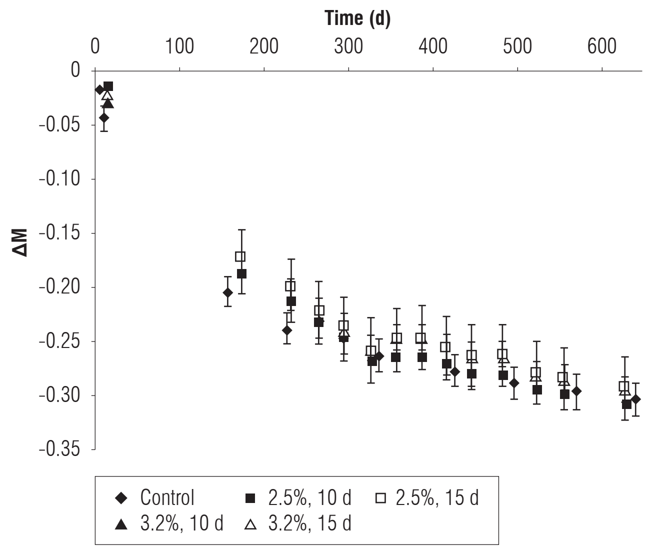

FIGURE 2. Mean values and standard deviation for the changes in total mass throughout the processing time.

Physicochemical characterization of dry-cured hams

There were no significant differences ($P>0.05$) for moisture content (x^w, w/w) or salt content (x^{NaCl} DM) between zones A and B of the samples and the control (Fig. 3). The

TABLE 1. Physicochemical parameters and microbial counting (log cfu g^{-1}) of the brine and hams after the salting stage. Mean values ± SD.

	Control	Dose (w/w) and salting time (days)			
		2.5		3.2	
		10	15	10	15
Brine (w/w of ham)	0.114 ± 0.012*	0.040 ± 0.006[a]	0.042 ± 0.004[a]	0.045 ± 0.005[a]	0.049 ± 0.006[a]
Moisture (g 100 g^{-1})	0.26*	0.147 ± 0.092[a]	0.074 ± 0.035[a]	0.184 ± 0.050[a]	0.177 ± 0.033[a]
NaCl (g 100 g^{-1})	0.644 ± 0.013[a]	0.659 ± 0.010[ab]	0.660 ± 0.007[b]	0.672 ± 0.011[b]	0.659 ± 0.003[ab]
Lipid (g 100 g^{-1})	0.083 ± 0.016[a]	0.092 ± 0.009[a]	0.087 ± 0.005[a]	0.084 ± 0.007[a]	0.085 ± 0.029[a]
x^{NaCl} (g 100 g^{-1}) DM	0.086 ± 0.005[bc]	0.060 ± 0.007[a]	0.076 ± 0.014[b]	0.083 ± 0.007[bc]	0.093 ± 0.022[c]
a_w	0.967 ± 0.004[a]	0.982 ± 0.006[b]	0.972 ± 0.005[a]	0.970 ± 0.001[a]	0.967 ± 0.000[a]
Z^{NaCl} (g NaCl mL^{-1})	0.031 ± 0.002[b]	0.022 ± 0.002[a]	0.032 ± 0.001[b]	0.029 ± 0.002[b]	0.038 ± 0.003[c]
Aerobic mesophiles	0.453 ± 0.785[a]	0.358 ± 0.620[a]	0.524 ± 0.908[a]	0.946 ± 0.827[a]	0.000 ± 0.000[a]
Salt-tolerant flora	0.887 ± 0.768[a]	0.399 ± 0.691[a]	1.312 ± 0.159[a]	0.892 ± 0.795[a]	1.283 ± 0.251[a]
Micrococcaceae	1.029 ± 0.893[a]	0.000 ± 0.000[a]	0.000 ± 0.000[a]	0.529 ± 0.000[a]	0.333 ± 0.577[a]

Means in a row with different letters are significantly different ($P<0.05$); Data obtained from Barat *et al.* (2006).

moisture content in the outer sampling zone (A) of the hams presented lower values than the inner zone (B); these results were expected because the outer zone is directly in contact with the salt. The moisture values agree with those established by other authors (Garrido-Novel *et al*., 2015).

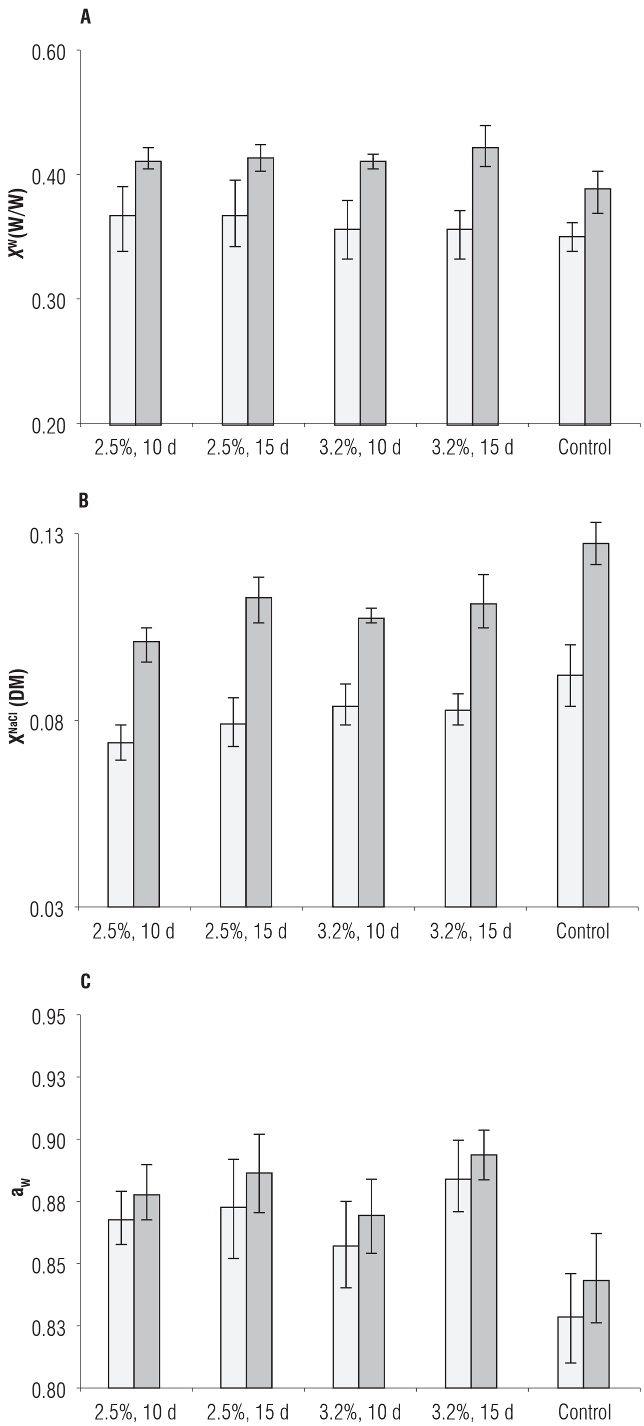

FIGURE 3. Mean values ± standard deviation of water content (x^w w/w), salt content on a dry matter basis (X^{NaCl} DM), and water activity in the sample zones A (lean surface, white bars) and B (inner point, black bars) at the end of the process.

The salt content values showed that the traditionally salted hams presented higher levels of sodium chloride than the hams salted in plastic bags. As expected, the salt content in the outer zone (A) presented lower values than in the inner zone (B) because salt diffuses to the zones with a greater amount of water (Aliño *et al*., 2010a). The highest salt contents were found in the control and the hams salted at 3.2% for 15 d. The water activity presented significant differences (P<0.05) with lower values in the outer zone (A) for all of the treatments and the control, which exhibited the lowest a_w values.

The multifactor ANOVA (Tab. 2) revealed that the amount of salt dosed and the sampling zone influenced the moisture and salt content. The effect of the sampling zone was greater as compared to the salt dose for all of the considered variables. The interactions between salt and zone did not affect the studied variables. Nevertheless, the effect of the interactions was less important than the factors considered individually.

TABLE 2. F-ratio values and significance levels obtained in the multifactor ANOVA for the physicochemical parameters according to the factors: salt dose (S) and zone of analysis (Z) and their interaction (SxZ).

	S	Z	SxZ
X^w	0.76^{ns}	17.91^{***}	0.31^{ns}
X^{NaCl}	32.14^{***}	27.47^{***}	2.23^{ns}
a_w	2.42^{ns}	3.50^{ns}	0.06^{ns}
Z^{NaCl}	0.45^{ns}	1.40^{ns}	0.33^{ns}

ns: not significant, *** P<0.001.

Texture and color analysis

There were no significant differences (P>0.05) between the treatments and the control for the TPA analysis (Tab. 3). On the other hand, it was observed that the lower moisture content in the control promoted higher values for hardness, chewiness and cohesiveness. However, these values may not be important at the consumption level because ham is consumed in slices and not in cubes. The results for the inner zone (B) did not show significant differences (P>0.05) between the treatments and the control; the values were similar for the texture attributes, except for the samples at 2.5% for 10 d, which presented a softer texture. Other authors (Gou *et al*., 2008; Andronikov *et al*., 2013) have found that a lower salt content in ham promotes proteolysis, which results in a softer texture in the final product (Gou *et al*., 2008; Andronikov *et al*., 2013).

The color parameters did not show significant differences (P>0.05) between the treatments and the control (Tab. 4). The mean values for luminosity and yellowness

TABLE 3. Mean values ± standard deviation of the texture parameters: hardness (g), chewiness (g), springiness, cohesiveness and adhesiveness in the *Semimembranosus* muscle (outer zone: A) and in the *Biceps femoris* muscle (inner zone: B) of the ham.

		Salt dose 2.5%		Salt dose 3.2%	
Outer (A)	**Control**	**10 d**	**15 d**	**10 d**	**15 d**
Hardness (g)	124.696 ± 21.145[b]	83.931 ± 26.361[a]	103.747 ± 38.003[ab]	91.615 ± 3.135[ab]	104.569 ± 31.207[ab]
Adhesiveness (g)	-0.778 ± 0.320[a]	-0.499 ± 0.437[a]	-0.807 ± 0.345[a]	-0.653 ± 0.308[a]	-0.644 ± 0.181[a]
Springiness	0.783 ± 0.032[a]	0.726 ± 0.111[a]	0.760 ± 0.057[a]	0.743 ± 0.022[a]	0.756 ± 0.044[a]
Chewiness (g)	60.199 ± 10.140[b]	37.786 ± 18.085[a]	49.644 ± 23.981[ab]	34.233 ± 14.712[ab]	50.669 ± 17.149[ab]
Cohesiveness	0.618 ± 0.023[ab]	0.576 ± 0.079[a]	0.603 ± 0.048[ab]	0.568 ± 0.021[ab]	0.634 ± 0.020[b]
Inner (B)					
Hardness (g)	109.770 ± 31.200[a]	98.499 ± 39.075[a]	114.532 ± 21.027[a]	124.920 ± 22.435[a]	118.542 ± 58.049[a]
Adhesiveness (g)	-0.504 ± 0.267[ab]	-0.372 ± 0.325[a]	-0.600 ± 0.391[a]	-0.409 ± 0.015[ab]	-0.458 ± 0.257[ab]
Springiness	0.747 ± 0.092[a]	0.721 ± 0.063[a]	0.734 ± 0.068[a]	0.730 ± 0.058[a]	0.736 ± 0.031[a]
Chewiness (g)	47.009 ± 13.797[a]	46.580 ± 21.754[a]	53.434 ± 10.850[a]	54.214 ± 17.845[a]	48.507 ± 21.536[a]
Cohesiveness	0.615 ± 0.115[a]	0.603 ± 0.058[a]	0.640 ± 0.083[a]	0.584 ± 0.093[a]	0.578 ± 0.061[a]

Means in a row with different letters are significantly different ($P<0.05$)

TABLE 4. Mean values ± standard deviation of color parameters L*: lightness, a*: redness, b*: yellowness, in the evaluated zones of the hams.

		Salt dose 2.5%		Salt dose 3.2%	
Outer	**Control**	**10**	**15**	**10**	**15**
L*	33.784 ± 3.472[a]	33.314 ± 3.747[a]	34.169 ± 4.279[a]	34.322 ± 1.094[a]	32.038 ± 2.907[a]
a*	10.187 ± 2.977[bc]	9.964 ± 2.166[b]	7.714 ± 1.476[a]	7.665 ± 0.512[a]	10.008 ± 2.125[bc]
b*	6.793 ± 2.976[a]	5.739 ± 1.830[a]	5.201 ± 0.656[a]	5.043 ± 1.065[a]	6.120 ± 1.182[a]
Inner					
L*	29.792 ± 2.281[bc]	25.593 ± 2.083[a]	30.883 ± 4.731[c]	25.288 ± 3.596[a]	26.622 ± 1.950[ab]
a*	7.131 ± 2.575[a]	6.014 ± 0.860[a]	5.680 ± 1.491[a]	5.420 ± 1.644[a]	6.620 ± 2.281[a]
b*	3.842 ± 0.969[b]	2.903 ± 0.726[ab]	3.179 ± 2.495[ab]	2.097 ± 1.147[a]	2.915 ± 1.445[ab]

Means in a row with different letters are significantly different ($P<0.05$)

were similar in the two sampling zones for all of the treatments and the control. Higher values for redness were observed for the samples salted at 3.2% for 15 d in both zones, which may have been due to the pH, salt concentration and moisture loss, which raised the pigment concentrations, such as myoglobin (Sanabria *et al*., 2004; Bermudez *et al*., 2014)

Sensory analysis of dry-cured hams

The overall scores marked by the assessors of the sensory attributes of the different evaluated samples are shown in figure 4. There were significant differences ($P<0.05$) between the samples for the evaluated attributes. The hams with lower acceptance rates were those salted with the highest concentration of salt; the treatment at 3.2% for 15 d presented the lowest acceptability; the hams presented similar behavior when salted at 3.2% for 10 d, except for the score of appearance and saltiness. The observed result can be attributed to the formation of a crust on the hams' surface, which was salted with a higher salt dose, affecting

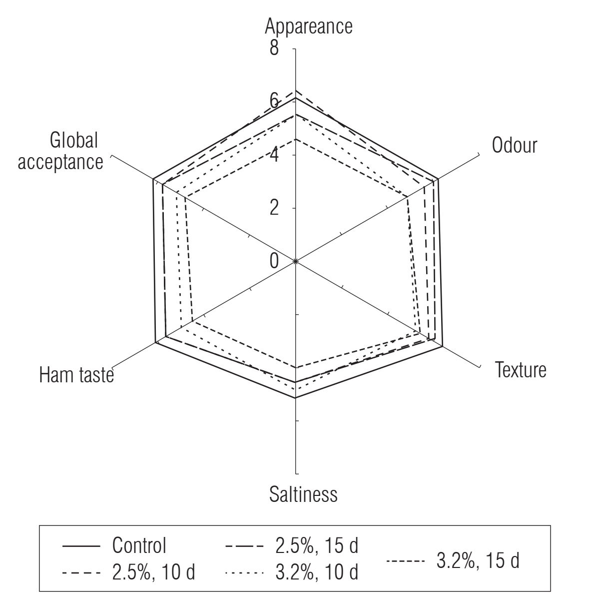

FIGURE 4. Score average for the different attributes evaluated in the control and the hams salted in plastic bags. 0: very unpleasant, 4: acceptable and 8: very pleasant.

the diffusion process and consequently deteriorating the organoleptic characteristics of the hams at the end of the process (Martínez-Onandi *et al*., 2016). On the other hand, the hams salted with a lower salt dose facilitated the diffusion process and improved their acceptance. In addition, it is important to emphasize that Spanish consumers highly value the salty taste of ham (Morales *et al*., 2013), which influenced the preference for the control over the other treatments.

Conclusions

The assessed methodology minimizes and reduces brine waste. Moreover, it provides hams with a lower salt content. The employed salt levels exhibited similar levels for the reference values for the physicochemical parameters. Among the salt doses, some samples with 3.2% (w/w) presented crust hardening, while the samples with the 2.5% salt dose presented acceptance close to the control. However, consumers should be made aware of the importance of reducing salt uptake, which is often high in cured products. Further research should be done in order to replicate the proposed methodology using biodegradable or reusable plastic bags or plastic or metal containers that can be used in the production line to make the whole productive process more environmentally friendly.

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SCIENTIFIC NOTE

Development of a new maize hybrid for the Ecuadorian lowland

Desarrollo de un nuevo híbrido de maíz para el Litoral Ecuatoriano

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ABSTRACT

Yellow maize (*Zea mays* L. var. indurata) is mainly produced in the Ecuadorian lowlands (less than 1,200 m a.s.l.), primarily for feed production. Although Ecuador has recently increased maize production, new genotypes are needed for self-sufficiency and in order to avoid costly imports. Maize is sown mostly from December to January during the rainy season. However, the irregularity of rainfall has become a constraint on production. "INIAP H-603 Superior" is a new single-cross maize hybrid developed for the Ecuadorian lowlands with improved yields, which could contribute to domestic food security. The new hybrid had an average yield of 8.48 t ha^{-1} with outstanding performance under the rainfall, sustained moisture, and irrigation conditions, outperforming the commercial hybrids INIAP H-602, DEKALB-7088 and INIAP H-553. Additionally, the new hybrid showed tolerance to the principal foliar diseases: leaf blight (*Exserohilum turcicum*), rust (*Puccinia sorghi*), and leaf spot (*Curvularia lunata*), as well as good adaptability and stability, with a regression coefficient (*bi*) of 0.98 when it was evaluated in 29 locations from 2010 to 2013. The greatest yield potential of the new hybrid (10.82 t ha^{-1}) was obtained with irrigation during the dry season.

Key words: breeding, food security, climate change, stability.

RESUMEN

El maíz amarillo duro (*Zea mays* L. var. indurata) es producido principalmente en las zonas bajas o costeras del Ecuador (menos de 1200 m s.n.m.) y está destinado mayoritariamente para la elaboración de alimento balanceado. A pesar de que la producción de este cereal en el Ecuador se ha incrementado en los últimos años, se requieren de nuevos genotipos que permitan la autosuficiencia y eviten importaciones de maíz. El cultivo de maíz es realizado principalmente en la época de lluvia, cuando las irregulares precipitaciones se convierten en las principales limitantes para la productividad del cultivo. "INIAP H-603 Superior" es un híbrido simple de maíz desarrollado para expresar su potencial bajo condiciones de riego, lluvia o humedad remanente. El nuevo híbrido obtuvo rendimientos promedios de 8.48 t ha^{-1}, superando a los híbridos comerciales INIAP H-602, DEKALB-7088 e INIAP H-553 y mostró tolerancia a las principales enfermedades que afectaron al cultivo: lancha (*Exserohilum turcicum*), roya (*Puccinia sorghi)*, y mancha por curvularia (*Curvularia lunata*); así como buena adaptabilidad y estabilidad de rendimiento, con un coeficiente de regresión (*bi*) de 0.98, basado en pruebas multi-ambiente en 29 localidades, realizadas del 2010 al 2013. El mayor potencial de rendimiento del nuevo híbrido (10.82 t ha^{-1}) se obtuvo en la época seca bajo riego.

Palabras clave: fitomejoramiento, seguridad alimentaria, cambio climático, estabilidad.

Introduction

Flint yellow maize is a crop of significant importance in Ecuador. During the rainy season of 2016, farmers sowed roughly 192,000 ha of flint yellow maize, with an average yield of 5.5 t ha^{-1} (Castro, 2016). Maize in Ecuador is a strategic crop for food security because of its multiple products and by-products, which are mainly used in the poultry, fish, and dairy feed industries.

Although this kind of maize production has increased in recent years in Ecuador, going from 540,000 t in 2002 to 1,730,000 t in 2015 (MAG, 2015), more high yielding hybrids that are adapted to the growing conditions in Ecuador are needed. Most of the maize (84% of the production area) is sown in December and January at the beginning of the rainy season (Aguilar *et al.*, 2015). The rainy season lasts three to five months, and the rainfall is irregularly distributed during this period (MAG, 2015).

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Accordingly, the objective of the National Maize Breeding Program of INIAP is to develop and release high yielding hybrids that are adapted to the local growing conditions, with resistance to the principal foliar diseases of this crop.

Materials and methods

The single-cross maize hybrid "INIAP H-603 Superior" was obtained in 2010, crossing selected lines generated by the Maize Breeding Program (MBP) of the National Institute for Agricultural Research (INIAP) and lines introduced by the International Center for Maize and Wheat Improvement (CIMMYT), Colombia (Alarcón *et al*., 2016).

Origin of parental lines

The female line POB.3.F4. 27-1-1-1 was developed by the MBP at the Experimental Station Portoviejo (EEP). It was derived from the Population POB.3 in 1994 using diallelic crossing between six genotypes: INIAP-541, INIAP-542, INIAP-526, Pool 26 *selección sequía* (CIMMYT), Pool 18 *selección sequía precoz* (CIMMYT), and Pacific 9205 (private company). After four cycles of mass selection, 256 inbred lines (S1) were developed with self-pollination. The lines were advanced to S4 and evaluated for adaptability and combining ability. This line was selected because of its good agronomic traits, combining ability and high yield. The male line CML-451 was introduced in 2007 from CIMMYT, Colombia. These lines are maintained at EEP by hand (plant to plant pollination), with controlled open pollination.

Experimental design and statistical analysis

This experiment was conducted in the provinces of Manabi (Santa Ana, Tosagua, Jipijapa), Guayas (Balzar, El Empalme), Los Rios (Mocahe, Valencia) and Loja (Zapotillo, Pindal, Paltas). Ten experimental hybrids, including "INIAP H-603 Superior", and five commercial checks (INIAP H-553, INIAP H-601, INIAP H-602, DEKALB-1596, and DEKALB-7088) were evaluated using a randomized complete block design with three replicates in 29 locations (trials) from 2010 to 2013. To establish the differences between the hybrids, an analysis of variance and significance tests were carried out per location and combined (McIntosh, 1983; González *et al*., 2010; Moore and Dixon, 2015) with the statistical package INFOSTAT v. 2015 (Di Rienzo *et al*., 2015). A Tukey t-test ($P<0.05$) was used to determine the differences in the mean yield between the hybrids, growing conditions and interactions. The model used to estimate yield was: $Yij = \mu+Gi+Aj+(GA)ij+Bk(j)+Eijk$, where: Yij = average performance of the i-th hybrid obtained in the j-th environment; μ = effect of the general median; Gi = effect of the i-th hybrid; Aj = effect of the j-th environment; (GA)ij = effect of the interaction between the i-th genotype and the j-th environment; Bk(j) = effect of the k-th repetition on the j-th environment, and Eijk = randomized effect of the experiment error associated with the i-th genotype on the j-th environment and k-th repetition.

The stability of the hybrids was estimated based on Eberhart and Russel's (1966) criteria (Lin *et al*., 1986). To evaluate the stability, the locations were grouped based on the three main maize Ecuadorian growing conditions: rainfall (20 trials), with irrigation during the dry season (four trials), and with water deficits (sustained moisture) during the dry season (five trials). The planting for the dry season occurred in June and July and, for the rainy season, in December and January. The experiment plots consisted of two rows (5 m long) with a distance between rows of 0.80 m, and 0.20 m between plants, for a population density of 62,500 plants ha^{-1}.

Agronomic management

The experiments were planted by hand. The fertilization and phytosanitary control of the crop were carried out according to Villavicencio and Zambrano (2009). The response of the hybrid to the prevalent foliar diseases: leaf blight (*Exserohilum turcicum*), rust (*Puccinia sorghi)*, and leaf spot (*Curvularia lunata*) was evaluated under natural infection conditions, along with the five commercial hybrids used as controls at the EEP, from 2010 to 2013. The disease symptoms were scored after silking (reproductive stage R4), based on a 1 to 5 scale developed by CIMMYT (1995), where 1 was no symptoms and 5 was complete infection. The plant and ear (cob) heights, male and female flowering time, and grain yield were also evaluated as described by CIMMYT (1995), while the corncob type and grain texture were scored according to IBPGR (1991).

Results and discussion

Agronomic characteristics

The plant architecture and phenology of "INIAP H-603 Superior" are similar to other maize hybrids developed by INIAP (Tab. 1). The index between the ear (cob) and the plant height (AM/AP) and male and female flowering times are important traits for breeders because they contribute to lodging resistance and complete grain filling of the cob. The index AM/AP for INIAP H-603 Superior (0.5) and flowering time (55 and 57 d) were good values for lodging resistance and grain filling. These values agree with those reported by Gordon and Deras (2017) for maize in Mesoamerica (AM/AP = 0.5, female flowering = 54 d, male flowering = 57 d), as well as the AM/AP index value reported by Vera *et al.* (2013), who evaluated eight hybrids in four locations in the central area of the Ecuadorian lowlands. Both authors observed variations caused by environmental effects, as seen in the present study.

TABLE 1. Morphological characteristics of the maize hybrid "INIAP H-603 Superior" developed for the lowlands of Ecuador.

Trait	Average and standard deviation	Range
Plant height	259 ± 26.8 cm	180-292
Ear height	127 ± 20.8 cm	75-155
Male flowering	55 ± 3.3 d	49-59
Female flowering	57 ± 2.8 d	51-62
Harvest time	120 d	118-135
Corncob type	Conic-cylindrical	
Grain texture	Flint	

Resistance to major foliar diseases

"INIAP H-603 Superior" was rated 3 (moderate) for leaf blight (*E. turcicum*), rated 1 (no symptoms) for rust (*P. sorghi)*, and rated 2 (mild) for leaf spot (*C. lunata*) as shown in Table 2. These are significant foliar diseases in the provinces of Manabi, Guayas and Los Rios (Villavicencio and Zambrano, 2009). The new hybrid showed equal or a better response (lower values) to foliar disease incidence than the other commercial hybrids. *P. sorghi* (1 to 8%) and *E. turcicum* (1 to 30%) were reported for Pergamino, Argentina by Parisi *et al.* (2014), who concluded that maize genotypes responded differently to these epiphytes. Vera *et al.* (2013) reported an average disease incidence of 3.31 and 2.44% for leaf spot and leaf blight, respectively, with an incidence not greater than 20% for maize hybrids evaluated in the Ecuadorian lowlands.

Yield performance

The combined analysis of variance for yield showed highly significant differences between the hybrids, environments and interactions (Tab. 3 and 4). A combined mean analysis showed that there were four ranges of significance for the hybrids, where "INIAP H-603 Superior", with 8.48 t ha^{-1} was the most stable (*bi* closest to 1) and shared the same range of significance for yield with DEKALB-1596 and INIAP H-601, with 8.20 t ha^{-1} and 7.95 t ha^{-1}, respectively.

There were significant differences between the yield obtained with irrigation during the dry season (9.93 t ha^{-1}) and the yields obtained during the rainy season (6.68 t ha^{-1}) and the dry season with residual humidity (6.65 t ha^{-1}) (Tab. 4). This suggests that, during the rainy season, this crop may not receive the amount of water required by the plant to express its yield potential at the appropriate time. These results support the concept that high maize yields

TABLE 2. Foliar disease ratings for "INIAP H-603 Superior" and five commercial maize hybrids evaluated at the Experimental Station Portoviejo, 2011-2013.

Disease*	INIAP H-603	INIAP H-553	INIAP H-601	INIAP H-602	DEKALB1596	DEKALB 7088
Leaf blight (*Exserohilum turcicum*)	3	3	3	3	4	4
Rust (*Puccinia sorghi*)	1	2	2	2	2	2
Leaf spot (*Curvularia lunata*)	2	3	3	3	4	4

* 1 to 5 scale, where 1 = no symptoms and 5 = severe infection (CIMMYT, 1995).

TABLE 3. Average yield, standard deviation, and yield stability of "INIAP H603 Superior" and five commercial maize hybrids evaluated in 29 locations in the Ecuadorian lowlands from 2010 to 2013.

Hybrids t ha^{-1}		Yield		
			Stability** bi	R^2
H1	INIAP H-603	8.48 ± 1.97 a*	0.98	0.84
H6	DEKALB-1596	8.20 ± 2.29 ab	0.62	0.65
H3	INIAP H-601	7.95 ± 1.98 abc	0.84	0.84
H4	INIAP H-602	7.66 ± 2.01 bc	1.04	0.83
H5	DEKALB-7088	7.29 ± 2.08 cd	1.26	0.57
H2	INIAP H-553	6.92 ± 1.69 d	0.73	0.84
Tukey test ($P<0.05$)		0.72		

* Means and standard deviations followed by the same letter do not differ statistically according to the Tukey's range test ($P<0.05$).

** Values close to 1 are the most stable, according to Eberhart and Russel (1966).

are associated with production under irrigation (Zambrano *et al.*, 2017) and coincide with the observations of Arellano *et al.* (2011) and Caicedo *et al.* (2017), who found that irrigation with high yield hybrids resulted in a differential or yield potential of 5.0 and 7.0 t ha^{-1}, respectively.

Stability analysis

The genotype X environment interaction (GxE) is an important input in the evaluation and development of new maize hybrids based on yield (Zambrano *et al.*, 2017) and resistance to diseases (Parisi *et al.*, 2014), where climatic, soil and management variations produce a significant effect on the differentiation of hybrids. This was first proposed by Finlay and Wilkinson (1963), Eberhart and Russell (1966), and Zobel *et al.* (1988), but nowadays there are several models for estimating GxE, which are widely used in maize, such as the reliability based on differences in yield with commercial controls (Camargo *et al.*, 2002) or the additive main effects and multiplicative interaction (AMMI) model (Caicedo *et al.*, 2017).

The Eberhart and Russel (1966) criterion for stability evaluates the relationship between environmental conditions and yield, where linear relationships are more stable. Alejos *et al.* (2006) showed how the Eberhart and Russel method is useful for evaluating genotypes with favorable or unfavorable responses to changing environments. In this study, "INIAP H-603 Superior" changed linearly with the change in the environment, with a regression coefficient (*bi*) of 0.98 and R^2 of 0.84 (Tab. 3), indicating that it is a genotype with good stability. These values are similar to those reported for new maize hybrids in Panama (Camargo *et al.*, 2002). Hybrids with *bi* values greater than 1, such as DEKALB-7088, which showed a *bi* of 1.26 and R^2 of 0.57,

TABLE 4. Average yield and standard deviation (t ha^{-1}) of the maize hybrids according to the growing conditions and hybrid X growing condition interaction evaluated in 29 locations in the Ecuadorian lowlands from 2010 to 2013.

Growing Condition		Yield (t ha^{-1})				
E2	Dry season (irrigated)	9.93 ± 1.37	a*			
E1	Rainy season	6.68 ± 1.87		b		
E3	Dry season (residual humidity)	6.65 ± 1.28		b		
Tukey test (*P*<0.05)		0.53				
Hybrid x Growing Condition						
	H1 X E2	10.82 ± 1.15	a			
	H6 X E2	10.48 ± 1.05	a			
	H3 X E2	10.43 ± 1.13	a			
	H4 X E2	9.69 ± 1.15	a			
	H5 X E2	9.18 ± 1.69	a	b		
	H2 X E2	8.95 ± 1.06	a	b	c	
	H1 X E3	7.58 ± 1.44		b	c	d
	H6 X E1	7.21 ± 2.26			c	d
	H1 X E1	7.04 ± 1.58			c	d
	H6 X E3	6.92 ± 1.20				d
	H4 X E3	6.79 ± 1.06				d
	H3 X E3	6.75 ± 1.12				d
	H3 X E1	6.68 ± 1.65				d
	H5 X E1	6.68 ± 2.04				d
	H4 X E1	6.50 ± 1.91				d
	H5 X E3	6.01 ± 1.15				d
	H2 X E1	5.95 ± 1.45				d
	H2 X E3	5.86 ± 1.06				d
Tukey test (*P*<0.05)		1.95				
CV (%)		23.6				

* Means and standard deviations followed by the same letter do not differ statistically according to the Tukey's range test (*P*<0.05).

H1 = INIAP H-603 Superior, H2 = INIAP H-553, H3 = INIAP H-601, H4 = INIAP H-602, H5 = DEKALB-7088, H6 = DEKALB-1596.

are very sensitive to environmental changes. In addition, “INIAP H-603 Superior” had high yields in all tested environments. These kinds of genotypes should have a good response to high-input agriculture (Sabaghania *et al.*, 2006).

Conclusions

The crossing of domestic (INIAP lines) and CIMMYT lines has allowed the development of a high yielding maize hybrid, with an average of 8.48 t ha^{-1}, that is adapted to the varying local growing conditions. “INIAP H-603 Superior” is a hybrid well suited for the Ecuadorian lowlands, with resistance to the three principal foliar diseases: leaf blight (*Exserohilum turcicum*), rust (*Puccinia sorghi)*, and leaf spot (*Curvularia lunata*), and good yield stability (*bi* = 0.98). The greatest yield potential of the new hybrid (10.82 t ha^{-1}) was obtained with irrigation during the dry season.

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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.

  E.g., 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

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