AGRONOMIA COLOMBIANA

Doi: 10.15446/agron.colomb

VOLUME XXXVI, No. 1 JANUARY-APRIL 2018 ISSN 0120-9965

Tarifa Postal Reducida No. 2015-404 4-72 La Red Postal de Colombia, vence 31 de Dic. 2018

Centro Editorial
Facultad de Ciencias Agrarias
Sede Bogotá

UNIVERSIDAD NACIONAL DE COLOMBIA

AGRONOMIA COLOMBIANA

VOLUME XXXVI | No. 1 | JANUARY-APRIL 2018 | ISSN (print): 0120-9965 / ISSN (online): 2357-3732

PUBLICATION OF A SCIENTIFIC-TECHNICAL NATURE BY THE FACULTY OF AGRICULTURAL SCIENCES OF THE UNIVERSIDAD NACIONAL DE COLOMBIA, BOGOTA

RECTOR
IGNACIO MANTILLA

DEAN
VÍCTOR JULIO FLÓREZ RONCANCIO

DIRECTOR-EDITOR
MAURICIO PARRA QUIJANO

EDITORIAL COMMITTEE

Juan Pablo Fernández Trujillo, Departamento de Ingeniería de Alimentos y del Equipamiento Agrícola, Universidad Politécnica de Cartagena, Murcia, Spain.

Hermann Restrepo, Faculty of Agricultural Sciences, Universidad Nacional de Colombia, Bogota, Colombia.

Leo Rufato, Centro Agroveterinário, Universidade do Estado de Santa Catarina, Lages, SC, Brazil.

Paulo César Tavares de Melo, Escola Superior de Agricultura Luiz de Queiroz, Universidade de São Paulo, Piracicaba, SP, Brazil.

Christian Ulrichs, Division Urban Plant Ecophysiology, Faculty of Life Sciences, Humboldt-Universität zu Berlin, Germany.

Helber Enrique Balaguera-López, Faculty of Agricultural Sciences, Universidad Pedagógica y Tecnológica de Colombia, Tunja, Colombia.

SCIENTIFIC COMMITTEE

Agim Ballvora, INRES-Plant Breeding and Biotechnology, University of Bonn, Germany.

Yoav Bashan, Environmental Microbiology and Biotechnology, Northwestern Center for Biological Research (CIBNOR), La Paz, Mexico.

Carmen Büttner, Division Phytomedicine, Faculty of Life Sciences, Humboldt-Universität zu Berlin, Germany.

Daniel G. Debouck, Genetic Resources Unit, International Center for Tropical Agriculture (CIAT), Cali, Colombia.

Derly J. Henriques da Silva, Departamento de Fitotecnia, Universidade Federal de Viçosa, Viçosa, MG, Brazil.

Idupulapati Rao, International Center for Tropical Agriculture (CIAT), Cali, Colombia.

Manuel Talón, Instituto Valenciano de Investigaciones Agrarias (IVIA), Moncada, Valencia, Spain.

Lorenzo Zacarías, Instituto de Agroquímica y Tecnología de Alimentos (IATA-CSIC), Burjassot, Valencia, Spain.

© Universidad Nacional de Colombia
Faculty of Agricultural Sciences

Publication registered at the Ministerio de Gobierno
Resolution No. 00862 of March 24, 1983

Information, correspondence, subscription and exchange:

Revista Agronomía Colombiana
Faculty of Agricultural Sciences,
Universidad Nacional de Colombia
P.O. Box 14490, Bogota-Colombia
Phone: (571) 316 5355 / 316 5000 ext. 10265
Fax: 316 5176
E-mail: agrocol_fabog@unal.edu.co

Electronic version available at:
http://www.scielo.org.co
http://www.revistas.unal.edu.co/index.php/agrocol
http://agronomia.unal.edu.co

ISSN: 0120-9965 (Print)
ISSN: 2357-3732 (Online)

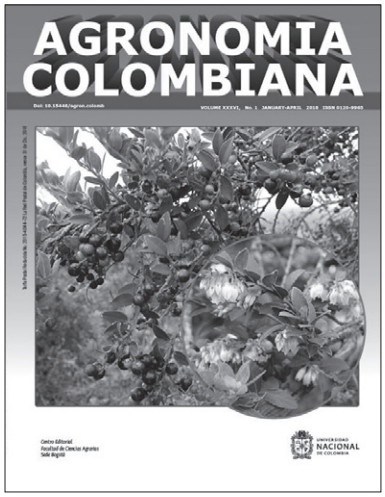
Published: Quarterly
Number of copies: 100

Editorial assistance
Luz Grass Bernal
Ana María Díaz
Stanislav Magnitskiy
César Albarracín

Translator and English proofreader
César Cabra

Electronic Design and Development
Isabel Sandoval

Printer
Digiprint



Our cover:
Effect of different sources of nitrogen on the vegetative growth of Andean blueberry (*Vaccinium meridionale* Swartz)
Article on pages: 58-67

Agronomía Colombiana is a technical-scientific publication classified by Colciencias in category A2 of the Índice Nacional de Publicaciones Seriadas y Científicas y Tecnológicas (Publindex) (Colombia). The journal is indexed in the Scientific Electronic Library Online (SciELO) and Scopus. Internationally, the journal is referenced in Redalyc, Latindex, AGRIS (FAO), ResearchGate, Family Farming Knowledge Platform (*Plataforma de Conocimientos sobre Agricultura Familiar*), and integrated in CABI Full Text and the following databases of CAB-ABSTRACTS: Agricultural Engineering Abstracts, Agroforestry Abstracts, Crop Physiology Abstracts, Field Crop Abstracts, Grasslands and Forage Abstracts, Horticultural Science Abstracts, Irrigation and Drainage Abstracts, Maize Abstracts, Nematological Abstracts, Ornamental Horticulture, Plant Breeding Abstracts, Plant Growth Regulator Abstracts, Postharvest News and Information, Potato Abstracts, Review of Agricultural Entomology, Review of Aromatic and Medicinal Plants, Review of Plant Pathology, Rice Abstracts, Seed Abstracts, Soils and Fertilizers, Sugar Industry Abstracts, Weed Abstracts y World Agricultural Economics and Rural Sociology Abstracts.

The authors of the articles submitted to the journal Agronomía Colombiana must be aware of and avoid scientific misconduct related to: scientific fraud in all or part of the data of the study and data falsification and manipulation; dishonesty due to fictitious authorship or gifting or exchange of co-authorship, duplicate publications, partial or complete, in different journals and self-plagiarism by reusing portions of previous writings; citation omission, citation copying without consultation and excessive self-citation, among others.

Reproduction and quotation of material appearing in the journal is authorized provided the following are explicitly indicated: journal name, author(s) name, year, volume, issue and pages of the source. The ideas and observations recorded by the authors are their own and do not necessarily represent the views and policies of the Universidad Nacional de Colombia. Mention of products or commercial firms in the journal does not constitute a recommendation or endorsement on the part of the Universidad Nacional de Colombia; furthermore, the use of such products should comply with the product label recommendations.

AGRONOMIA COLOMBIANA

VOLUME XXXVI | No. 1 | JANUARY-APRIL 2018 | ISSN (print): 0120-9965 / ISSN (online): 2357-3732

TABLE OF CONTENTS

The Faculty of Agricultural Sciences of the Universidad Nacional de Colombia is pleased to present the first issue of Volume 36, 2018 of the Agronomia Colombiana journal. This issue is composed of 10 articles on various aspects of agricultural and food research, conducted particularly in the Neotropics. The areas covered in this issue are: Plant Breeding, Genetic Resources and Molecular Biology (with the highest number of papers), Crop Protection, Soils, Fertilization and Water Management, Physiology and Postharvest Technology, Economics and Rural Development, and Food Science and Technology.

In the Genetic Improvement section our readers will find a paper about hybrid vigor in *Cucurbita moschata*. Additionally, two studies on characterization, one of a virus and the other of soybean genotypes, are included as advances in molecular biology, although the former is closely related to crop protection.

Two papers on Crop Protection, particularly on Malherbology and Entomology, are included as well. The first paper relates the application of fertilization to an herbicide in carrot. The second focuses mainly on the preference of a thrips species for cotton structures.

The Soils and Fertilization section includes two studies. The first one highlights the role of the boron-zinc interaction on the nutrition of cotton, and the second one focuses on the nitrogen fertilization of a species that has been recently cultivated, *Vaccinium meridionale*.

The Physiology and Postharvest Technology section presents a paper that evaluates the quality of the *Acca sellowiana* fruit, known as feijoa or pineapple guava, produced in the Department of Cundinamarca, Colombia.

The Economy and Rural Development section contributes in this issue with a research on the experience of peasant markets, and how the access of small-scale farmers to market systems and commercialization has been improved by this program.

La Facultad de Ciencias Agrarias de la Universidad Nacional de Colombia se complace en entregar el primer número del volumen 36 de 2018 de la revista Agronomía Colombiana. Este número está compuesto de 10 artículos sobre diversos aspectos de la investigación agraria y alimentaria realizada particularmente en el neotrópico. Las áreas que abarca el presente número son fitomejoramiento, recursos genéticos y biología molecular (con el mayor número de publicaciones), protección de cultivos, Suelos, fertilización y manejo de aguas, Fisiología y tecnología poscosecha, economía y desarrollo rural y ciencia y tecnología de alimentos.

Sobre mejoramiento genético se publica un artículo sobre vigor híbrido en *Cucurbita moschata*. Dos artículos de caracterización, uno en virus y otro en genotipos de soya, se publican como avances en biología molecular, si bien el primero tiene una relación estrecha con protección de cultivos.

Adicionalmente se publican dos artículos sobre protección de cultivos, en particular sobre malherbología y entomología. Uno de ellos relaciona la aplicación de fertilización y un herbicida en zanahoria. El segundo trata de la preferencia de un trip sobre estructuras de la plata de algodón.

En temas relacionados con suelos y fertilización se presentan dos artículos. El primero de ellos poner en relieve el papel de la relación boro-zinc en la nutrición del cultivo del algodón. El segundo se enfoca en la fertilización nitrogenada de una especie que se ha empezado a cultivar recientemente, *Vaccinium meridionale*.

Respecto al tema de tecnología y fisiología poscosecha se presenta un artículo donde se evalúa la calidad del fruto de *Acca sellowiana* conocido como feijoa o guayabo del país, producido en Cundinamarca, Colombia.

Economía y desarrollo rural contribuye en este número con un artículo sobre la experiencia de los mercados campesinos y como a través de ella se ha mejorado el acceso de pequeños productores al mercado y la comercialización.

Finally, the Food Science and Technology section presents a paper that describes the response surface methodology for the fermentation optimization of *Capsicum frutescens*, one of the cultivated *Capsicum* species, along with *C. anuum*, *C. pubescens* and *C. Baccatum,* which are also of American origin.

Finalmente respecto al tema de procesamiento de alimentos, se presenta un artículo que describe la metodología de superficie de respuesta en la optimización de la fermentación de *Capsicum frutescens*, una de las especies de *Capsicum* cultivadas, junto con *C. anuum*, *C. pubescens* y *C. baccatum* también de origen americano.

Mauricio Parra Quijano
Editor en jefe
Revista Agronomía Colombiana

Yield heterosis and average fruit weight as a function of inbreeding in *Cucurbita moschata* Duch. ex Poir.

Heterosis del rendimiento y peso promedio de fruto en función de la endogamia en *Cucurbita moschata* Duch. ex Poir.

Javier Restrepo[1]*, Franco Vallejo[1], and Edwin Restrepo[2]

ABSTRACT

In order to estimate the mean heterosis, mid parent heterosis and heterobeltiosis (HB), three diallel crossings of *Cucurbita moschata* were evaluated, each formed by six parents with three levels of inbreeding (S_0, S_1, S_2). A randomized complete block experimental design was used with four replicates, arranged in split plots. The variables yield per plant (YPP) and average fruit weight (AFW) were analyzed. The hybrids between S_1 or S_2 inbred lines presented heterotic superiority regarding to those between S_0 parents for the variables YPP and AFW. Likewise, the hybrids between S_2 inbred lines reported heterotic superiority in comparison to those among S_1 inbred lines, for such variables. The hybrids between S_2 inbred lines that reported the highest expression levels of HB for YPP were P1xP3, P2xP6 and P1xP2, with values ranging between 131.42 and 98.24%; while the hybrids among S_1 inbred lines that recorded the highest values of HB, for this same variable, were P3xP5 and P1xP5 with values of 191.71 and 139.29%, respectively. Furthermore, the hybrids between S_2 and S_1 inbred lines that registered the highest level of HB for AFW were P1xP3 and P1xP5 with values of 108 and 83.7%, respectively.

Key words: butternut squash, selection, heterobeltiosis, diallel crossing, inbred lines, hybrids.

RESUMEN

Para estimar la heterosis promedia, heterosis relativa y heterobeltiosis (HB) se evaluaron tres cruzamientos dialélicos de *Cucurbita moschata*, conformados cada uno por seis progenitores con tres niveles de endogamia (S_0, S_1, S_2). Se utilizó el diseño experimental de bloques completos al azar con cuatro repeticiones y arreglo en parcelas divididas. Se analizaron las variables producción por planta (PFP) y peso promedio del fruto (PPF). Los híbridos producidos entre líneas endogámicas S_2 o S_1 presentaron superioridad heterótica en comparación a los híbridos formados entre padres S_0 para PFP y PPF. Igualmente, los híbridos entre líneas endogámicas S_2 reportaron para dichas variables superioridad heterótica con respecto a los híbridos entre líneas endogámicas S_1. Los híbridos entre líneas endogámicas S_2 que presentaron los mayores niveles de expresión de HB para PFP fueron P1xP3, P2xP6 y P1xP2 con valores entre 131.42 y 98.24%, respectivamente; mientras que los híbridos entre líneas endogámicas S_1 que registraron los niveles más altos de HB para dicha variable fueron P3xP5 y P1xP5 con 191.71 y 139.29%, respectivamente. Por otro lado, los híbridos entre líneas endogámicas S_2 y S_1 que registraron los niveles más altos de HB para PPF fueron P1xP3 y P1xP5, con valores de 108 y 83.7%, respectivamente.

Palabras clave: zapallo, selección, heterobeltiosis, cruzamientos dialélicos, líneas endogámicas, híbridos.

Introduction

The butternut squash *Cucurbita moschata* (Duch. ex Lam.) Duch. ex Poir. is grown and consumed in the tropical and subtropical regions of the American continent and other places in the world. In Colombia, this is the most cultivated and consumed species of the genus *Cucurbita*, with a planted area and an average production rate of 11,723 ha and 107,839 t, respectively (Minagricultura, 2016). It has a high genetic diversity and its center of domestication is located, possibly, around the northwest region of South America (Restrepo and Vallejo, 2008). It is characterized due to its nutritional, industrial and combustible properties (Restrepo, 2015). Among others, the butternut squash medicinal benefits include anti-carcinogenic (Zhang *et al.*, 2012), anti-diabetic (Chang *et al.*, 2014), anti-oxidant (Wu *et al.*, 2014) and hypolipidemic properties (Zhao *et al.*, 2014).

Heterosis is known as the hybrid vigor expressed in hybrids. It represents the superiority in performance of hybrid individuals compared with their parents (Hallauer *et al.*, 2010). According to the referent used to compare the behavior of the produced hybrid, heterosis can be expressed as three different terms: mid parent, standard variety and better

Received for publication: 13 July, 2017. Accepted for publication: 14 February, 2018 Doi: 10.15446/agron.colomb.v36n1.66312

[1] Facultad de Ciencias Agrarias, Universidad Nacional de Colombia. Palmira (Colombia).
[2] Facultad de Ingeniería, Universidad Pontificia Bolivariana. Palmira (Colombia).
* Corresponding author: jarestrepos@palmira.unal.edu.co

CC BY NC ND

parent heterosis. The standard variety is designated as standard heterosis and the better parent heterosis is better known as heterobeltiosis (Alam *et al*., 2004).

Heterotic superiority has been demonstrated for the yield per plant (YPP) of *C. moschata* on F_1 hybrids produced from inbred lines with different degrees of inbreeding in contrast to the hybrids produced from the crosses between S_0 parents (Espitia *et al*., 2006; Ortiz *et al*., 2013). For the variables YPP and the average fruit weight (AFW), there was a greater magnitude and statistical significance in the estimates of mid parent heterosis (MPH), heterobeltiosis (HB) and standard variety heterosis (STH) in the hybrids between S_1 inbred lines compared to those from S_0 (Espitia *et al*., 2006). In contrast, for these variables a significant decrease in the estimates of mid parent heterosis and heterobeltiosis was recorded in the hybrids between S_1 inbred lines related to the hybrids between S_0 parents (Vallejo and Gil, 1998).

The main objective of this research was to estimate the degree of MPH and HB of the resulting hybrids from three diallel crossings of *Cucurbita moschata*. Each crossing was composed by six progenitors with different degrees of inbreeding (S_0, S_1, and S_2) for the variables YPP and AFW. As a final product, the research results are intended to contribute significantly to the research program "Genetic Improvement, Agronomy and Production of Vegetables Seeds" of the Universidad Nacional de Colombia, Palmira Campus, committed to produce and release butternut squash hybrids to the market. Furthermore, the hypothesis of this study was that the level of inbreeding of parents can influence yield heterosis and average fruit weight in *C. moschata*.

Materials and methods

The research was carried out at the Experimental Center of the Universidad Nacional de Colombia, Palmira Campus (CEUNP, its Spanish acronym), located in Candelaria, department of Valle del Cauca, Colombia (3°25'34.42" N and 76°25'47.57" W, 980 m a.s.l., average annual temperature of 26°C, average annual precipitation of 1,100 mm and average relative humidity of 76%).

Three diallel crosses of pumpkin *C. moschata* were analyzed. Each cross was composed by six parents with varying levels of inbreeding (S_0 parents, S_1 and S_2 inbred lines). The following S_0 parents were selected considering their outstanding features such as size, external fruit color, pulp color and thickness and due to their diverse geographic origin: UNAPAL-Abanico-75-1 (P1), UNAPAL-Abanico-75-2 (P2), UNAPAL-Dorado (P3), an introduction from Costa Rica denominated IC3A (P4), UNAPAL-Llanogrande-1 (P5) and UNAPAL-Llanogrande-2 (P6). In the current research, the S_1 and S_2 inbred lines were produced from S_0 open-pollinated parents previously selected. A weighted selection index that included variables such as AFW (2.0-4.0 kg), pulp thickness (3.5-5.0 cm) and Salmon colored pulp was used to select the fruit. The selection ranges indicated for each variable correspond to optimum fruit values for fresh consumption market, where consumers prefer to buy small and whole fruits instead of sliced fruit. Twenty-one genotypes (six parents and fifteen direct crosses) were evaluated for each diallel cross.

The variables YPP (kg) and AFW (kg) were analyzed. The agronomic evaluation of 63 genotypes of three diallel crossings was carried out during the second semester of 2011. A randomized complete block experimental design with four replicates was used. Field treatments were arranged in split plots, with the main plot corresponding to the diallel cross (level of inbreeding) and the subplot corresponding to the evaluated genotypes (six parents and 15 F_1 hybrids, in each of the diallel crosses). Planting distance was 2.5 m between lines and 3.0 m between plants. Each experimental plot consisted on a furrow of five plants (37.5 m^2), with three central plants set as useful plot area. Genetic and statistical analysis were performed using the method proposed by Hallauer and Miranda in 1981 (Hallauer *et al*., 2010). The statistical model associated with the experimental design for each diallel crossing was:

$$Y_{ijkl} = \mu + g_{ij} + b_k + D_l + (Db)_{lk} + (1/rn)\Sigma\Sigma e_{ijkl},$$

where:

i, j: 1, 2.....p parents; p = 6;

k: 1, 2…...r replicates; r = 4;

μ: population mean of all genotypes;

g_{ij}: effect of genotype ij – th;

b_k: effect of block k - th;

D_l: effect of inbreeding generation l;

$(Db)_{lk}$: effect of interaction of inbreeding generation by blocks;

$(1/rn)\ \Sigma\Sigma e_{ijkl}$: experimental error associated with the observation Yijkl;

Y_{ijkl}: phenotypic average value observed of the variable under study for the genotype (ij) in inbreeding generation l.

Simultaneously, the source of genotypes variation (g_{ij}) was partitioned into three new sources of variation: parents, crosses and the contrast between crosses and parents following the methodology suggested by Hallauer *et al.* (2010). The mid parent heterosis (MPH) and heterobeltiosis (HB) were estimated as follows:

MPH: ratio between the mean value of the particular hybrid (F_1) and the mean value of the two parents for that hybrid (MP), expressed in percentage.

$$MPH = [(F_1 / MP) \times 100] - 100.$$

HB: ratio between the mean value of the particular hybrid (F_1) and the best parent mean value for that hybrid (BP), expressed as a percentage.

$$HB = [(F_1 / BP) \times 100] - 100$$

The two types of heterosis were statistically tested using the Student's T-test. The analysis of variance was performed using the SAS® software (SAS / STAT® package, version 9.4 of the SAS® system for Windows) from SAS Institute Inc. 2012 (Cary, North Carolina, USA). For the estimation of the different types of heterosis, the Excel® 2013 program version 15.0 of Microsoft® Office was used.

Results and discussion

Analysis of variance (ANOVA)

The Mean Squares from the ANOVA for the variables YPP and AFW in three generations of inbreeding of *C. moschata* are presented in Table 1. There were significant differences for YPP and AFW in the sources of variation genotypes and generations, indicating that at least one of the generations or one of the genotypes is different from the others. By partitioning the genotypes source of variation into their components in each generation of inbreeding, we observed for YPP and AFW the existence of significant differences in all sources of variation considered, except for the parental source in the S_0 generation of inbreeding. This exception was due, possibly, to the fact that in this work we used a selection index that included, among other variables, the AFW. Therefore, we selected the six S_0 parents, with similar values for this variable (2-4 kg). This range of selection corresponds to the optimal values of AFW for the fruits commercialization targeted to the fresh consumption market, mainly established by consumers who prefer buying the whole fruit.

Further analysis exposed a statistical significance in YPP and AFW for the parents vs. crosses contrast (P vs. C) in the three generations of inbreeding. This statistical difference showed that the mean performance of all F_1 crosses (between S_0 parents and between S_1 or S_2 inbred lines) was higher than the average performance of their parents as a whole, indicating significant heterotic effects in all three generations for these traits (Tab. 1).

The mean performances of the hybrids produced between six S_0 parents, between six S_1 inbred lines and between six S_2 inbred lines are presented in Table 2. It was observed that on average for YPP, the hybrids between S_2 inbred lines presented higher values compared to the hybrids between S_1 lines or between S_0 progenitors. For the AFW trait, it was found that hybrids between S_1 or S_2 lines presented higher means than those obtained between S_0 parents.

TABLE 1. Mean Squares (MS) from the ANOVA for the variables Yield per plant (YPP) and average fruit weight (AFW) in three generations of inbreeding of *C. moschata*, according to the method of Hallauer *et al.* (2010).

Sources of Variation	DF	Variables	
		YPP (kg)	AFW (kg)
		MS	MS
Replicates (R)	3	3.47	0.73
Generations (D)	2	34.60 *	7.56 **
R*D	6	10.18	1.02 *
Genotypes (G)	60	57.24 **	3.28 **
Genotypes (S_0 G)	20	26.59 **	1.51 **
Parents (P_0)	5	14.22	1.20
Crosses (C_0)	14	16.87 *	1.34 **
P_0 vs. C_0	1	224.54 **	5.55 **
Genotypes (S_1 G)	20	61.43 **	3.71 **
Parents (P_1)	5	31.62 **	1.81 **
Crosses (C_1)	14	44.23 **	2.85 **
P_1 vs. C_1	1	451.29 **	25.13 **
Genotypes (S_2 G)	20	83.70 **	4.62 **
Parents (P_2)	5	21.92	1.15 *
Crosses (C_2)	14	32.21 **	2.44 **
P_2 vs. C_2	1	1113.43 **	52.55 **
Error	180	9.63	0.40
Means		12.28	3.39
CV (%)		25.27	18.72

*, **: significant at 5% and 1% levels of probability, respectively, according to F-test.

In the S_0 generation, the hybrids P4xP5 (15.42 kg/plant) and P1xP3 (15.00 kg/plant) presented the highest mean values for YPP. In the S_1 inbreeding generation, the hybrids P1xP5 (20.50 kg/plant) and P3xP5 (19.08 kg/plant) had the best performances; while in the S_2 generation the hybrid P1xP3 (20.87 kg/plant) was highlighted. The hybrids P1xP5 (S_1

TABLE 2. Mean performance of fifteen hybrids from six S_0 parents, fifteen hybrids from six inbred lines S_1 and fifteen between six inbred lines S_2 of squash *C. moschata* for the variables Yield per plant (YPP) and average fruit weight (AFW).

Hybrids	YPP (kg)			AFW (kg)		
	S_0	S_1	S_2	S_0	S_1	S_2
P1xP2	10.27 bcd	12.08 cde	17.88 abc	2.62 ef	4.19 cdef	4.84 abc
P1xP3	15.00 a	12.32 cde	20.87 a	4.02 ab	3.64 fgh	4.98 a
P1xP4	13.89 ab	14.75 bc	15.33 bcd	3.90 ab	4.15 def	4.53 abc
P1xP5	13.05 abc	20.50 a	15.00 bcd	3.16 cde	5.73 a	3.50 def
P1xP6	10.71 bcd	10.33 def	14.00 de	2.60 ef	3.20 hi	2.89 efg
P2xP3	12.46 abc	17.19 ab	16.00 bcd	3.36 bcd	5.16 ab	4.79 abc
P2xP4	12.04 abcd	13.67 bcde	16.73 bcd	3.21 cde	3.82 fgh	4.22 bcd
P2xP5	12.04 abc	15.38 bc	15.56 bcd	2.69 def	4.86 bcd	4.15 cd
P2xP6	12.25 abcd	14.88 bc	18.47 ab	3.00 cde	4.56 bcde	4.89 ab
P3xP4	14.95 a	10.13 ef	13.73 de	4.28 a	3.32 gh	3.76 d
P3xP5	14.45 a	19.08 a	14.98 bcd	3.53 bc	4.01 efg	3.84 d
P3xP6	13.38 ab	13.08 cde	14.81 cd	3.37 bcd	3.41 gh	3.73 d
P4xP5	15.42 a	13.83 bcd	14.30 cd	3.40 bcd	3.49 fgh	4.17 cd
P4xP6	8.63 d	12.54 cde	10.46 ef	2.95 cde	3.49 fgh	2.91 fg
P5xP6	9.71 cd	8.03 f	9.47 f	2.17 f	2.47 i	2.49 g
Means	12.55	13.85	15.17	3.22	3.97	3.98
CV (%)	16.36	24.01	18.73	17.91	21.18	19.62
LSD (5%)		3.63			0.74	

P1 = Unapal-Abanico-75-1; P2 = Unapal-Abanico-75-2; P3 = Unapal-Dorado; P4: IC3A Central American introduction; P5 = Unapal-Llanogrande-1; P6 = Unapal-Llanogrande-2. Means within a column followed by the same letter are not significantly different, according to least significant differences multiple range test.

generation) and P1xP3 (S_2 generation) were the genotypes that registered the highest AFW mean values (5.73 and 4.98 kg, respectively); thus, they are considered as the most indicated genotypes to enhance the features required by the industry and fresh market consumers (big and whole fruit with no weight limit). Furthermore, regarding the market of fresh consumption conformed by consumers who prefer to purchase small and whole fruits instead of sliced fruit, the most indicated hybrid is P1xP6 (S_2 generation), since this genotype presented the highest mean value of YPP of all those genotypes that registered an optimal AFW value for this market.

Estimates of heterosis

The expression of the mid parent heterosis (MPH) and heterobeltiosis (HB) for YPP showed a greater magnitude and statistical significance in the hybrids of S_2 inbred lines than those obtained from the S_1 inbred lines (Tab. 3). Similar results were reported by Ortiz *et al.* (2013) in *C. moschata*, who found higher values of MPH for YPP in the hybrids of S_2 inbred lines. In turn, the MPH and HB were of greater magnitude and significance in the hybrids of S_1 inbred lines than in those produced from the S_0 parents (Tab. 3). Likewise, Ortiz *et al.* (2013) recorded higher estimates of MPH for YPP in the hybrids of S_1 inbred lines than those of S_0 lines. Furthermore, and regarding *C. moschata*, Espitia *et al.* (2006) found a greater magnitude and statistical significance after calculating the MPH and HB values in the hybrids from S_1 inbred lines compared to those from S_0 parental lines.

In the diallel crossings between S_2 inbred lines for YPP, the MPH varied from 69.35 to 229.02%, thus the statics value varied from zero across the 100% of hybrid lines. In the diallel crosses among S_1 inbred lines, the MPH ranged between 9.31 and 209.25% showing in the 60.00% of the hybrids statics values different from zero. In contrast, for diallel crossings between S_0 parents the MPH only fluctuated from 2.02 to 81.90%, recording 60.00% of the hybrids with statics values different from zero. Regarding the mean heterosis (MH), the estimations in the diallel crosses among S_1 and S_2 inbred lines represented the 46.79 and 182.29%, a higher value, compared to the estimation made in the diallel crossings between S_0 parents, indicating that as the inbreeding progresses, the MH also goes forward gradually (Tab. 3).

TABLE 3. Mid parent heterosis (MPH), heterobeltiosis (HB) and mean heterosis (MH) of F_1 hybrids from diallel crossings between S_0 parents and between S_1 and S_2 inbred lines, in squash *C. moschata* for the variable yield per plant (YPP).

YPP (kg)						
Generation	**S_0**		**S_1**		**S_2**	
F_1 Hybrids	**MPH**	**HB**	**MPH**	**HB**	**MPH**	**HB**
P1xP2	14.16	-7.77	9.31	-10.76	104.38**	98.24**
P1xP3	33.33*	32.06*	63.04*	43.77*	147.43**	131.42**
P1xP4	37.03*	24.68	57.12**	44.48*	76.40**	69.96**
P1xP5	37.67	17.12	185.38**	139.29**	96.50**	66.35**
P1xP6	13.21	-3.88	27.31	20.62	137.96**	55.26*
P2xP3	36.77	9.68	71.20**	26.95*	96.01**	88.79**
P2xP4	50.59*	31.84	15.08	0.92	98.71**	97.34**
P2xP5	80.01**	68.97**	58.98**	13.53	111.31**	83.57**
P2xP6	67.42*	57.55*	40.35*	9.90	229.02**	117.89**
P3xP4	45.91*	31.62	20.99	-0.73	69.35**	64.20**
P3xP5	50.71*	27.21	209.25**	191.71**	112.52**	90.87**
P3xP6	39.80*	17.75	84.16**	70.65**	179.40**	88.64**
P4xP5	81.90**	68.79**	72.82**	35.51	95.77**	71.08**
P4xP6	2.02	-5.56	40.32	22.85	88.29*	25.12
P5xP6	24.53	24.20	19.18	4.67	110.40 *	51.46
MH (%)	40.09		58.85		113.17	

* and **: significant at 5% and 1% levels of probability, respectively, according to the Student's T-test. P1: UNAPAL-Abanico-75-1; P2: UNAPAL-Abanico-75-2; P3: UNAPAL-Dorado; P4: IC3A Central American introduction; P5: UNAPAL-Llanogrande-1; and P6: UNAPAL-Llanogrande-2.

At an individual level, the hybrids among S_2 inbred lines for YPP that registered the highest levels of HB were: P1xP3, P2xP6, P1xP2, P2xP4 and P3xP5, with values of 131.42, 117.89, 98.24, 97.34 and 90.87%, respectively. On the other hand, hybrids between S_1 inbred lines that recorded the highest expression levels of HB for YPP were the P3xP5, P1xP5 and P3xP6 with values of 191.71, 139.29 and 70.65%, respectively; the hybrids among S_0 parents that presented the highest values of HB were P2xP5 and P4xP5 (68.97 and 68.79%, respectively) (Tab. 3). Most of these hybrids were produced by crossings among parents that were developed from accessions collected in distant geographical regions (departments of Cauca and Magdalena in Colombia and Costa Rica). A high genetic differentiation (Fst = 0.17) between the Colombian accessions collected in the departments of Cauca and Magdalena had been reported in a previous study by Restrepo and Vallejo (2008), indicating a high possibility to find important levels of genetic divergence among several parents selected to produce the hybrids of this study. The other factor that must have occurred in hybrids expressing heterosis or hybrid vigor was the existence of unidirectional dominance levels in most of loci that controlled the trait YPP.

Regarding the variable AFW, the expression of the MPH and HB also registered a higher magnitude and significance in the hybrids of S_2 inbred lines than those of the S_1 inbred lines (Tab. 4). Similarly, the hybrids of S_1 inbred lines presented a greater heterotic superiority compared with those of the S_0 parents. According to Espitia *et al.* (2006) in *C. moschata*, AFW values presented a higher magnitude and statics significance in the MPH and HB estimations in the S_1 inbred lines compared to the S_0 parents as well.

In the diallel crosses among S_1 inbred lines for AFW the MPH fluctuated between 10.84 and 122.54%, presenting statics values different from cero in the 73.33% of the hybrids. In the diallel crossings between S_2 inbred lines the MPH varied between 49.96 and 147.00%, showing statics values different from zero in the 100.00% of the hybrids. In contrast, the diallel crosses between S_0 parents the MPH ranged between -8.01 and 35.54%, and only the 46.67% of the hybrids registered values different from zero. The MH value showed a gradual increase in its estimation as long as the inbreeding process continued, in such way that the diallel crosses among S_1 or S_2 inbred lines represented 105.73 and 265.24% more, compared to the estimation made in the diallel crosses between S_0 parents (Tab. 4). Furthermore, the HB in the diallel crossings between S_1 inbred lines ranged between 2.51 and 83.70%, showing static values different from zero in the 46.67% of the hybrids' lines. In the diallel crosses among S_2 inbred lines the HB fluctuated between

TABLE 4. Mid parent heterosis (MPH), heterobeltiosis (HB) and mean heterosis (MH) of F_1 hybrids from diallel crossings between S_0 parents and between S_1 and S_2 inbred lines, in squash *C. moschata* for the variable average fruit weight (AFW).

AFW (kg)						
Generation	**S_0**		**S_1**		**S_2**	
F_1 Hybrids	**MPH**	**HB**	**MPH**	**HB**	**MPH**	**HB**
P1xP2	6.97	-12.40	22.49*	12.47	88.30**	76.06**
P1xP3	29.54*	24.88*	34.37*	16.69	110.90**	108.00**
P1xP4	28.21*	26.11*	31.92*	30.86*	86.35**	83.32**
P1xP5	22.93	5.72	122.54**	83.70**	49.96**	46.45**
P1xP6	-6.34	-13.10	20.12	2.51	60.47**	20.89
P2xP3	31.01*	4.22	71.12**	38.30**	88.73**	74.19**
P2xP4	28.59	3.91	10.84	2.53	61.41**	53.28**
P2xP5	32.80	25.13	68.62**	30.22**	64.94**	50.87**
P2xP6	34.59*	17.33	53.63**	22.23*	147.00**	77.93**
P3xP4	35.54**	32.80**	21.49	4.79	56.83**	52.18**
P3xP5	31.53*	9.71	85.11**	74.32**	66.48**	64.84**
P3xP6	16.74	4.78	51.48**	48.39**	111.00**	60.44**
P4xP5	29.75*	10.06	34.38*	10.23	75.49**	68.66**
P4xP6	4.35	-4.58	29.71*	9.96	75.49**	17.82
P5xP6	-8.01	-15.40	16.80	12.18	75.49*	9.34
MH (%)	21.29		43.80		77.76	

* and **: significant at 5% and 1% levels of probability, respectively, according to the Student's T-test. P1: UNAPAL-Abanico-75-1; P2: UNAPAL-Abanico-75-2; P3: UNAPAL-Dorado; P4: IC3A Central American introduction; P5: UNAPAL-Llanogrande-1; and P6: UNAPAL-Llanogrande-2.

9.34 and 108.00%, showing values significantly different from zero in the 80.00% of the hybrids. Finally, the HB value varied from -15.40 to 32.80% in the diallel crosses between S_0 parents, registering only the 20.00% of the hybrids with statics values different from zero.

The hybrids produced between S_2 inbred lines for AFW that presented the highest levels of HB were: P1xP3, P1xP4, P2xP6, P1xP2 and P2xP3, with values of 108.00, 83.32, 77.93, 76.06 and 74.19%, respectively. On the other hand, hybrids between S_1 inbred lines that registered the highest values of HB for AFW were P1xP5, P3xP5 and P3xP6 with values of 83.70, 74.32 and 48.39%, respectively. The hybrid among S_0 parents that showed the highest expression of HB was P3xP4 with a value of 32.80% (Tab. 4). Most of these hybrids were also formed by crossings between parents that were developed from accessions collected in distant geographic regions (departments of Cauca and Magdalena in Colombia and Costa Rica), indicating that it was very probable to find important levels of genetic divergence as it was mentioned before.

The greater expression of the types of heterosis previously mentioned in the hybrids of S_2 or S_1 inbred lines compared to those produced from the S_0 parental lines found in this research for YPP and AFW, confirmed the hypothesis proposed in this study. Furthermore, the results obtained concur with those reported by Hallauer *et al*. (2010) and Falconer and Mackay (1996), who stated that the progenies of inbred lines express a higher heterosis compared with those expressed by the progenies with a broad genetic base. This higher heterosis condition is only possible if important levels of genetic divergence are present among the parents and there is an existence of unidirectional dominance levels in most of loci that control the trait in the parental lines.

Most of the hybrids produced in this study by crossings among S_0 parents and between S_1 or S_2 inbred lines, presented positive MPH for the variables YPP and AFW. Similar results were obtained in hybrids of *C. moschata* produced from lines with different level of inbreeding for both of the variables by Vallejo and Gil (1998), Espitia *et al*. (2006), Jahan *et al*. (2012), Ortiz *et al*. (2013), El-Tahawey *et al*. (2015), Begun *et al*. (2016), Ahmed *et al*. (2017), and Darrudi *et al*. (2018). In contrast, Du *et al*. (2011) in *C. moschata* for AFW found negative estimations for MPH in most of the hybrids produced among S_8 inbred lines. On the other hand, Li *et al*. (2013) reported hybrids of *C. moschata* produced from inbred lines for AFW, positive and negative MPH values. Pandey *et al*. (2010) in *C. moschata* for AFW registered similar results in the hybrids formed from S_1 inbred lines.

Regarding HB, most of hybrids formed between S_0 parents and among S_1 or S_2 inbred lines, reported also positive values for the variables YPP and AFW. These results concur to those reported in hybrids of *C. moschata* produced between lines with different levels of inbreeding for both of the variables by Mohanty and Mishra (1999), Espitia *et al*. (2006), Jha *et al*. (2009), Ortiz *et al*. (2013), El-Tahawey *et al*. (2015), Begun *et al*. (2016), Ahmed *et al*. (2017), and Darrudi *et al*. (2018). In contrast, Vallejo and Gil (1998) and Jahan *et al*. (2012) obtained negative estimations of the HB for the same variables in the same species. Furthermore, Sirohi *et al*. (2002) reported positive and negative estimations for both variables in the HB value for *C. moschata*. Pandey *et al*. (2010), also found positive and negative values of HB in *C. moschata* for the variable YPP.

Conclusions

A direct relationship between levels of inbreeding and heterotic superiority of hybrids for the variables yield per plant and average fruit weight was established.

The hybrids from S_2 inbred lines that reported the highest expression levels of HB for YPP were P1xP3, P2xP6 and P1xP2, while the hybrids among S_1 inbred lines that recorded the highest values of HB, for this same variable, were P3xP5 and P1xP5.

The hybrids among S_2 inbred lines that registered the highest levels of HB for AFW were P1xP3, P1xP4, P2xP6 and P1xP2, while the hybrids from S_1 inbred lines that showed the highest values of HB for this same variable were P1xP5 and P3xP5.

The genetic materials selected in this study are experimental hybrids with a high potential to be used in the Research Program "Genetic Improvement, Agronomy and Production of Vegetables Seeds" of the Universidad Nacional de Colombia, Palmira campus.

Literature cited

Ahmed, B., M.A.T. Masud, M. Zakaira, M.M. Hossain, and M.A.K. Mian. 2017. Heterosis and combining ability of pumpkin (*Cucurbita moschata* Duch. Ex Poir.). J. Agric. Stud. 5(3), 132-139. Doi: 10.5296/jas.v5i3.11920

Alam, M.F., M.R. Khan, M. Nuruzzaman, S. Parvez, A.M. Swaraz, I. Alam, and N. Ahsan. 2004. Genetic basis of heterosis and inbreeding depression in rice (*Oryza sativa* l). J. Zhejiang Univ. Sci. 5(4), 406-411. Doi: 10.1631/jzus.2004.0406

Begum, F., A.M. Akanda, M.A.T. Masud, M.G. Rasul, and M.A. Islam. 2016. Combining ability and heterosis for PRSV-W resistance in pumpkin (*Cucurbita moschata*). J. Int. Acad. Res. Multidiscip. 4(1), 92-102.

Chang, C.I., C.M. Hsu, T.S. Li, S.D. Huang, C.C. Lin, C.H. Yen, C.H. Chou, and H.L. Cheng. 2014. Constituents of the stem of *Cucurbita moschata* exhibit antidiabetic activities through multiple mechanisms. J. Funct. Foods 10, 260-273. Doi: 10.1016/j.jff.2014.06.017

Darrudi, R., V. Nazeri, F. Soltani, M. Shokrpour, and M.R. Ercolano. 2018. Evaluation of combining ability in *Cucurbita pepo* L. and *Cucurbita moschata* Duchesne accessions for fruit and seed quantitative traits. J. Appl. Res. Med. Aromat. Plants 9, 70-77. Doi: 10.1016/j.jarmap.2018.02.006

Du, X., Y. Sun, X. Li, J. Zhou, and X. Li. 2011. Genetic divergence among inbred lines in *Cucurbita moschata* from China. Sci. Hortic. 127, 207-213. Doi: 10.1016/j.scienta.2010.10.018

El-Tahawey, M.A.F.A., A.M. Kandeel, S.M.S. Youssef, and M.M.M. Abd El-Salam. 2015. Heterosis, potence ratio, combining ability and correlation of some economic traits in diallel crosses of pumpkins. Egypt. J. Plant Breed. 19(2), 419-439. Doi: 10.12816/0011721

Espitia, C.M.M., C.F.A. Vallejo, and G.D. Baena. 2006. Depresión en vigor por endogamia y heterosis para el rendimiento y sus componentes en zapallo *Cucurbita moschata* Duch. Ex Poir. Rev. Fac. Nal. Agron. Medellín 59(1), 3089-3103.

Falconer, D.S. and T. Mackay. 1996. Introduction to Quantitative Genetics. Fourth edition. Longmans Green, Harlow, Essex, UK.

Hallauer, A.R., M.J. Carena, and J.B. Miranda. 2010. Quantitative genetics in maize breeding. Springer, New York, Dordrecht, Heidelberg, London. Doi: 10.1007/978-1-4419-0766-0

Jahan, T.A., A. Islam, M.G. Rasul, M. Mian, and M.M. Haquez. 2012. Heterosis of qualitative and quantitative characters in sweet gourd (*Cucurbita moschata* Duch. ex Poir.). Afr. J. Food Agric. Nutr. Dev. 12(3), 6186-6199.

Jha, A., S. Pandey, M. Rai, D.S. Yadav, and T.B. Singh. 2009. Heterosis in relation to combining ability for flowering behaviors and yield parameters in pumpkin. Veg. Sci. 36 (3 Suppl.), 332-335.

Li, X.Z., Z.W. Liu, L. Sun, X.H. Du, and J.G. Zhou. 2013. The Combining ability analysis and the heterosis performance of pumpkin. Acta Agric. Boreali Sinica. 28(S1), 61-68. Doi: 10.7668/hbnxb.2013.S1.013

Minagricultura. 2016. Red de Información y comunicación del sector agropecuario colombiano. URL: http://www.agronet.gov.co/Documents/AHUYAMA2016.pdf; (accessed 10 May 2018).

Mohanty, B.K. and R.S. Mishra. 1999. Heterosis for yield and yield components in pumpkin (*Cucurbita moschata* Duch. ex. Poir.). Indian J. Genet. Pl. Br. 59(4), 505-510.

Ortiz, S., F.A. Vallejo, G.D. Baena, E.I. Estrada, and M.P. Valdes. 2013. Zapallo para consumo en fresco y fines agroindustriales investigación y desarrollo. Universidad Nacional de Colombia-Sede Palmira-Facultad de Ciencias Agropecuarias. Palmira, Colombia.

Pandey, S., A. Jha, S. Kumar, and M. Rai. 2010. Genetics and heterosis of quality and yield of pumpkin. Indian J. Hort. 67(3), 333-338.

Restrepo, J.A. 2015. Heterosis y habilidad combinatoria en función de la endogamia para el rendimiento y características del fruto

de zapallo *Cucurbita moschata* Duch. ex Poir. Tesis Doctoral en Ciencias Agrarias-Mejoramiento Genético Vegetal. Universidad Nacional de Colombia-Sede Palmira-Facultad de Ciencias Agropecuarias. Palmira, Colombia.

Restrepo-Salazar, J.A. and F.A. Vallejo-Cabrera. 2008. Caracterización molecular de introducciones Colombianas de Zapallo *Cucurbita moschata*. Acta Agron. 57(1), 9-17.

Sirohi, P.S., Y.S. Reddy, and T.K. Behera. 2002. Heterosis for yield and its attributing traits in pumpkin (*Cucurbita moschata* Duch. ex Poir.). Veg. Sci. 29(2), 178-179.

Vallejo, C.F.A. and M.O.V. Gil. 1998. Efecto de la endocría sobre algunos caracteres agronómicos del zapallo, *Cucurbita moschata* Poir. Acta Agron. 48(1/2), 46-50.

Wu, H., J. Zhu, W. Diao, and C. Wang. 2014. Ultrasound-assisted enzymatic extraction and antioxidant activity of polysaccharides from pumpkin (*Cucurbita moschata*). Carb. Pol. 113, 314-324. Doi: 10.1016/j.carbpol.2014.07.025

Zhang, B., H. Huang, J. Xie, C. Xu, M. Chen, C. Wang, A. Yang, and Q. Yin. 2012. Cucurmosin induces apoptosis of BxPC-3 human pancreatic cancer cells via inactivation of the EGFR signaling pathway. Oncol. Rep. 27, 891-897. Doi: 10.3892/or.2011.1573

Zhao, X.H., L. Qian, D.L. Yin, and Y. Zhou. 2014. Hypolipidemic effect of the polysaccharides extracted from pumpkin by cellulase-assisted method on mice. Int. J. Biol. Macromol. 64, 137-138. Doi: 10.1016/j.ijbiomac.2013.12.001

Molecular characterization of Potato virus Y (PVY) and Potato virus V (PVV) isolates naturally infecting cape gooseberry (*Physalis peruviana*) in Antioquia, Colombia

Caracterización molecular de aislamientos del virus Y (PVY) y virus V (PVV) de la papa infectando naturalmente uchuva (*Physalis peruviana*) en Antioquia, Colombia

Natalia Álvarez[1], Helena Jaramillo[1], Yuliana Gallo[1,2], Pablo Andrés Gutiérrez[1], and Mauricio Marín[3*]

ABSTRACT

Due to the increase of the international demand for functional fruits, cape gooseberry (*Physalis peruviana*) has become one of the crops of highest expansion in Colombia and the Andean region of South America. Unfortunately, the emergence of fungal and unidentified viral diseases has slowed down the cultivation of cape gooseberry in Colombia and, particularly, in the department of Antioquia. In this work, a next-generation sequencing virome analysis of cape gooseberry plants from eastern Antioquia was performed, using leaves exhibiting symptoms such as mosaics, leaf deformation and greening of veins. The complete genomes of Potato virus Y (PVY) and Potato virus V (PVV) were obtained in the assembled data. The presence of both viruses was confirmed in the samples obtained at two commercial cape gooseberry fields by real time RT-PCR (RT-qPCR) and partial Sanger sequencing of the coat protein (CP). Sequence analysis revealed significant sequence similarity between PVY and PVV isolates infecting *P. peruviana* to previously identified strains infecting potato (*Solanum tuberosum* and *S. phureja*) and tomato (*Solanum lycopersicum*) in the same geographical region. This study suggests that cape gooseberry could be an alternate host to viruses of other economically important solanaceous crops in the Andean region of South America.

Key words: genomics, potyviruses, *Solanaceae*, virus diseases.

RESUMEN

Dado el incremento en la demanda internacional por frutas con características funcionales, la uchuva (*Physalis peruviana*) ha sido uno de los cultivos con mayor expansión en los últimos años en Colombia y otros países suramericanos. Desafortunadamente, la emergencia de enfermedades micóticas y virales ha reducido dichos planes de siembra, especialmente en el departamento de Antioquia. En este trabajo, se realizó un análisis de secuenciación de nueva generación del viroma asociado a tejidos foliares de plantas de uchuva del oriente antioqueño, con síntomas de mosaicos, deformación de brotes y verdeamiento de venas. Los genomas completos de los virus Y (PVY) y V (PVV) de la papa fueron obtenidos a partir de las secuencias ensambladas. La presencia de ambos virus fue confirmada en dos cultivos comerciales de uchuva, utilizando RT-PCR en tiempo real y secuenciación Sanger de una porción del gen de la cápside. Los análisis filogenéticos de dichas secuencias revelaron la existencia de altos niveles de similitud entre los aislamientos de PVY y PVV obtenidos en uchuva y cepas previamente identificadas para esta misma región geográfica en cultivos de papa (*Solanum tuberosum* y *S. phureja*) y tomate (*Solanum lycopersicum*). Este estudio sugiere que las plantas de uchuva pueden servir como hospedantes alternos de virus de importancia económica en cultivos de solanáceas de la region Andina de Suramérica.

Palabras clave: enfermedades virales, genómica, potyvirus, *Solanaceae*.

Introduction

Cape gooseberry (*Physalis peruviana* L.) is a solanaceous fruit crop native to the South American Andes that has recently become one of the most promising agricultural export trades in Latin American countries such as Colombia, Ecuador and Perú (Fisher *et al.,* 2014). The cape gooseberry plant produces a fruit with excellent nutritional properties, which is also a good source of phosphorous, dietary fiber and vitamins A, B and C (Ramadan, 2011). Due to its high content of phenolic acids, flavonoids and other bioactive compounds with antibacterial, anti-inflamatory, anti-tumorigenic and antioxidant properties, *P. peruviana* is also considered to be a functional fruit (Wu *et al.,* 2005; Ramadan, 2011). In Colombia, the cape gooseberry crop comprises a total cultivated area of 952 ha with an estimated

Received for publication: 17 May, 2017. Accepted for publication: 16 February, 2018 Doi: 10.15446/agron.colomb.v36n1.65051

[1] Laboratory of Industrial Microbiology, Faculty of Sciences, Universidad Nacional de Colombia. Medellín (Colombia)
[2] Faculty of Medicine, Universidad CES, Medellín (Colombia)
[3] Laboratory of Cellular and Molecular Biology, Faculty of Sciences, Universidad Nacional de Colombia. Medellín (Colombia).
* Corresponding author: mamarinm@unal.edu.co

CC BY NC ND

yield of about 13,260 t per year; the departments of Boyacá, Antioquia and Cundinamarca are the main producers, accounting for 58.4, 17.4 and 17.5 percent of the national production (Agronet, 2016). Recently, the production of cape gooseberry has declined from 13.76 t ha^{-1} yr^{-1} in 2010 down to 9.81 t $ha^{-1}yr^{-1}$ in 2014 (Agronet, 2016). This drop has been attributed to several factors, which include climate change, the increase in the incidence and severity of fungal diseases caused by *Fusarium oxysporum* and *Phoma* sp. (Fisher *et al.,* 2014) and the infection by several viruses inducing chlorosis, mosaics, leaf deformation, dwarfism and greening of veins (Zapata *et al.,* 2005; Aguirre *et al.,* 2014; Gutiérrez *et al.,* 2015; Rodríguez *et al.,* 2016).

P. peruviana can be host to a wide range of viruses such as tobamovirus (Tobacco mosaic virus, TMV) (Capoor and Sharma, 1965; Gómez *et al.,* 1997), polerovirus (Potato leafroll virus, PLRV) (Natti *et al.,* 1953), cucumovirus (Cucumber mosaic virus, CMV) (Chamberlain, 1939; Gupta and Singh, 1996; Daza and Rodríguez, 2006), potexvirus (Potato virus X, PVX) (Horvath, 1970; Zapata *et al.,* 2005; Gutiérrez *et al.,* 2015), crinivirus (Tomato chlorosis virus, ToCV) (Trenado *et al.,* 2007), tospovirus (Tomato chlorotic spot virus, TCSV and Tomato spotted wilt virus, TSWV) (Da-Graça *et al.,* 1985; Eiras *et al.,* 2012), several potyviruses (Peru tomato mosaic virus, PTV; Colombian datura virus, CDV; Potato virus Y, PVY and Bean yellow mosaic virus, BYMV) (Horvath, 1970; Salamon and Palkovics, 2005; Kaur *et al.,* 2014; Kisten *et al.,* 2016; Cutler *et al.,* 2018) and the viroid Potato spindle tuber viroid (PSTVd) (Hadidi *et al.,* 1976; Verhoeven *et al.,* 2010). The capacity of *Physalis* species to serve as virus hosts was investigated by Horváth (1996), who demonstrated the susceptibility of *P. alkekengi* to 10 viruses, *P. ixocarpa* to 14 viruses and *P. pubescens* to two viruses. This work also showed that *P. peruviana* is systemically susceptible to Alfalfa mosaic virus (AMV), Potato aucuba mosaic virus (PAMV) and PVY, and locally susceptible to Tobacco rattle virus (TRV). The role of *P. peruviana* as an alternate host of viruses affecting economically important crops has been demonstrated for PVY, PSTVd, ToCV in tomato (*Solanum lycopersicum*) (Trenado *et al.,* 2007; Verhoeven *et al.,* 2009; Kisten *et al.,* 2016) and CDV in tobacco (*Nicotiana tabacum*) (Salamon and Palkovics, 2005).

In the Andean region of Colombia, cape gooseberry is frequently inter-cultivated with other solanaceous crops, such as tamarillo (*S. betaceum*), tomato, bell pepper (*Capsicum annuum*) and potato (*S. tuberosum* and *S. phureja*) and can also grow as a weed within these crops (Fischer *et al.,* 2014). A recent next generation sequencing (NGS) study suggested that *P. peruviana* could be a natural reservoir host of PVX (Gutiérrez *et al.,* 2015); in this work, the role of cape gooseberry as an alternative virus host to other solanaceous crops in the municipality of La Unión (Antioquia) was furtherly investigated using NGS and RT-qPCR tests on cape gooseberry plants exhibiting mosaics, leaf deformation and greening of veins.

Materials and methods

Sample collection

Ten samples were collected at two commercial cape gooseberry plots in the municipality of La Unión (Antioquia) (5°58′22′′ N, 75°21′40′′ W and 2500 m a.s.l.), where some plants exhibited typical symptoms of viral infection. In the first plot, rugose mosaic and leaf deformation symptoms were detected in leaves; in the second plot, rugose mosaics and greening of veins were observed (Fig. 1). Six

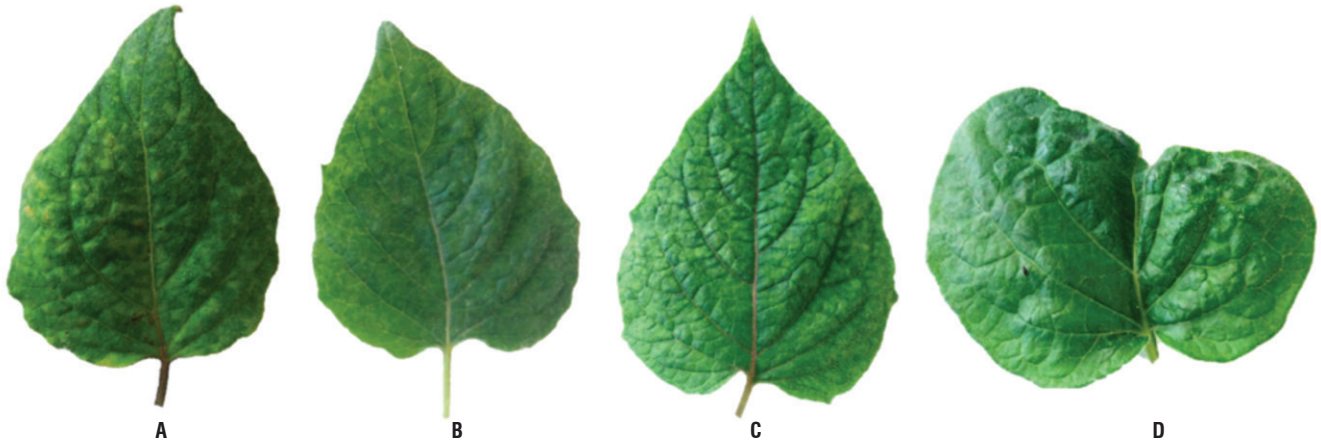


FIGURE 1. Symptoms of viral infection in the cape gooseberry leaves observed in this work. Rugose mosaics (**A** and **B**), greening of veins (**C**) and leaf deformation (**D**).

additional asymptomatic leaf samples were included for the RT-qPCR tests.

Next generation sequencing

High-throughput sequencing of the *P. peruviana* transcriptome was performed on a bulked sample of symptomatic leaves. The total RNA was extracted by the Trizol method (Chomczynski, 1993) following the manufacturer's instructions (ThermoFisher Scientific, Waltham, MA, USA) and the integrity was determined with a 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). The ribosomal RNA was removed with the TruSeq Stranded Total RNA with a Ribo-Zero Plant kit (Illumina, San Diego, CA, USA). The TruSeq Stranded Total RNA LT Sample Prep Kit (Illumina, San Diego, CA, USA) was used for the preparation of cDNA libraries and the ligation of adapters. Sequencing was performed with the Illumina HiSeq 2000 system service provided by Macrogen (South Korea).

Once the sequencing data was obtained, adapter sequences and low quality bases (Phred score < 30) were removed from the dataset with SeqTK v.r82 (GitHub, 2015). Sequences were assembled with Trinity (Grabherr *et al.,* 2011) and viral contigs were identified with a local BLASTN (Gish and States, 1993) search against a database containing all plant virus species currently accepted by the International Committee on Taxonomy of Viruses (ICTV). Genome assemblies were verified by mapping them against the reads with Bowtie2 (Langmead and Salzberg, 2012) and checked for inconsistencies and assembly artifacts with Tablet (Milne *et al.,* 2010). Protein coding regions were annotated using BLASTX (Gish and States, 1993) against reference PVV (NC_004010) and PVY (NC_001616) genomes. Putative protease cleavage sites were confirmed by comparison to published P1, HC-Pro and NIa-Pro consensus cleavage sites (Adams *et al.,* 2005). The complete genome sequences were deposited in GenBank under accession codes KY711363 and KY711364 with PVY_physalis and PVV_physalis as isolate names, respectively.

RT-qPCR tests

Primer specificity was first evaluated in bulk samples comprising either symptomatic (SL1 or SL2) or asymtomatic (AL1 and AL2) leaves collected at each plot. For these bulk samples, the total RNA was extracted from 100 mg of ground tissue with the GeneJET Plant RNA Purification kit following the manufacturer´s protocol (ThermoFisher Scientific, Waltham, MA, USA) and eluted in 40 µL of DEPC treated water. Purity and concentration were determined by absorbance readings at 260 and 280 nm using a Nanodrop 2000C (ThermoFisher Scientific, Waltham, MA, USA).

Synthesis of cDNA and RT-qPCR were performed using the method reported by Muñoz-Escudero *et al.* (2016a). PVY was detected with the primers PVY-1 FP (5´-CCAATCGTTGAGAATGCAAAAC-3´) and PVY-1 RP (5´-ATATACGCTTCTGCAACATCTGAGA-3´) (Singh *et al.,* 2013) after amplifying a 74 bp segment of the CP region. The primers PVV_phu_F (5'-ATGCTGGAAAAGATCCAGC-3') and qPVV_phu_R (5'-CATCCCGCTCCTCAAC-3') were used to target an 89 bp region of CP for PVV (Álvarez *et al.,* 2016).

After primer validation, each leaf sample was tested individually by Immunocapture Real-Time RT-PCR (IC-RT-qPCR) using an antigen-coated ELISA plate (ACP-ELISA, SRA 27200/0096) containing the PTY 1 monoclonal antibody for a generic detection of the potyvirus. Positive (LPC 27200) and negative (LNC 27200) controls were purchased from Agdia (Elkhart, IN, USA). Absorbance was measured at 405 nm in a Multiskan plate reader (ThermoFisher Scientific, Waltham, MA, USA). Samples were considered positive for ELISA when the absorbance value has higher than the cut-off value defined by the formula: Cut-off = (average OD405 + 3 s.d.) x 1.1 as recommended by Bioreba (Reinach, Switzerland).

For the RT-qPCR step, virus particles were released from the ELISA plate with 70 µl of a 10 mM Tris-HCl buffer (pH 8.0) containing 1% Triton X 100 and incubated at 70°C for 10 min (Wetzel *et al.,* 1992). RT-qPCR reactions included a negative control lacking template cDNA and a positive control containing cDNA from infected potato leaf tissue. Samples were considered positive after exhibiting fluorescence values higher than the threshold before the 35th cycle. Amplicon specificity was verified by High Resolution Melting (HRM) in the 50 and 99°C range and confirmed by Sanger sequencing of three samples plus the positive control.

To confirm the phylogenetic affinity of PVY and PVV isolates from *P. peruviana*, the RT-PCR amplification was performed on three positive RT-qPCR samples using primers to target the CP region. These amplicons were sequenced afterwards. The RT-PCR reaction was performed following the procedure reported by Henao-Díaz *et al*. (2013) with the primers PVYCPF (5´-ACCATCAAGSAAATGACACA-3´) and PVYCPR (5´-CGGAGAGACACTACATCACA-3´) (Glais *et al.,* 2002) for PVY. For PVV, the primers PVV_phu_F and PVV_phu_R

(5'-TGAAAGTGGGCTTTGCG-3') were used instead (Álvarez *et al.,* 2016). In each case, amplicons of the expected size were obtained (PVY: 801 pb; PVV: 459 pb). Samples were gel purified using the QIAquick Gel Extraction kit (Qiagen, Hilden, Germany) and sequenced at Macrogen using an ABI Prism 3730xl sequencer (PE Applied Biosystems, Foster City, CA, EEUU). PVY and PVV CP partial sequences were deposited in GenBank under accesion codes KY711356-62.

Phylogenetic analyses

Phylogenetic trees using the polyprotein coding segments of PVY and PVV were inferred by the Maximum Likelihood method using the General Time Reversible model (Nei and Kumar, 2000) and were modelled with a discrete Gamma distribution with 5 categories plus invariable sites and a gamma parameter of 1.02. The phylogenetic analysis using the polyprotein coding segments of PVY and PVV was inferred by the Maximum Likelihood method based on the Tamura-Nei model (Tamura and Nei, 1993) with a discrete Gamma distribution of 5 categories plus invariable sites and a gamma parameter of 1.42. Positions with less than 95% site coverage were eliminated in each case. Nucleotide substitution models were selected with MODELTEST (Posada and Crandall, 1998) and sequences were aligned with MUSCLE (Edgar, 2004). Evolutionary analyses were conducted in MEGA6 (Tamura *et al.,* 2013).

Results and discussion

Next generation sequencing

Sequencing of the *P. peruviana* transcriptome resulted in a paired-end library of 13,420,698 reads (101nt/read) for a total of 1,355,490,498 sequenced nucleotides. Two potyviruses, Potato virus Y (PVY) and Potato virus V (PVV) were identified in the assembled data. The PVY contig (PVY_physalis) comprised 9,675 nt and had an average sequence depth of 1,683x (Fig. 2A). A total of 219,155 reads were mapped after the PVY assembly for an abundance of 1,679 reads per kilobase per million reads (RPKM). Forty one polymorphic sites (39 transitions and 2 transversions) were identified in the assembled genome. The sequence identified as PVV (PVV_physalis) corresponded to a contig of 9,832 nucleotides assembled from 108,629 reads (823 RPKM) and an average sequence coverage of 1,091x (Fig. 3A). In contrast to PVY_physalis, the PVV_physalis assembly did not contain any polymorphic sites.

Characterization of the PVY_physalis isolate

The ORF encoding the potyviral polyprotein in PVY_physalis was identified at the nucleotide positions 169-9,354, and corresponds to a protein of 3,061 a.a. (Fig. 2A). The PVY_physalis polyprotein is cleaved into a ten mature proteins product by the action of potyviral proteases. The P1 and HC-Pro cleavage sites were identified at positions 284 (RRMVQF/S) and 740 (KHYRVG/G), respectively. P3, 6K1, CI, 6K2, VPg, NIa-Pro, NIb and CP are cleaved by NIa-pro at the positions 1,105, 1,157, 1,791, 1,843, 2,031, 2,275 and 2,794, respectively, These are the sites with the consensus sequence V-x-[HE]-[QE]/[AGSR] (Revers and García, 2015). The GA_7T sequence inducing frameshift protein product P3N-PIPO (247 a.a.) was identified at the nucleotide position 2,899 within the P3 segment (Chung *et al.,* 2008) (Fig. 2A). Six nucleotide polymorphisms in the PVY_physalis assembly were translated into amino acid changes within the HC-Pro (T326A, I335M), 6K1 (V1118I), VPg (G1944S, H1964N), and CP (A2809E) segments. A BLASTN search against the complete nucleotide collection at GenBank revealed that PVY_physalis is closely related to the isolates LaUnionT (99.3%, KX531041), mar7 (99.1%, KR270797), VarA (98.7%, KT290511) and VarB (96.7%, KT290512) that are infecting *S. tuberosum* and *S. lycopersicum* in the department of Antioquia (Muñoz-Baena *et al.,* 2016; Muñoz-Escudero *et al.,* 2016a, b).

A phylogenetic analysis of complete PVY genomes clustered the sequences into well-defined clades corresponding to the strains PVY^{N}, PVY^{C}, PVY^{O} and PVY^{NP} plus the recombinant strains PVY^{NTN} and $PVY^{N:O/N-Wi}$ (Fig. 2B). PVY_physalis was part of a clade that includes some of the Colombian isolates identified using BLAST. This clade is sister to a group comprising isolates RRA-1, SASA-61, NTNHO90, NTND6 and NTNO92, which are non-recombinant PVY strains, but some of them have shown to induce the tuber necrotic ringspot disease in potato (Lorenzen *et al.,* 2006; Ogawa *et al.,* 2008). PVY_physalis is clearly different from recombinant PVY isolates identified in potato crops in northern (Yarumal_varB) and eastern Antioquia (La_Union) (Muñoz-Escudero *et al.,* 2016a, b). A phylogenetic analysis using partial CP sequences revealed a similar topology for the complete genome tree with some clades collapsing as a result of recombination as shown in a previous research (Karasev and Gray, 2013). The PVY_physalis is different to isolate PVY-KZNU from South Africa, and it was identified in *P. peruviana* plants exhibiting mottling, mosaic, and chlorosis symptoms on a tomato farm moderately infested with cape gooseberry weeds (Kisten *et al.,* 2016). The PVY-KZNU was identified as a recombinant PVY^{C} strain with spliced PVY^{O}-type RNA fragments in the coat protein region (Kisten *et al.,* 2016), a result that agreed with the phylogenetic analysis performed in this research (Fig. 2C). The identification of

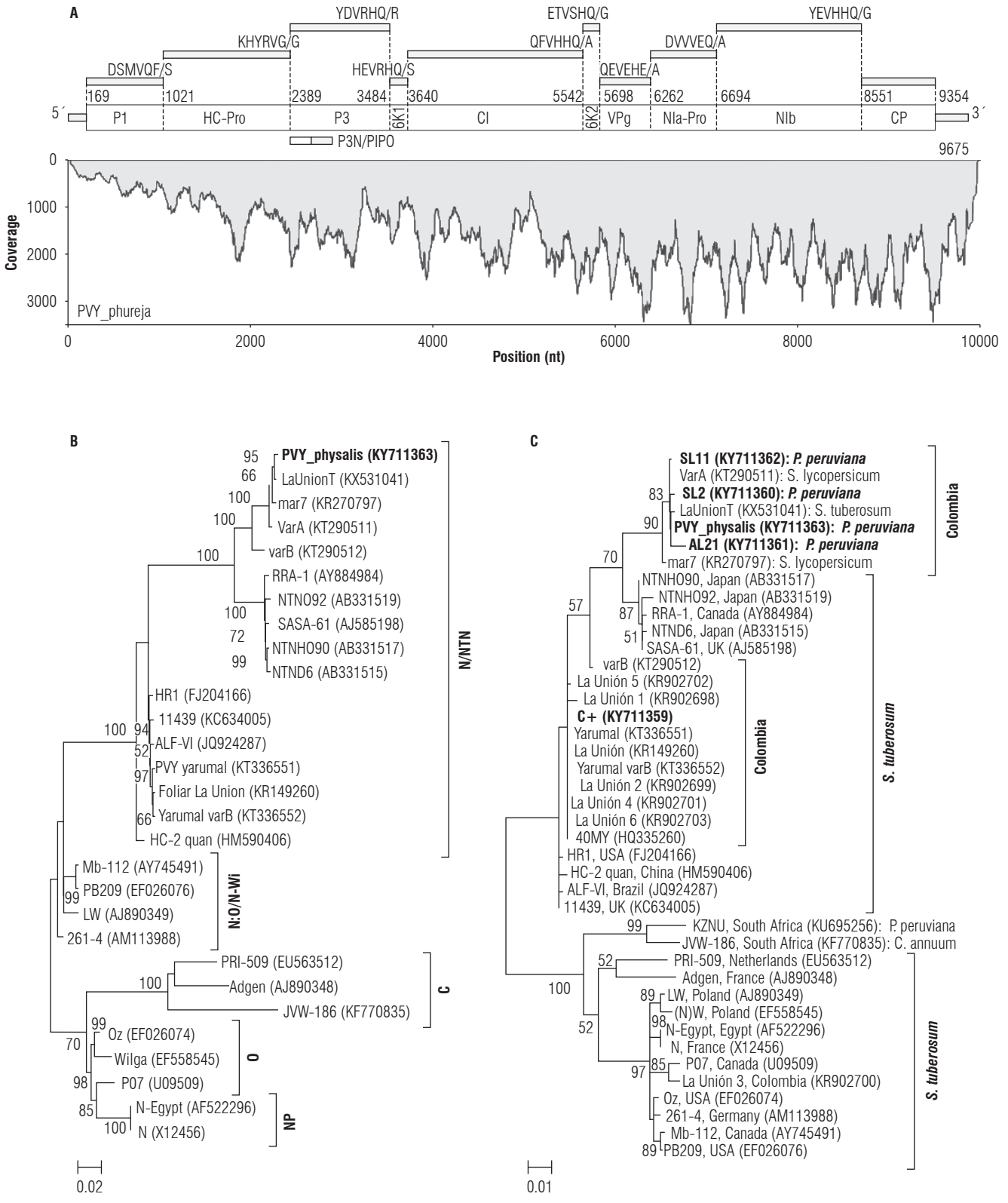


FIGURE 2. Genome annotation of the PVY_phureja genome and the phylogenetic relationship to other PVY isolates. **A)** Sequence coverage of PVY_phureja assembly and location of some important molecular features. The relative position of each mature protein with their corresponding protease cleavage sites is shown. Phylogenetic analysis of PVY isolates using complete (**B**) and partial CP (**C**) sequences confirms the close relationship between PVY_physalis and non-recombinat PVY isolates infecting *S. lycopersicum* and *S. tuberosum* in eastern Antioquia. Trees are drawn to scale with branch lengths in units of number of base substitutions per site as indicated in the bar at the bottom. Bootstrap values are shown above the branches.

these different PVY variants clearly demonstrates that *P. peruviana* can serve as a reservoir to PVY strains infecting other solanaceous crops of economic importance such as tomato and potato. So far, and according to different sources, this genome represents the first complete PVY sequence naturally infecting *P. peruviana*.

Characterization of PVV_physalis

The PVV_physalis polyprotein (3,065 a.a.) was encoded at nucleotide positions 186-9,383. P1 and HC-Pro cleavage sites were identified at positions 289 (RRMVQF/S) and 745 (IKHRVG/G), respectively. NIa-Pro cleavage sites contained the same V-x-[HE]-[QE]/[AGSD] motif observed in PVY_physalis and were located at the amino acid positions: 1102 (P3), 1,154 (6K1), 1,788 (CI), 1,840 (6K2), 2,028 (VPg), 2,274 (NIa-Pro) and 2,793 (NIb). P3N-PIPO (231 a.a.) is predicted to result from the frameshifting at the GA_7C sequence at the nucleotide position 2,907 with the P3 coding region (Chung *et al.,* 2008) (Fig. 3A). The closest homologs to PVV_physalis in GenBank are the PVV isolates La Union_varA (99.9%, KT985458), phureja (99.8%, KP849483) and La Union_varB (99.6%, KT985459) infecting *S. phureja* in Antioquia (Álvarez *et al.,* 2016; Gutiérrez *et al.,* 2016), isolate KER.LAL.P (83.6%, KC433411) from Iran and isolate DV 42 (83.0%, AJ243766) from Scotland (Oruetxebarria *et al.,* 2000; Shamsadden-Saeed *et al.,* 2014). A comparison of PVV polyproteins reveals two aminoacid changes unique to PVV_ physalis. In the first change, at the position 1,393 within the CI, a serine residue replaced a threonine/alanine observed in the PVV isolates infecting potato; this was followed by a second change at the position 2,903 within the CP, where a glutamic acid observed in the PVV strains infecting potato was replaced by lysine in the PVV_physalis.

A phylogenetic analysis of PVV genomes confirms that the PVV_physalis isolate has higher affinity to the PVV^{phu} lineage infecting *S. phureja* in eastern Antioquia and, more distantly, to the PVV isolates DV42 and KER.LAL.P infecting *S. tuberosum* in Eurasia (Scotland and Iran). Both clades are cleary differentiated and supported by a 100% bootstrap (Fig. 3B). The phylogenetic analysis of the partial CP sequences was in agreement with the complete genome analysis. Again, the CP sequences isolated from *P. peruviana* (PVV_physalis, SL1 and SL2) formed a distinct clade, along with the sequences infecting *S. phureja* (PVV_phureja) (Fig. 3C). All these sequences have been isolated in the municipality of la Unión in eastern Antioquia. The *S. phureja* / *P. peruviana* group is sister to a divergent PVV isolate identified in *S. tuberosum* cv. Papa Amarillo, which has distinct serological properties from other isolates infecting *S. tuberosum* (Shiel *et al.,* 2004).

The PVV^{phu} genetic lineage was first identified in a 454 GS-FLX pyrosequencing study of *S. phureja* root tissue in Colombia (Gutiérrez *et al.,* 2014) and its existence was confirmed with follow up genome sequencing studies (Álvarez *et al.,* 2016; Gutiérrez *et al.,* 2016). The PVV^{phu} lineage was originally believed to be exclusive to *S. phureja,* but its detection here in *P. peruviana* suggests that this lineage might be present in a wider range of hosts. Finally, a recently discovered PVV strain, TamarilloEc, was found to infect tamarillo (*S. betaceum*) in Ecuador (Insuasti *et al.,* 2016) and proposed to be the first PVV isolate infecting a host different from potato; our analysis contradicts this claim as PVV_TamarilloEc seems to be more closely related to the Ecuatorian rocoto virus than to any member of the PVV group. We believe that this strain has been misclasified and should be renamed.

Detection of PVV and PVY by RT-qPCR

Infection of *P. peruviana* by PVY and/or PVV was confirmed by RT-qPCR using specific primers for each species. In a preliminary experiment, the amplification reaction was performed on total RNA extracted from bulks containing either symptomatic (SL1 and SL2) or asymptomatic (AL1 and AL2) samples from each plot (L1 and L2) (Tab. 1). PVY was detected in all four samples with Ct values between 9.97 and 27.12 and very similar melting temperatures (77.5±0.5°C). A Ct of 13.39 was observed in the potato sample used as positive control with slightly lower Tm (76.48), (Fig. 4A). Individual amplification reactions using IC-RT-PCR confirmed the PVY results using the bulked samples. In this case, all samples tested positive, with higher Ct values (26.55-32.47) but with the same distribution of Tm (77.5±0.5°C). Regarding symptoms, leaves exhibiting mosaics (S1-L1 and S1-L2) had the lowest Ct values (26.55-27.52), followed by the samples with mottling (S2-L1, Ct=28.60) and greening of veins (S2-L2, Ct= 28.75). As expected, the highest Ct values were observed in the majority of asymptomatic samples (28.30-32.47) (Tab. 1). Previous work on PVY infecting potato (Medina *et al.,* 2016) and tomato (Muñoz-Baena *et al.,* 2016) in Colombia by RT-qPCR using the same primers reported in this research also resulted in similar Tm values (77.5°C±0.5°C). The identity of RT-qPCR amplification product was confirmed by the Sanger sequencing of four samples which were identical to the CP region of PVY isolates from Colombia and Cuba isolated from potato, tamarillo, tomato and pepper (KT290511, JF939837, HQ335262, HQ335245, KY050811).

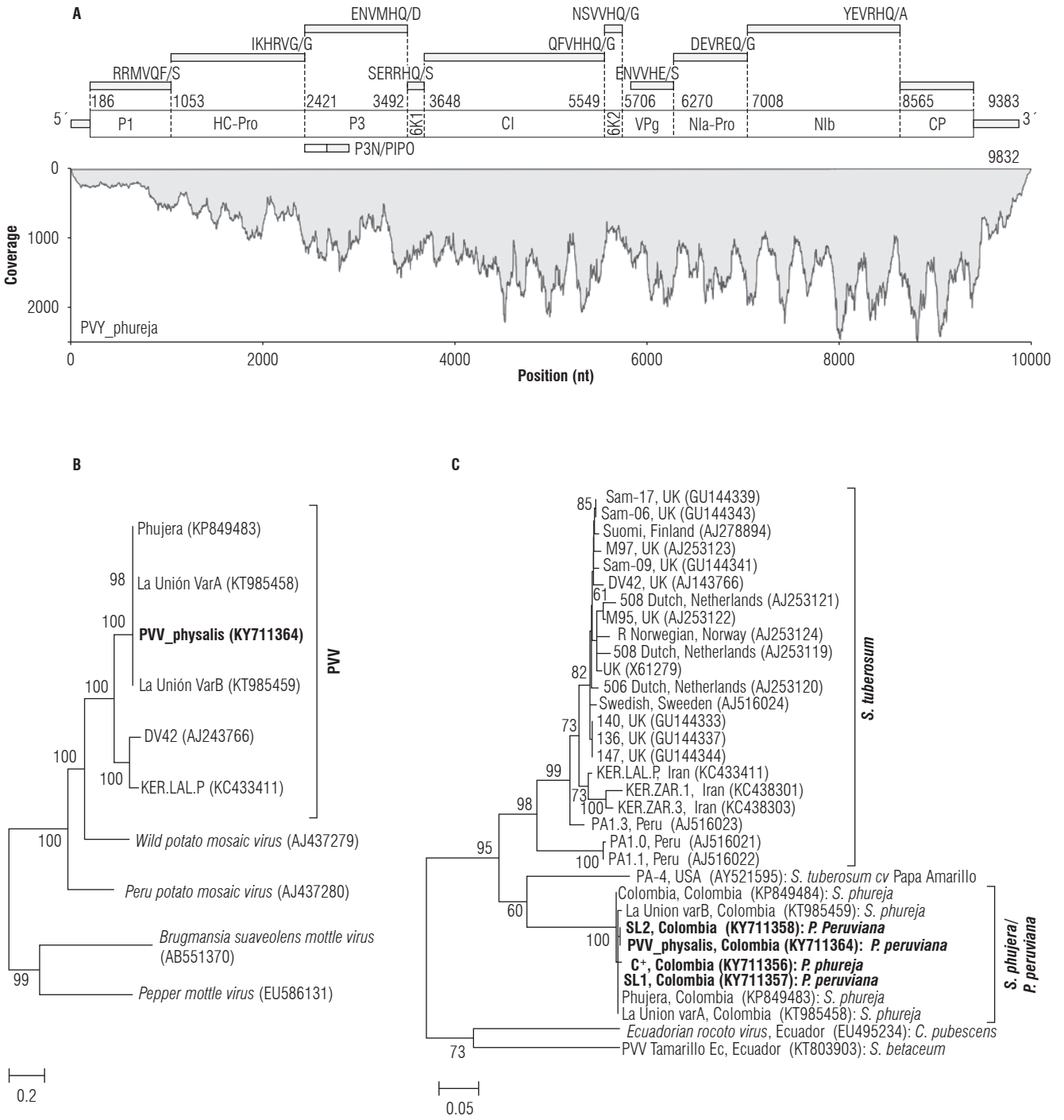


FIGURE 3. Genome annotation of the PVV_phureja genome and phylogenetic relationship to other PVV isolates. **A)** Sequence coverage of PVV_phureja assembly and location of some important molecular features. The relative position of each mature protein with their corresponding protease cleavage sites is shown. Phylogenetic analysis of PVV isolates using complete (**B**) and partial CP (**C**) sequences confirms the close relationship between PVV_physalis and PVV isolates infecting *S. phureja*. Trees are drawn to scale with branch lengths in units of number of base substitutions per site as indicated in the bar at the bottom. Bootstrap values are shown above the branches.

In contrast to PVY, the incidence of PVV was lower in both plots. PVV was detected in the asymptomatic and symptomatic bulks from the first plot but tested negative in both samples from the second plot. Surprisingly, a lower Ct value was observed in the asymptomatic sample (A-L1, Ct=22.38) than in the symptomatic one (S-L1, Ct=28.69) (Fig. 4B). Tm values were in good agreement with the positive control suggesting a sequence similarity. IC-RT-PCR

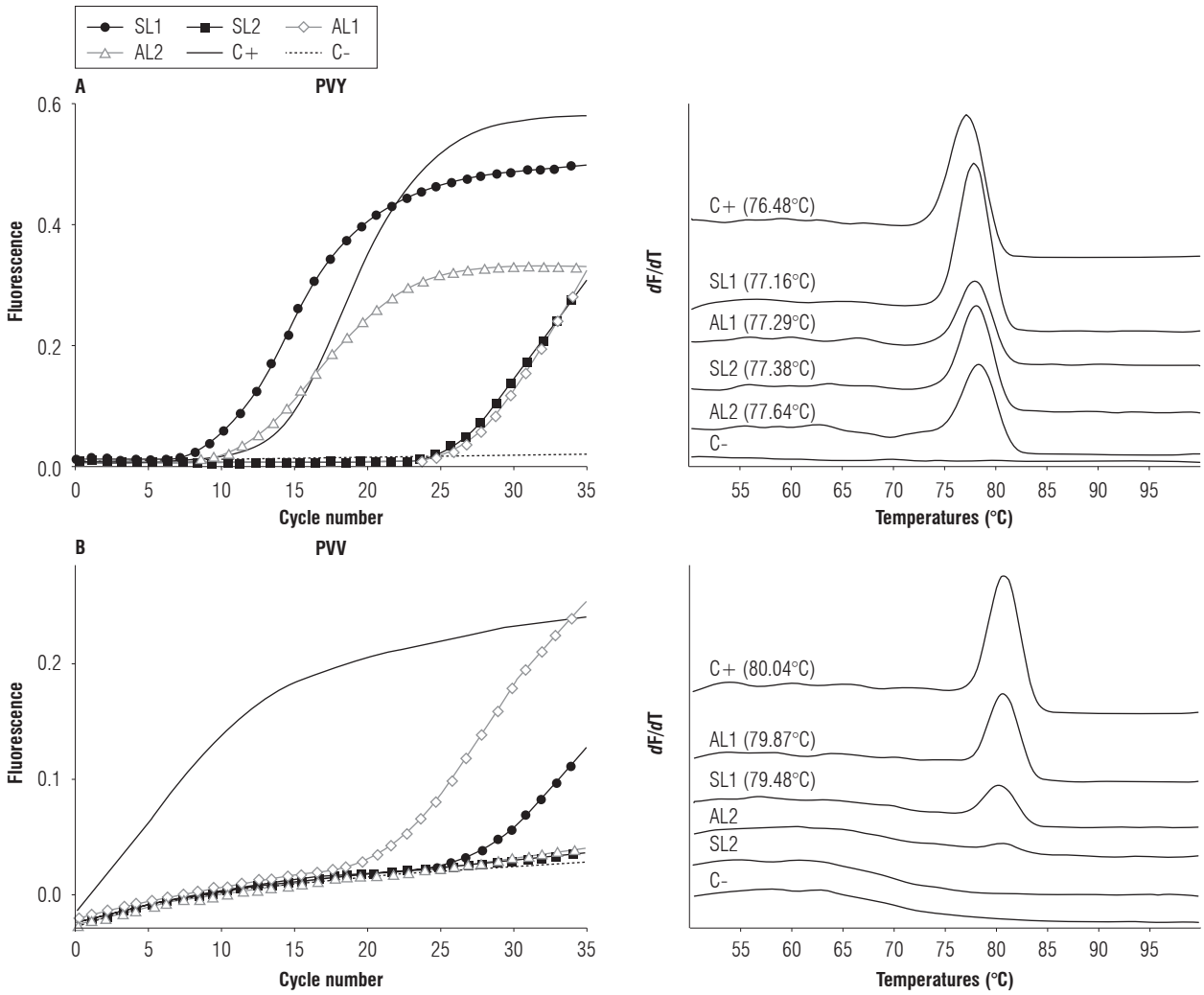


FIGURE 4. Molecular detection of Potato virus Y (PVY) and Potato virus V (PVV) in *P. peruviana* samples from La Unión (Antioquia). RT-qPCR amplification curves of PVY (**A**) and PVV (**B**) of symptomatic and asymptomatic leaf samples SL1-2 and AL1-2. Corresponding melting curve profiles are shown to the right with Tm values indicated in parentheses.

results differed from the RT-qPCR as only one sample in the first plot (S2-L1, Ct=26.83) tested positive for PVV, contrasting three positive samples in the second plot (A2-L2, A3-L2 and S1-L2) (Fig. 4B). There was no significant correlation between the symptoms and Ct values for PVV. The Tm values of 80±0.5°C are in good agreement with a previous study on PVV isolates infecting *S. phureja* isolates in Colombia (Álvarez *et al.,* 2016). Sequencing of RT-qPCR amplicons reveals a nucleotide sequence identity between 95 to 99% to PVV GenBank accesions KT985459, KT985458 and KC438304.

The natural ocurrence of potyviruses in cape gooseberry was first reported in Hawaii in 1953 (Sakimura, 1953) and later confirmed by serological and electron microscopy studies in India (Prakash *et al.,* 1988). *P. peruviana* has been thoroughly shown to be an alternate host to several viruses of tomato (Trenado *et al.,* 2007; Verhoeven *et al.,* 2010; Kisten *et al.,* 2016), tobacco (Schubert *et al.,* 2006) and potato (Prakash *et al.,* 1988; Gutiérrez *et al.,* 2015) and there is an increasing number of reports of viruses infecting *P. peruviana* in comercial plots all over the world such as the tospovirus TCSV in Rio Grande do Sul State of Brazil (Eiras *et al.,* 2012) and the potyvirus BYMV in Barabanki (India) (Kaur *et al.,* 2014). In Colombia, PVY was first identified in the department of Cundinamarca in a study of *P. peruviana* plants with leaf mosaics and mottling in 2006 (Daza and Rodríguez, 2006). In the mentioned research, PVY was detected using a combinantion of serological assays and electron microscopy and it was demonstrated to be transmitted through the aphid *Myzus persicae* acting as vectors from the cape gooseberry plants and by mechanical

TABLE 1. RT-q PCR detection of Potato virus Y (PVY) and Potato virus V (PVV) in leaf samples from *P. peruviana* plots in Antioquia (Colombia).

RT-qPCR (Total RNA)		PVY		PVV	
Sample name	**Type of sample**	**Ct***	**Tm****	**Ct**	**Tm**
C-		**>35**			
C+	Infected Potato leaf	13.39	76.48	80.04	4.66
SL1	Symptomatic bulk	9.97	77.16	28.69	79.48
SL2	Symptomatic bulk	26.37	77.38	>35	
AL1	Asymptomatic bulk	27.12	77.29	22.38	79.87
AL2	Asymptomatic bulk	12.49	77.64	>35	
RT-qPCR (Immunocapture)		**PVY**		**PVV**	
Sample name	**Type of sample**	**Ct***	**Tm****	**Ct**	**Tm**
C-		**>35**			
C+	Infected Potato leaf	27.02	76.99	5.02	79.78
A1-L1	Asymptomatic	30.28	77.29	>35	
A2-L1	Asymptomatic	31.07	76.56	>35	
A3-L1	Asymptomatic	28.30	77.46	>35	
S1-L1	Mosaic	26.55	77.35	>35	
S2-L1	Mottling	28.60	77.39	26.83	79.27
A1-L2	Asymptomatic	30.48	77.56	>35	
A2-L2	Asymptomatic	31.21	77.34	29.97	78.62
A3-L2	Asymptomatic	32.47	77.29	26.82	79.79
S1-L2	Mosaic	27.52	77.51	28.59	79.44
S2-L2	Greening of veins	28.75	77.64	>35	

*Threshold cycle. **Melting temperature.

infection to indicator plants. A later investigation also detected potyvirus infecting cape gooseberry plants in the municipality of Mosquera (Cundinamarca) using generic antibodies for aphid transmitted potyvirus and confirmed their results by electron microscopy (Aguirre *et al*., 2014). Based on ELISA tests, PVY was also recently reported in three *P. peruviana* samples from Cundinamarca and Boyaca (Cutler *et al*., 2018).

The great diversity of viruses shown to infect cape gooseberry highlights the importance to continue the virome research on this host, including different geographical regions and growth conditions such as mixed cropping, crop rotation systems and even considering this species as a weed for other crops. Our results support the notion that mixed cultivation of *P. peruviana* with other solanaceous plants should be avoided and its presence as weed should be controlled as vectors transmitting potyviruses, such as *M. persicae*, *Aphis gossypii* and *Macrosiphum euphorbiae* are insects frequently associated with *P. peruviana* (Afsah, 2015). Future work should address the cross pathogenicity of PVV and PVY in the South American Andes and other places where there is coexistence between *P. peruviana* and other solanaceous crops as well as their effect on yield, plant longevity and physicochemical properties of the cape goosberry fruit.

Conclusions

The analysis of next generation sequencing data from *P. peruviana* leaf samples and the symptoms of the viral disease revealed an infection caused by the potyviruses PVY and PVV in the municipality of La Unión (Antioquia). These results were confirmed by real time RT-PCR (RT-qPCR) and the Sanger sequencing of the capsid region. Phylogenetic analysis confirmed these potyvirus isolates to be closely related to PVY and PVV isolates identified previously in tomato and potato crops in Antioquia, respectively, which suggests that cape gooseberry could be an alternate host to viruses of other economically important solanaceous crops in the Andean region of South America.

Acknowledgments

This work was supported by The Universidad Nacional de Colombia (VRI Grant 34579) and Colciencias (706-2015, VRI project 30498).

Literature cited

Adams, M.J., J.F. Antoniw, and F. Beaudoin. 2005. Overview and analysis of the polyprotein cleavage sites in the family *Potyviridae*. Mol. Plant Pathol. 6(4), 471-487. Doi: 10.1111/j.1364-3703.2005.00296.x

Afsah, A.F.E. 2015. Survey of insects & mite associated cape gooseberry plants (*Physalis peruviana* L.) and impact of some selected safe materials against the main pests. Ann. Agric. Sci. 60(1), 183-191. Doi: 10.1016/j.aoas.2015.04.005

Agronet. 2016. Red de información y comunicación del sector agropecuario Colombiano. URL: http:// www.agronet.gov.co/Paginas/default.aspx (accessed 15 November 2016).

Aguirre-Ráquira, W., D. Borda, and L. Hoyos-Carvajal. 2014. Potyvirus affecting Uchuva (*Physalis peruviana* L.) in Centro Agropecuario Marengo, Colombia. Agr. Sci. 5(10), 897-905. Doi: 10.4236/as.2014.510097

Álvarez, D., P.A. Gutiérrez, and M. Marín. 2016. Caracterización molecular del Potato virus V (PVV) infectando *Solanum phureja* mediante secuenciación de nueva generación. Acta Biol. Colomb. 21(3), 521-531. Doi: 10.15446/abc.v21n3.54712

Capoor, S.P. and D.C. Sharma. 1965. Purple leaf disease of beet. Indian Phytopathol. 18, 88-89.

Chamberlain, E.E. 1939. Cucumber-mosaic (Cucumis virus 1 of Smith 1937). N.Z. J. Sci. Tech. 21, 74-90.

Chomczynski, P. 1993. A reagent for the single-step simultaneous isolation of RNA, DNA and proteins from cell and tissue samples. BioTechniques 15(3), 532-537.

Chung, B.Y., W.A. Miller, J.F. Atkins, and A.E. Firth. 2008. An overlapping essential gene in the *Potyviridae*. Proc. Natl. Acad. Sci. U S A. 105(15), 5897-902. Doi: 10.1073/pnas.0800468105

Cutler, J., J. Langer, S. Von Bargen, O. Acosta-Losada, F. Casierra-Posada, A. Castañeda-Cárdenas, M. Betancourt-Vásquez, W. Cuellar, E. Arvydas-Stasiukynas, D. Altenbach, and C. Büttner. 2018. Preliminary evaluation of associated viruses in production systems of cape gooseberry, purple passion fruit, and rose. Rev. Colomb. Cienc. Hortic. 12(2), 390-396. Doi: 10.17584/rcch.2018v12i2.7799

Da Graça, J.V., T.N. Trench, and M.M. Martin. 1985. Tomato spotted wilt virus in commercial cape gooseberry (*Physalis peruviana*) in Transkei. Plant Pathol. 34(3), 451-453. Doi: 10.1111/j.1365-3059.1985.tb01390.x

Daza, P.A. and P.A. Rodríguez. 2006. Enfermedades de origen viral en plantas de uchuva (*Physalis peruviana* L.) en el Departamento de Cundinamarca. Biol. Thesis. Pontificia Universidad Javeriana, Bogotá, Colombia.

Edgar, R.C. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res. 32(5), 1792-1797. Doi: 10.1093/nar/gkh340

Eiras, M., I.F. Costa, A.L. Chaves, A. Colariccio, and R. Harakava. 2012. First report of a tospovirus in a commercial crop of cape gooseberry in Brazil. New Dis. Rep. 25, 25. Doi: 10.5197/j.2044-0588.2012.025.025

Fisher, G., P.J. Almanza-Merchán, and D. Miranda. 2014. Importancia y cultivo de la uchuva (*Physalis peruviana* L.). Rev. Bras. Frutic. 36(1), 1-15. Doi: 10.1590/0100-2945-441/13

Gish, W. and D.J. States. 1993. Identification of protein coding regions by database similarity search. Nat. Genet. 3(3), 266-272. Doi: 10.1038/ng0393-266

Glais, L., M. Tribodet, and C. Kerlan. 2002. Genomic variability in Potato potyvirus Y (PVY): evidence that PVY^{NW} and PVY^{NTN} variants are single to multiple recombinants between PVY^{O} and PVY^{N} isolates. Arch. Virol. 147(2), 363-378. Doi: 10.1007/s705-002-8325-0

Gómez, J.E., F. Morales, and J. Arroyave. 1997. Mosaic disease of *Physalis peruviana* in Colombia. ASCOLFI Informa. 23, 52.

GitHub. 2015. SeqTK.URL: https://github.com/lh3/seqtk/ (accessed 15 November 2016).

Grabherr, M.G., B.J. Haas, M. Yassour, J.Z. Levin, D.A. Thompson, I. Amit, X. Adiconis, L. Fan, R. Raychowdhury, Q. Zeng, Z. Chen, E. Mauceli, N. Hacohen, A. Gnirke, N. Rhind, F. di Palma, B.W. Birren, C. Nusbaum, K. Lindblad-Toh, N. Friedman, and A. Regev. 2011. Full-length transcriptome assembly from RNA-seq data without a reference genome. Nat. Biotechnol. 29(7), 644-652. Doi: 10.1038/nbt.1883

Gupta, S.P. and B.R. Singh. 1996. Severe mosaic of cape gooseberry due to Cucumber mosaic virus. Indian J. Virol. 12(2), 155-156.

Gutiérrez, P.A., J.F. Alzate, and M. Marín. 2014. Caracterización del viroma de ARN de tejido radical de *Solanum phureja* mediante pirosecuenciación 454 GS-FLX. Bioagro 26(2), 89-98.

Gutiérrez, P.A., J.F. Alzate, and M.M. Montoya. 2015. Complete genome sequence of an isolate of Potato virus X (PVX) infecting cape gooseberry (*Physalis peruviana*) in Colombia. Virus Genes. 50(3), 518-522. Doi: 10.1007/s11262-015-1181-1

Hadidi, A., D. Jones, D. Gillespiet, F. Wong-Staalt, and T. Dienert. 1976. Hybridization of Potato spindle tuber viroid to cellular DNA of normal plants. Proc. Nat. Acad. Sci. 73(7), 2453-2457.

Henao-Díaz, E., P. Gutiérrez-Sánchez, and M. Marín-Montoya. 2013. Análisis filogenético de aislamientos del Potato virus Y (PVY) obtenidos en cultivos de papa (*Solanum tuberosum*) y tomate de árbol (*Solanum betaceum*) en Colombia. Actual. Biol. 35(99), 219-232.

Horvath, J. 1970. Reaction of *Physalis* species to plant viruses. I. The cape gooseberry as a symptomless carrier of Potato virus X and Y. Acta Phytopathol. Acad. Sci. Hungaricae. 5, 65-72.

Horváth, J. 1996. Ornamental *Physalis* species as perennial virus hosts. Acta Hortic. 432, 204-211. Doi: 10.17660/ActaHortic.1996.432.25

Insuasti, M.L., J.B. Ochoa, R.R. Martin, R.A. Alvarez, and F. Quito-Avila. 2016. First report of Potato virus V and Peru tomato mosaic virus on tamarillo (*Solanum betaceum*) orchards of Ecuador. Plant Dis. 100(4), 868. Doi: 10.1094/PDIS-09-15-1063-PDN

Karasev, A. and S. Gray. 2013. Continuous and emerging challenges of Potato virus Y in potato. Annu. Rev. Phytopathol. 51, 571-586. Doi: 10.1146/annurev-phyto-082712-102332

Kaur, C., R. Raj, S. Kumar, and S.K. Raj. 2014. First report of Bean yellow mosaic virus on cape gooseberry in India. New Dis. Rep. 29, 17. Doi: 10.5197/j.2044-0588.2014.029.017

Kisten, L., V. Moodley, and A. Gubba. 2016. First report of Potato virus Y (PVY) on *Physalis peruviana* in South Africa. Plant Dis. 100(7), 1511. Doi: 10.1094/PDIS-12-15-1442-PDN

Langmead, B. and S. Salzberg. 2012. Fast gapped-read alignment with Bowtie 2. Nat. Methods. 9(4), 357-359. Doi: 10.1038/nmeth.1923

Lorenzen, J.H., T. Meacham, P.H. Berger, P.J. Shiel, J.M. Crosslin, P.B. Hamm, and H. Kopp. 2006. Whole genome characterization of Potato virus Y isolates collected in the western USA and their comparison to isolates from Europe and Canada. Arch. Virol. 151(6), 1055-1074. Doi: 10.1007/s00705-005-0707-6

Medina, H.C., P.A. Gutiérrez, and M. Marín. 2015. Detección del Potato virus Y (PVY) en tubérculos de papa mediante TAS-ELISA y qRT-PCR en Antioquia (Colombia). Bioagro 27(2), 83-92.

Milne, I., M. Bayer, L. Cardle, P. Shaw, G. Stephen, F. Wright, and D. Marshall. 2010. Tablet-next generation sequence assembly visualization. Bioinformatics 26(3), 401-402. Doi: 10.1093/bioinformatics/btp666

Muñoz-Baena, L., P. Gutiérrez, and M. Marín. 2016. Detección y secuenciación del genoma del Potato virus Y (PVY) que infecta plantas de tomate en Antioquia, Colombia. Bioagro. 28(2), 69-80.

Muñoz-Escudero, D., P.A. Gutiérrez, and M. Marín. 2016a. Detección y caracterización molecular del Potato virus Y (PVY) en cultivos de papa (*Solanum tuberosum* L.) del norte de Antioquia, Colombia. Rev. Protección Veg. 31(1), 9-19.

Muñoz-Escudero, D., P.A. Gutiérrez, and M. Marín. 2016b. Detection and genome characterization of Potato virus Y isolates infecting potato (*Solanum tuberosum* L.) in La Unión (Antioquia, Colombia). Agron. Colomb. 34(3), 317-328. Doi: 10.15446/agron.colomb.v34n3.59973

Natti, J.J., H.C. Kirkpatrick, and A.F. Ross. 1953. Host range of Potato leaf roll virus. Amer. Potato J. 30(3), 55-64. Doi: 10.1007/BF02859918

Nei, M. and S. Kumar. 2000. Molecular Evolution and Phylogenetics. Oxford University Press, New York. 352 p.

Ogawa, T., Y. Tomitaka, A. Nakagawa, and K. Ohshima. 2008. Genetic structure of a population of Potato virus Y inducing potato tuber necrotic ringspot disease in Japan; comparison with North American and European populations. Virus Res. 131(2), 199-212. Doi: 10.1016/j.virusres.2007.09.010

Oruetxebarria, I., T. Kekarainen, C. Spetz, and J.P. Valkonen. 2000. Molecular characterization of Potato virus V genomes from Europe indicates limited spatiotemporal strain differentiation. Phytopathol. 90(4), 437-444. Doi: 10.1094/PHYTO.2000.90.4.437

Posada, D. and K.A. Crandall. 1998. MODELTEST: testing the model of DNA substitution. Bioinformatics 14(9), 817-818.

Prakash, O., A.K. Misra, S.J. Singh, and K.M. Srivastava. 1988. Isolation, purification and electron microscopy of mosaic virus of cape gooseberry. Int. J. Tropical Plant Diseases. 6(1), 85-87.

Ramadan, M.F. 2011. Bioactive phytochemicals, nutritional value, and functional properties of cape gooseberry (*Physalis peruviana*): An overview. Food Res. Int. 44(7), 1830-1836. Doi: 10.1016/j.foodres.2010.12.042

Revers, F. and J.A. García. 2015. Molecular biology of potyviruses. Adv. Virus Res. 92, 101-199. Doi: 10.1016/bs.aivir.2014.11.006

Rodríguez, M.H., N.E. Niño, J. Cutler, J. Langer, F. Casierra-Posada, D. Miranda, M. Bandte, and C. Büttner. 2016. Certificación de material vegetal sano en Colombia: un análisis crítico de oportunidades y retos para controlar enfermedades ocasionadas por virus. Rev. Colomb. Cienc. Hortic. 10(1), 164-175. Doi: 10.17584/rcch.2016v10i1.4921

Salamon, P. and L. Palkovics. 2005. Occurrence of Colombian Datura virus in Brugmansia hybrids, *Physalis peruviana* L. and *Solanum muricatum* Ait. in Hungary. Acta Virol. 49(2), 117-122.

Sakimura, K. 1953. Potato virus Y in Hawaii. Phytopathol. 43(4), 217.

Shamsadden-Saeed, F., H. Massumi, S. Moradi, M. Maddahian, J. Heydarnejad, A.H. Pour, and A. Varsani. 2014. Incidence and characterization of Potato virus V infections in Iran. Virus Disease. 25(1), 78-84. Doi: 10.1007/s13337-013-0178-4

Shiel, P.J., L. Miller, S.A. Slack, and P.H. Berger. 2004. Isolation and partial nucleic acid characterization of a new isolate of Potato virus V with distinct biological and serological properties. Plant Dis. 88(4), 368-372. Doi: 10.1094/PDIS.2004.88.4.368

Singh, M., R.P. Singh, M.S. Fageria, X. Nie, R. Coffin, and G. Hawkins. 2013. Optimization of a Real-Time RT-PCR assay and its comparison with ELISA, conventional RT-PCR and the grow-out test for large scale diagnosis of Potato virus Y in dormant potato tubers. Am. J. Potato Res. 90(1), 43-50. Doi: 10.1007/s12230-012-9274-z

Tamura, K. and M. Nei. 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Mol. Biol. Evol. 10(3), 512-526.

Tamura, K., G. Stecher, D. Peterson, A. Filipski, and S. Kumar. 2013. MEGA6: Molecular evolutionary genetics analysis. Mol. Biol. Evol. 30(12), 2725-2729. Doi: 10.1093/molbev/msT197

Trenado H.P, I.M. Fortes, D. Louro, and J. Navas-Castillo. 2007. *Physalis ixocarpa* and *P. peruviana*, new natural hosts of Tomato chlorosis virus. Eur. J. Plant Pathol. 118, 193-196.

Verhoeven, J.T.J., M. Botermans, J.W. Roenhorst, J. Westerhof, and E.T.M. Meekes. 2009. First report of Potato spindle tuber viroid in cape gooseberry (*Physalis peruviana*) from Turkey and Germany. Plant Dis. 93(3), 316. Doi: 10.1094/PDIS-93-3-0316A

Wetzel, T., T. Candresse, G. Macquaire, M. Ravelonandro, and J. Dunez. 1992. A highly sensitive immunocapture polymerase chain reaction method for plum pox potyvirus detection. J. Virol. Methods. 39(1-2), 27-37. Doi: 10.1016/0166-0934(92)90122-T

Wu, S.J., L.T. Ng, Y.M. Huang, D.L. Lin, S.S. Wang, S.N. Huang, and C.C. Lin. 2005. Antioxidant activities of *Physalis peruviana*. Biol. Pharm. Bull. 28(6), 963-966.

Zapata, J.L., A. Saldarriaga, M. Londoño, and C. Díaz. 2005. Las enfermedades limitantes en cultivo y poscosecha de la uchuva y su control. pp. 97-110. In: Fischer, G., D. Miranda, W. Piedrahita and J. Romero (eds.). Avances en cultivo, poscosecha y exportación de la uchuva (*Physalis peruviana* L.) en Colombia. Universidad Nacional de Colombia, Bogotá, Colombia.

Screening of Colombian soybean genotypes for *Agrobacterium* mediated genetic transformation conferring tolerance to Glyphosate

Tamizaje de genotipos de soya colombianos para transformación genética mediada por *Agrobacterium* confiriendo tolerancia a glifosato

Adriana Rojas[1], Silvio Lopez-Pazos[2], and Alejandro Chaparro-Giraldo[1]*

ABSTRACT

Soybean is a very important crop worldwide due to its multiple uses as raw material for industry and to its high nutritional value. Colombia consumes a large amount of imported soybean because domestic production does not supply demand. There are soybean varieties adapted to the environmental conditions in the Colombian territory, but none of them have been enhanced by genetic engineering to confer competitive advantages compared to imported product. In this research, the Colombian soybean varieties SK7, P29 and Soyica P34 ability to be genetically transformed by *Agrobacterium tumefaciens* strains AGL0 and EHA105 using a Glyphosate tolerance cassette was tested. It was found that SK7 variety presented a better regeneration performance from the cotyledonary node, and also had the highest transformation frequency with AGL0 strain. The P29 variety was also transformed, but a lower efficiency was registered. It was not possible to transform Soyica P34 variety under the established parameters. This research is an advance towards the construction of a platform to enhance the generic transgenic crops in Colombia.

Key words: *Glycine max*, herbicide, genetically modified organism, *in vitro* regeneration, transgenic plant.

RESUMEN

La soya es un cultivo muy importante a nivel mundial debido a sus múltiples usos en la industria y a su alto valor nutricional. Colombia consume una gran cantidad de soya, principalmente importada, porque la producción interna no suple la demanda. Existen variedades de soya adaptadas a las condiciones medioambientales del territorio colombiano, pero ninguna de ellas ha sido mejorada por ingeniería genética para conferir ventajas competitivas al cultivo frente a las importaciones. En este trabajo se evaluaron las variedades de soya SK7, P29 y Soyica P34 respecto a su capacidad para ser transformadas genéticamente por las cepas de *Agrobacterium tumefaciens* AGL0 y EHA105, utilizando un casete de tolerancia a glifosato. Se encontró que la variedad SK7 presentó un mejor desempeño en regeneración a partir de nudo cotiledonar, e igualmente tuvo la mayor frecuencia de transformación con la cepa AGL0. La variedad P29 también fue transformada, aunque con una eficiencia menor. No fue posible transformar la variedad Soyica P34 bajo los parámetros establecidos. Este trabajo fue un avance hacia la construcción de una plataforma de generación de cultivos transgénicos genéricos en Colombia.

Palabras clave: *Glycine max*, herbicida, organismo genéticamente modificado, regeneración *in vitro*, planta transgénica.

Introduction

Soybean (*Glycine max*) is a Fabaceae plant, whose seeds contain sugars (~30%), protein (~35%), edible oil (~20%), fiber, vitamins, and minerals. Soybean is a source of protein comparable to meat or eggs. Soybean cake is used as animal feed or industrial substrate (Widholm *et al*., 2010). The soybean production for 2016 was calculated at 320 million t. United States and Brazil are major soybean producer countries. United States has an estimated soybean production of 108 million t, and their harvested area is estimated at 33 million ha. Brazil has a harvested area of 33 million ha with a production of 100 million t (USDA, 2017). Colombia with an average production rate of 75,000 t in 2016 is considered a small soybean producer, occupying the 37th position worldwide, and the 6th in South America (Fenalce, 2017). Colombian soybean is developed mainly in the Eastern Plains region, which is a key area for agricultural development due to its plain geography suitable for technification, vast land extensions, and development opportunities, especially in the post-conflict period. Considering that soybean is a plant originated and cultivated in temperate latitudes, and due that Colombia is a tropical country, several Colombian breeding programs have been developed since the 80s producing varieties adapted to local soil and weather conditions, including relevant differences in plant physiology parameters as the plant photoperiod

Received for publication: 4 September, 2017. Accepted for publication: 1 March, 2018 Doi: 10.15446/agron.colomb.v36n1.67440

[1] Plant Genetic Engineering Research Group, Biology Department, Universidad Nacional de Colombia, Bogota (Colombia).
[2] Sciences Faculty, Universidad Antonio Nariño, Bogota (Colombia).
* Corresponding author: achaparrog@unal.edu.co

CC BY NC ND

and flowering processes that are correlated to the production rates (Valencia and Ligarreto, 2010). Currently, there are several soybean varieties with a great adaptation to local conditions, and high production (over 25,000 kg ha^{-1}) which are used by local farmers.

The genetic modification by transgenesis (GM) in commercial plants has been a useful technology providing farmers with a tool to increase crop yield, reduce the quantity of pesticides, and increase the farmer profit (Klümper and Qaim, 2014). Soybean has been bred by transgenesis, including different traits as herbicide tolerance, insect pest resistance, and improvement of oil quality (ILSI Research Foundation, 2017). Particularly, herbicide tolerance has been a very widespread and successful trait on soybean, having adoption rates of 94% in USA, 96.5% in Brazil, and 100% in Argentina in 2016 (ISAAA, 2016). GM soybean enhanced with herbicide tolerance has several advantages compared to the regular varieties. Among them, it allows the use of one herbicide per crop, a longer period of weed control, a lower Glyphosate concentration in soil, less herbicide application events, the use of low toxicity herbicides such as Glyphosate, and higher profits to the producer as an expression of all the above. Also, herbicide tolerant soybean is compositionally equivalent to the conventional genotypes and finally, the expiration of patents protecting herbicide tolerant soybeans can be a base for generic GM crops (Bonny, 2009). In Colombia, the government policies allow growers to produce transgenic plants and there are no restrictions to the consumers. Last reports indicate that in 2016 100,000 ha of GM maize were planted, of which 9,800 ha were GM cotton and 12 ha were GM flowers (ISAAA, 2016). Governments are committed to assess and manage the risks associated with the development and release of genetically modified crops. There is an established regulation of GM soybean specially attending the food safety affairs, including a maximum limit of herbicide, substantial equivalence, and varieties description. Countries have developed protocols by GM detection based on phenotypic or molecular assays (Tillmann *et al*., 2004).

Agrobacterium tumefaciens is a relevant microorganism used in transgenesis of crops. This process is based on the transfer of a DNA segment (T-DNA) from a tumor-inducing (Ti) plasmid, which is incorporated into the plant genome with its resultant expression. The T-DNA and Vir proteins form a molecular set that delivers a single strand of this T-DNA into the cell (Bourras *et al*., 2015). Soybean is a recalcitrant plant for *Agrobacterium*-mediated transformation producing low transformation efficiencies and requiring the use of hypervirulent bacterial strains and specific plant genotypes to allow its transformation (Atif *et al*., 2013). Some protocols have been applied to improve the transformation efficiency using *A. tumefaciens;* these methodologies include the modification of certain sanitation and infestation procedures toward observing possible differences among genotypes (Liu *et al*., 2013).

Glyphosate, whose chemical name is N-(phosphonomethyl) glycine ($C_3H_8NO_5P$), is an odorless white strong acid. It is a crystalline powder with a fusion point of 184.5°C and molecular mass of 169.1 $gmol^{-1}$. Glyphosate is a systemic herbicide and can be used with practically any type of crop to control weeds worldwide. Enzyme 5-enolpyruvyl-shikimate-3-phosphate synthase (EPSPS) is the target of Glyphosate, which is directly involved in the synthesis of the aromatic residues: phenylalanine, tyrosine, and tryptophan (Shikimate pathway). In 1996, the first GM soybean with Glyphosate tolerance was introduced in USA, expressing an EPSPS protein from a CP4 strain of *A. tumefaciens*, which has no affinity for Glyphosate and allows the normal perform of the shikimate pathway (Duke and Powles, 2008; Duke and Cerdeira, 2010). Glyphosate tolerance is a successful trait in soybean crop, and it currently offers the possibility to develop generic transgenic crops. Considering that patents that cover the development process of this particular trait recently expired (Jefferson *et al*., 2015), there is an increase in the freedom to operate (FTO) of the commercial plants related. Colombian soybean varieties have not been bred by transgenesis despite the fact that they are approved as commercial crops with Glyphosate tolerance (ICA, 2010) and that imported GM soybean seeds have low possibilities to grow well in tropical conditions. The main objective of this study was to evaluate the capacity of Colombian soybean varieties to be transformed by *A. tumefaciens*, using a Glyphosate tolerance expression cassette with FTO in Colombian territory, as a stage to produce and commercialize generic GM crops in Colombia.

Materials and methods

Plant genotypes

Three Colombian genotypes of soybean were used in transformation experiments: SK7 and P29 (bred by Kamerun and Panorama companies respectively) and Soyica P-34 (bred by the Colombian Agricultural Institute - ICA). These genotypes were chosen due to its high cultivation rate in the Eastern Plains region. SK7 genotype is adapted to grow between 300 and 1,200 m a.s.l., a vegetative stage of 110-112 d, height of 101 cm, white flowers, brown pubescence, oil content of 20.47%, and a yield rate of 2,600 kg ha^{-1}. P29

genotype is adapted to grow between 300 and 1,200 m a.s.l. as well, has a vegetative stage of 105-115 d, height of 100 cm, purple flowers, brown pubescence, an oil content of 21.37%, and a yield rate of 2,600 kg ha^{-1}. Soyica P-34 is equally adapted to the same altitude (300 to 1,200 m a.s.l.), a vegetative stage of 110 d, height of 69 cm, white flowers, brown pubescence, an oil content of 20%, and a yield rate of 2,700 kg ha^{-1}. As start material field conditions seed were used, without any fungicidal or insecticidal treatment, and a moisture between 11 to 12%. These varieties were chosen because they were the most demanded by farmers at the time in which the experiment was performed.

Agrobacterium strains, expression cassette and vector

A previously reported cassette designated as E-IGP was used; this cassette contains a polyubiquitin promoter from soybean (GmUbi), followed by a transit peptide from *Petunia hybrida*, a codon optimized *cp4 epsps* gene for expression in soybean tissues, and a nopaline synthase (NOS) terminator codon (Jiménez, 2014). The E-IGP cassette was introduced into a pCAMBIA1301 vector on which the GUS reporter gene and the hygromycin selection gene were excised in such a way that only the E-IGP cassette could be transferred to the plant genome (Jiménez, 2014). The vector containing the E-IGP cassette was introduced into *A. tumefaciens* strains AGL0 (bought from an American Type Culture Collection, under ATCC®BAA-100™ denomination) and EHA105 (acquired by a donation from the Cenicaña institution). Recombinant strains were maintained in Luria Bertani (LB) medium containing 50 mg L^{-1} kanamycin. *Agrobacterium* cultures used for infection of explants were grown in LB medium. The observed optical density at 650 nm (OD_{650}) ranged from 0.8 to 1.0, at 28°C and 200 rpm. A bacterial pellet was obtained by centrifugation of 30 ml of bacterial culture at 8000 rpm for 4 min at 20°C. The pellet was resuspended in 25 ml of co-cultivation liquid medium (CCLM) (1X Gamborg vitamins, 0.1X B5 salts (Gamborg *et al*., 1968), 1.67 mg L^{-1} benzylaminopurine (BAP), 0.25 mg L^{-1} gibberellic acid (GA_3), 3% sucrose, 20 mM2-[N-Morpholino] ethanesulfonic acid (MES), 200 µ macetosyringone, pH 5.7) and then was used as inoculum for the plant tissues.

Explant preparation and *A. tumefaciens* infection

Soybean seeds were selected considering their appearance, choosing those that did not have lacerations or spots, and had a homogeneous size. The seeds surface was sterilized following the chlorine gas technique (Paz *et al*., 2004; Paz *et al*., 2006; Song *et al*., 2013) for 16 h, generating gas from a mix of 4.1 ml 10 N HCl with 100 ml 5% NaClO. Sterilized seeds were germinated on 0.7% Plant Tissue Culture (PTC) agar medium (water plus agar), pH 5.7, with the hilum proximal to the media for 5 d, and incubated under a 16/8 (light/dark) photoperiod at 26°C.

Once seeds were germinated, the seed coat was eliminated and a cutting was done 5 mm below the cotyledons junction to eliminate the hypocotyl. After that, cotyledons were separated by a longitudinal cut on the remaining piece of hypocotyl. Plumule was eliminated from both cotyledons, and some incisions (7-12) were made on the cotyledonary node. Each cotyledon with its own cotyledonary node was considered as an explant to transformation. Explants were infected with *A. tumefaciens* strains by submerging them in a CCLM solution containing bacterial biomass for 30 min, followed by cultivation on a co-cultivation medium (CCM) (CCLM added with 0.7% PTC agar), with the adaxial side down, and incubated in the dark for 3 d at 28°C (Zhang *et al*., 1999).

Regeneration test of soybean varieties

A first trial was performed to observe the behavior of soybean varieties in an *in vitro* system intended for *Agrobacterium*-mediated transformation. For such task, a regeneration ability assay was carried out for every variety without *Agrobacterium* infection. In this essay, 200 seeds of each variety were selected and germinated obtaining 240 explants that were prepared as described above. These explants were cultivated on CCM without *Agrobacterium* strain for 3 d, and later were cultivated on a shoot induction medium (SIM) (1X B5 salts, 1X Gamborg vitamins (Gamborg *et al*., 1968), 3% sucrose, 1,67 mg L^{-1} BAP, 3mM MES, 0.7% PTC agar, pH 5.7) for 4 weeks, with a medium replacement at the end of the second week.

After four weeks on SIM, the number of explants producing at least one shoot (regenerating explants) was recorded as well as the number of shoots produced by each regenerating explant.

Transformation, regeneration and selection

Transformation assays were performed following the methodology described by Zhang *et al.* (1999), plus some modifications. To assess the ability of each vegetal variety to be transformed with E-IGP cassette, the assay was divided in two treatments and two controls as follows: Treatment 1: three varieties transformed with an EHA105 strain containing a E-IGP cassette, and selected *in vitro* using the herbicide Glyphosate; Treatment 2: three varieties transformed with an AGL0 strain containing a E-IGP cassette, and selected *in vitro* using the herbicide Glyphosate; Control 1 (relative control): three varieties without transformation,

and selected *in vitro* using Glyphosate; and Control 2 (absolute control): three varieties without transformation, and without *in vitro* selection.

For each treatment and control, 130 explants of each variety as described above were prepared. Treatments were inoculated with *Agrobacterium* strains and co-cultivated as described above. Controls were cultivated on CCM in the same way as treatments, but without bacteria. After co-cultivation, all explants were rinsed in sterile water added with 50 mg L^{-1} cabenicillin, on a rotary shaker at 410 rpm for 40 min, three times. After this rinse, the explants were transferred to SIM mixed with antibiotics (250 mg L^{-1} cefotaxime, 100 mg L^{-1} timentin), and they were incubated for two weeks, under a 16/8 (light/dark) photoperiod at 26°C. After this procedure, SIM was renewed with a fresh SIM plus antibiotics solution and mixed with a 148 µM Glyphosate reagent grade (Phytotechnology Laboratories®, Lenexa, KS, USA). This methodology was followed in Treatments 1, 2 and in Control 1; Glyphosate was not added in Control 2. During the process to transfer explants to fresh SIM, the remaining hypocotyl of each explant was cut to allow the fresh tissue to directly contact the growth medium. Growing process was performed for two additional weeks under the same conditions.

Once the shoot induction period was finished, it was followed by a shoot elongation period (SEP). All explants were cut to eliminate remaining cotyledon and thus allowing fresh tissue to be in contact with the medium. Explants developing at least one shoot were transferred to Shoot Elongation Medium (SEM) (1X B5 salts, 1X Gamborg vitamins (Gamborg *et al*., 1968), 3% sucrose, 0.5 mg L^{-1} GA_3, 0.1 mg L^{-1} indole acetic acid (IAA), 0.7 mg L^{-1} BAP, 50 mg L^{-1} glutamine, 50 mg L^{-1} asparagine, 3 mM MES, 250 mg L^{-1} cefotaxime, 100 mg L^{-1} timentin, 0.7% PTC agar, pH 5.7) added with a 35 µM Glyphosate reagent grade (Phytotechnology Laboratories®, Lenexa, KS, USA) in Treatments 1, 2 and in Control 1, and without Glyphosate in Control 2. The SEM solution was replaced in the explants every two weeks for fresh SEM. In treatments and control containing Glyphosate, the herbicide was added during four weeks, removing it between the fifth week and until the end of SEP. The explants were allowed to grow until shoots reached a height of 3 cm, for a maximum SEP of 10 weeks, under 16/8 (light/dark) photoperiod to 26°C.

Shoots that reached the required height (3 cm) were individualized, labeled as "KJ" for SK7 variety, "PJ" for P29 variety and "SJ" for Soyica P34 variety, and marked by a number to indicate a consecutive individualization, thus discriminating the lines obtained from different treatments. Each individual line was transferred to a Propagation Medium (PM) (MS salts 0.66X (Murashige and Skoog, 1962), (1X Gamborg vitamins (Gamborg *et al*., 1968), 3% sucrose, 0.7% PTC agar, pH 5.7), and it was propagated to obtain biomass to consume in molecular and phenotypical analysis.

Molecular and phenotypical analysis

Individualized lines that were successfully propagated were subjected to the polymerase chain reaction (PCR) analysis to detect transgene insertion. Genomic DNA was extracted from each line following CTAB buffer methodology (Doyle, 1991), and it was quantified spectrophotometrically using NanoDrop equipment (Thermo Fisher Scientific, Waltham, MA USA). A 205 bp region of transgene was amplified using the pair of primers 5'-CTTTGCTGAAGGAGCTACCG-3' and 5'-GTGATCGAGATGCGTAGCAA-3' along with reagents included in Kapa3G Plant PCR kit (Sigma-Aldrich Corp. St. Louis, MO, USA) following manufacturer's instructions. PCR products were separated by electrophoresis on a 1% agarose gel. Transformation frequency was calculated as number of positive PCR lines / total number of transformed explants × 100.

To detect EPSPS expression, the vegetal biomass of propagated lines was subjected to the commercial Enzyme Linked Immunosorbent Assay (ELISA) Roundup Ready CP4 EPSPS (Agdia Inc. Elkhart, IN, USA), following manufacturer's instructions. Absorbance of each sample was measured using iMark™ Microplate Reader (Biorad, Hercules, CA, USA) at 655 nm wavelength. Percentage of functional transformants was calculated as number of positive ELISA lines / total number of transformed explants × 100.

On each positive PCR sample, a Southern Blotting assay was performed following recommendations of digoxigenin (DIG) applications platform for filter hybridization (Sigma-Aldrich Corp. St. Louis, MO, USA) (Eisel *et al*., 2008). A DIG-labeled probe was synthesized using the PCR DIG Probe Synthesis kit (Sigma-Aldrich Corp., St. Louis, MO, USA) with the same primers described above for PCR. Twenty µg of genomic DNA were digested with *PvuII* and *NdeI* enzymes (New England Biolabs® Inc., Ipswich, MS, USA) in a double digest reaction. Restriction fragments were separated on 0.7% agarose gel by electrophoresis and transferred onto a positively charged nylon membrane (Sigma-Aldrich Corp., St. Louis, MO, USA). Prehybridization, hybridization, membrane washing, and detection were conducted following the platform instruction manual (Eisel *et al*., 2008). The detection of probe-target hybrids was done by chemiluminescence using CDP-Star substrate (Sigma-Aldrich Corp., St. Louis, MO, USA).

Only a small amount of possible transgenic lines were allowed to grow on soil-type substrate. On hardened lines, a "plant painting" was performed using a 0.5% dilution of commercial Glyphosate (Victorius® 48 SL, Sodiak SA, Bogota, Colombia), over the shoot of the plant. Herbicide affectation was recorded after 10 d based on plant survival. Parallel to application on transformed lines, the same Glyphosate solution (0.5%) was applied on a non-transformed plant as positive control for test herbicide activity. In contrast, solvent (tap water) was applied on another non-transformed plant as a negative control.

Statistical analysis

A completely randomized design was used in biological assays, non-parametric comparison tests were used by statistical differences determination using a *P* value of 0.05. R software (R Development Core Team, 2008) was applied for the calculation.

Results

Regeneration capacity of soybean varieties

Three Colombian soybean varieties were evaluated regarding their regeneration capacity as a preliminary clue of how their behavior on an *Agrobacterium*-mediated transformation system will be. Starting from 240 explants of each variety, 212 explants of Soyica P34 (88.3%), 181 explants of SK7 (75.4%) and 231 explants of P29 (96.2%) developed regenerated shoots. After counting the number of shoots per explant (on regenerative explants) in each variety, it was found that, on average, Soyica P34 regenerates 11.5 shoots per explant (± 3.9 shoots), SK7 regenerates 17.7 shoots per explant (± 7.73 shoots) and P29 regenerates 12.7 shoots per explant (± 5.2 shoots) (Fig.1).

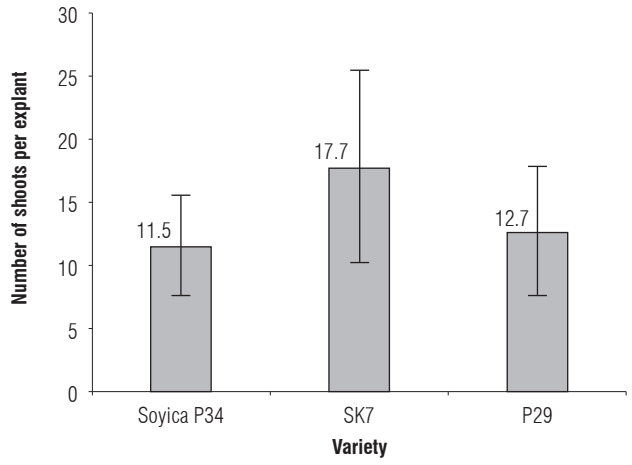


FIGURE 1. Regeneration capacity of vegetal varieties as number of shoots per explant.

At first the non-parametric Kruskal-Wallis test was applied using R software, to compare if varieties outputs have equality in the middle range or if at least one of them is different (Hollander *et al.*, 2014). In statistical terms, some of the following hypothesis systems were assessed:

H_0: $\mu_{Soyica\ P34} = \mu_{SK7} = \mu_{P29}$ vs H_a: $\mu_{Soyica\ P34} \neq \mu_{SK7} = \mu_{P29}$

H_0: $\mu_{Soyica\ P34} = \mu_{SK7} = \mu_{P29}$ vs H_a: $\mu_{Soyica\ P34} = \mu_{SK7} \neq \mu_{P29}$

H_0: $\mu_{Soyica\ P34} = \mu_{SK7} = \mu_{P29}$ vs H_a: $\mu_{Soyica\ P34} \neq \mu_{P29} = \mu_{SK7}$

H_0: $\mu_{Soyica\ P34} = \mu_{SK7} = \mu_{P29}$ vs H_a: $\mu_{Soyica\ P34} \neq \mu_{SK7} \neq \mu_{P29}$

When the test was carried out, a *P*-value of 0 was obtained, and the null hypothesis was rejected, suggesting that at least one of these average ranges is different from the others. Therefore, the non-parametric Wilcoxon test was used, in order to compare the differences on the average ranges of two samples, and to identify which is higher (Hollander *et al.*, 2014). The test was carried out two by two, with $\alpha = 0.1$, as follows:

H_1: $\mu_{Soyica\ P34} = \mu_{SK7}$ vs H_a: $\mu_{Soyica\ P34} < \mu_{SK7}$

H_2: $\mu_{P29} = \mu_{Soyica\ P34}$ vs H_a: $\mu_{P29} < \mu_{Soyica\ P34}$

H_3: $\mu_{P29} = \mu_{SK7}$ vs H_a: $\mu_{P29} < \mu_{SK7}$

The p-values associated with the tests performed were 0.00 for H_1, 0.99 for H_2 and 0.00 for H_3. For any p-value smaller than α, the null hypothesis is rejected, indicating that the mean range of shoots of the SK7 variety is greater than the mean range of the Soyica P34 and P29 varieties.

Agrobacterium-mediated transformation

The three Colombian soybean varieties described above were subjected to a genetic modification. Such methodology consisted in an insertion of a transgene designed to express a CP4 EPSPS protein to confer tolerance to the herbicide Glyphosate. This process was performed using the *A. tumefaciens* bacteria as a transgene's vehicle, assessing two bacterial strains, AGL0 and EHA105.

To assess the behavior of treatments and controls in each variety, the explant number in each process stage prior line individualization was recorded. Each treatment and control had initially 130 explants, and this number was decreasing in successive stages (Fig. 2). It was observed that in all three plant varieties, addition of Glyphosate to the culture medium reduced the explants regeneration compared to the control without herbicide addition. Taking as a reference the explants subjected to absolute control that reached the shoot elongation 3 stage (SE-3) in each

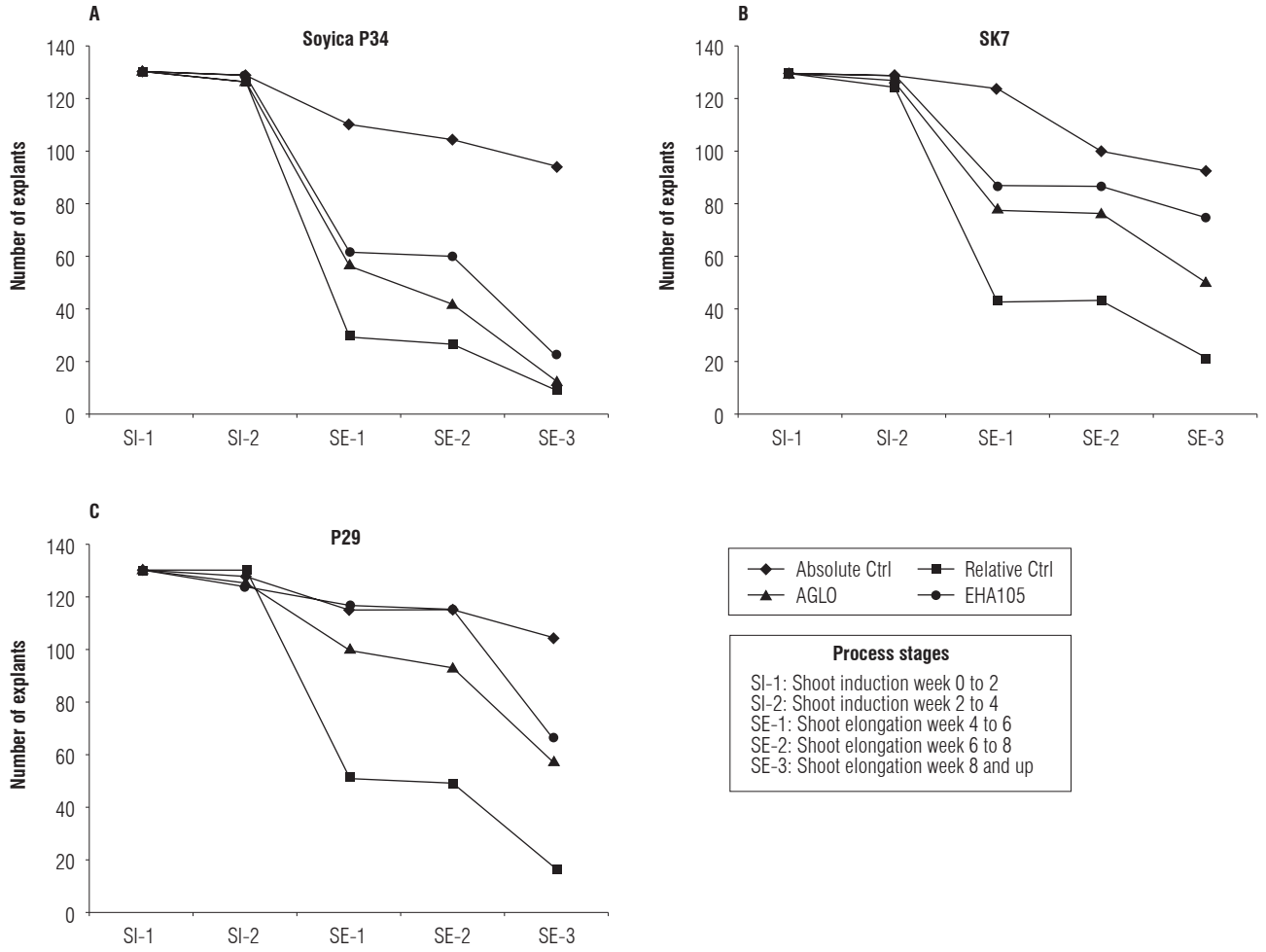


FIGURE 2. Recovered explants per variety on different stages of transformation process.

variety (100%), it was observed that the highest damage by herbicide was presented in Soyica P34. For this variety, 8.5% of explants in relative control, 12.8% in transformation with strain AGL0 and 22.3% in transformation with strain EHA105 reached the SE-3 stage. Furthermore, for P29 16% of explants in relative control, 55.8% in transformation with strain AGL0 and 64.4% in transformation with strain EHA105 reached the SE-3 stage. The most tolerant variety to herbicide addition was SK7, in which 23.7% of explants in relative control, 54.8% in transformation with AGL0 and 80.6% in transformation with strain EHA105 reached SE-3 stage (Fig. 2).

After reaching the SE-3 stage, the shoots that grew above 3 cm were labeled and marked. These Lines were propagated, and only those that were successful were subjected to molecular and phenotypical analysis. A total of 91 possible primary transformants of all varieties and treatments were labeled (Tab. 1), but only 53 lines were effectively propagated, so DNA extraction was performed exclusively on those 53 lines. PCR was done on extracted DNA and separated by electrophoresis (Fig. 3).

SK7 showed the highest transformation frequency (10.8%) when it was combined with AGL0 strain (Tab. 1). P29 was also efficiently transformed in a lower proportion, (5.4%) when it was combined with AGL0 strain, and 6.1% when it was combined with EHA105 strain. It was not possible to transform Soyica P34 variety under the procedures followed in this research (Tab. 1).

To assess the protein expression, an ELISA test on biomass of the propagated plants from possible primary transformants was performed. In this test positive results by 12 lines of SK7 and P29 varieties were obtained (Tab. 1). Thus, the highest percentage of functional transformants was obtained for SK7 when it was combined with AGL0 (3.8%). For P29 variety, the highest percentage of functional transformants was obtained also by using AGL0 strain (2.3%) (Tab. 1).

TABLE 1. Results of transgene presence / absence test and CP4 EPSPS protein detection on generated lines.

Plant genotype	Bacterial strain	Primary transformants	PCR (+)	ELISA (+)	Transformation frequency (PCR (+))	Functional transformants (ELISA (+))
SK7	AGL0	22	14	5	10.8%	3.8%
	EHA105	31	6	3	4.6%	2.3%
P29	AGL0	14	7	3	5.4%	2.3%
	EHA105	23	8	1	6.1%	0.8%
Soyica P34	AGL0	1	0	0	0%	0%
	EHA105	0	0	0	0%	0%

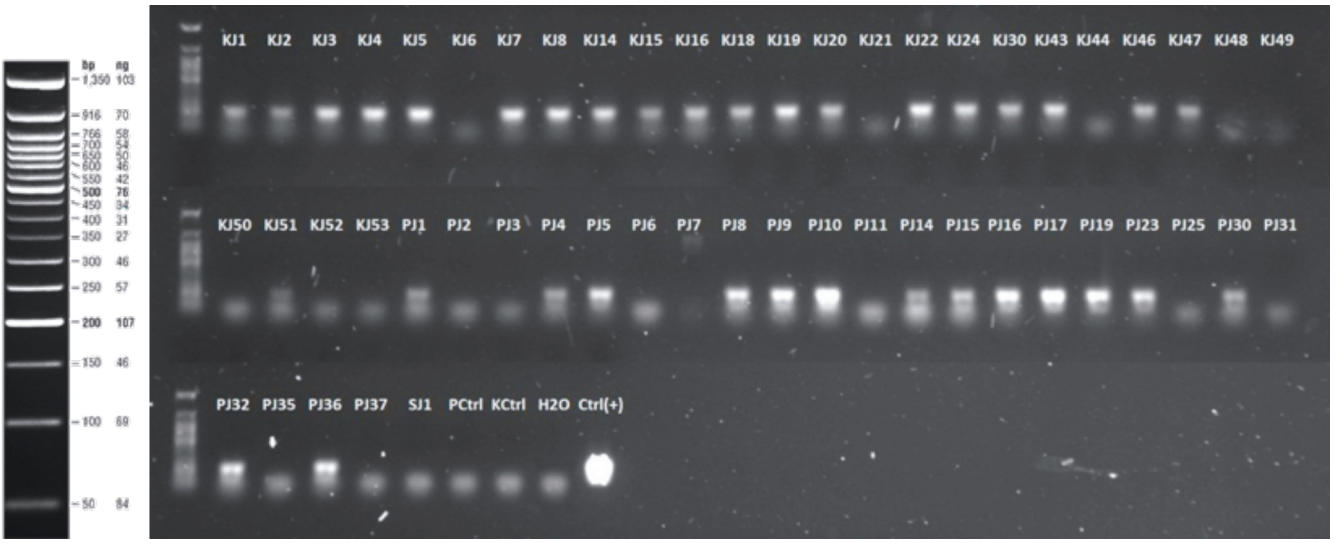


FIGURE 3. Detection of the E-IGP cassette on possible primary transformants. The name of each line is indicated at the top of each lane. PCtrl: Non transformed P29 variety, KCtrl: Non transformed SK7 variety, H2O: absolute control with primers, Ctrl (+): positive control, corresponding to plasmid vector E-IGP extracted by miniprep. The molecular weight marker corresponds to 50 bp DNA Ladder (New England Biolabs).

A Southern blot was performed on positive PCR samples. All of them showed a single insertion band, ranging between 6.5 to 24 kbp at a different size in every DNA sample (Fig. 4).

Some lines allow their hardening on soil-type substrate, supplying material to perform some greenhouse phenotypical tests. After applying a 0.5% solution of the commercial Glyphosate dose in a time period of 10 d, tolerance was assumed as the survival of treated plants. In contrast, susceptibility was considered as death of treated plants. The KJ7, KJ8, KJ15, KJ18 and PJ32 lines were found to effectively tolerate the herbicide, as there was no plant death after 10 d (Fig. 5C), whereas lines KJ24, KJ47 and SJ1 did not tolerate the herbicide application and died (Fig. 5D).

Discussion

Transformation efficiency in soybean using *A. tumefaciens* is, in general terms low, mainly due to difficulties in the T-DNA transfer efficiency. To obtain transformed plants in regeneration systems, it is necessary to use hypervirulent strains of *Agrobacterium* (Atif *et al.*, 2013). In the present study, three soybean varieties adapted to cropping conditions in Colombian territory were used, and considering that it is the first research carried out in the country on soybean genetic transformation, it was absolutely necessary to establish a transformation platform for this species, evaluating diverse factors that could affect the process, as the predisposition of local varieties to be genetically transformed.

The transformation efficiency in soybean is highly genotype-dependent (Atif *et al.*, 2013), which may be linked to its regeneration capacity, among other factors. Paz *et al.* (2004) reported that Williams variety had a higher rate of regeneration within a set of 10 different soybean varieties with 100% regeneration. Within this set, the lowest rate of regeneration was obtained by the Harosoy variety, with 68%. In this study, the selected plant genotypes had different performances in each of the developed tests. In evaluation of regeneration capacity, it was observed that the P29 variety had the highest percentage of regeneration (96.2%), while SK7 had the lowest percentage (75.4%).

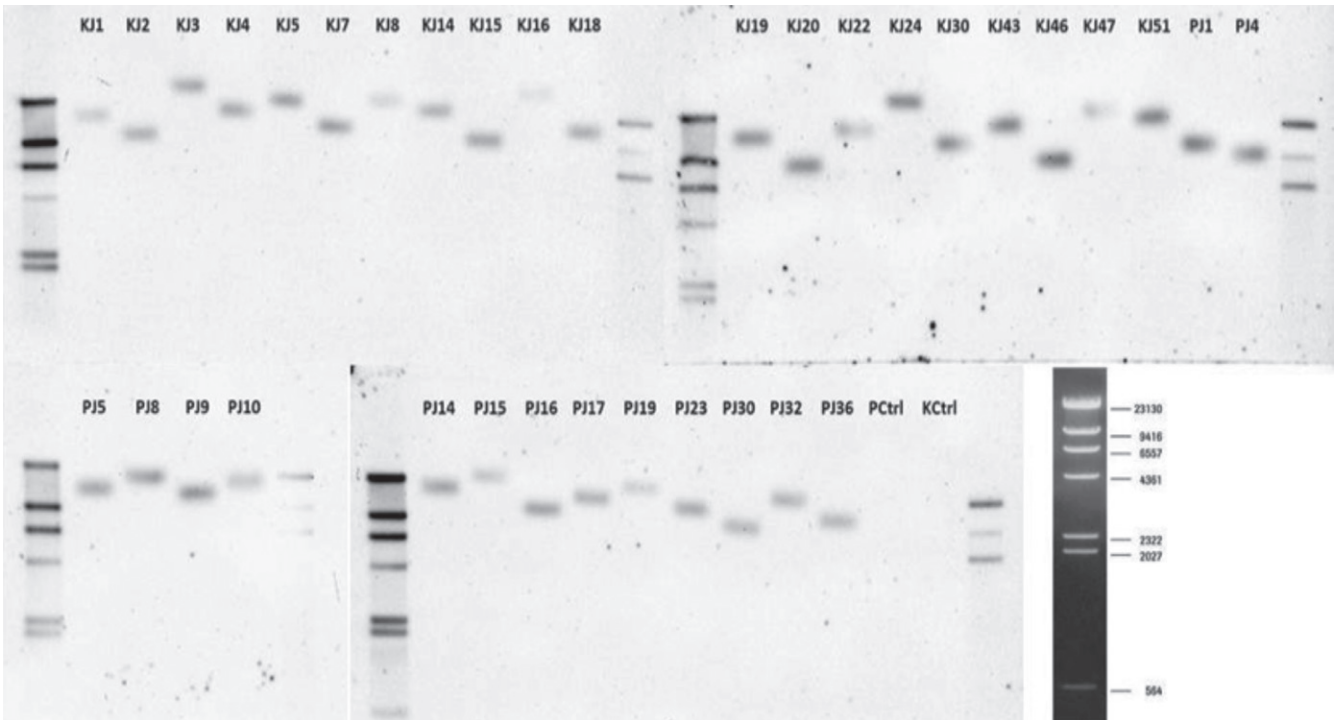


FIGURE 4. Southern blotting on positive PCR samples. The name of each transformant is indicated on the top of each lane. PCtrl: Non transformed P29 variety, KCtrl: Non transformed SK7 variety. Each membrane has, on its left side, a molecular marker, corresponding to DNA Molecular Weight Marker II, DIG-labeled (Sigma-Aldrich Corp. St. Louis, MO, USA), and, on its right side, a positive control, corresponding to E-IGP cassette integrated into pCAMBIA1301 non-linearized vector.

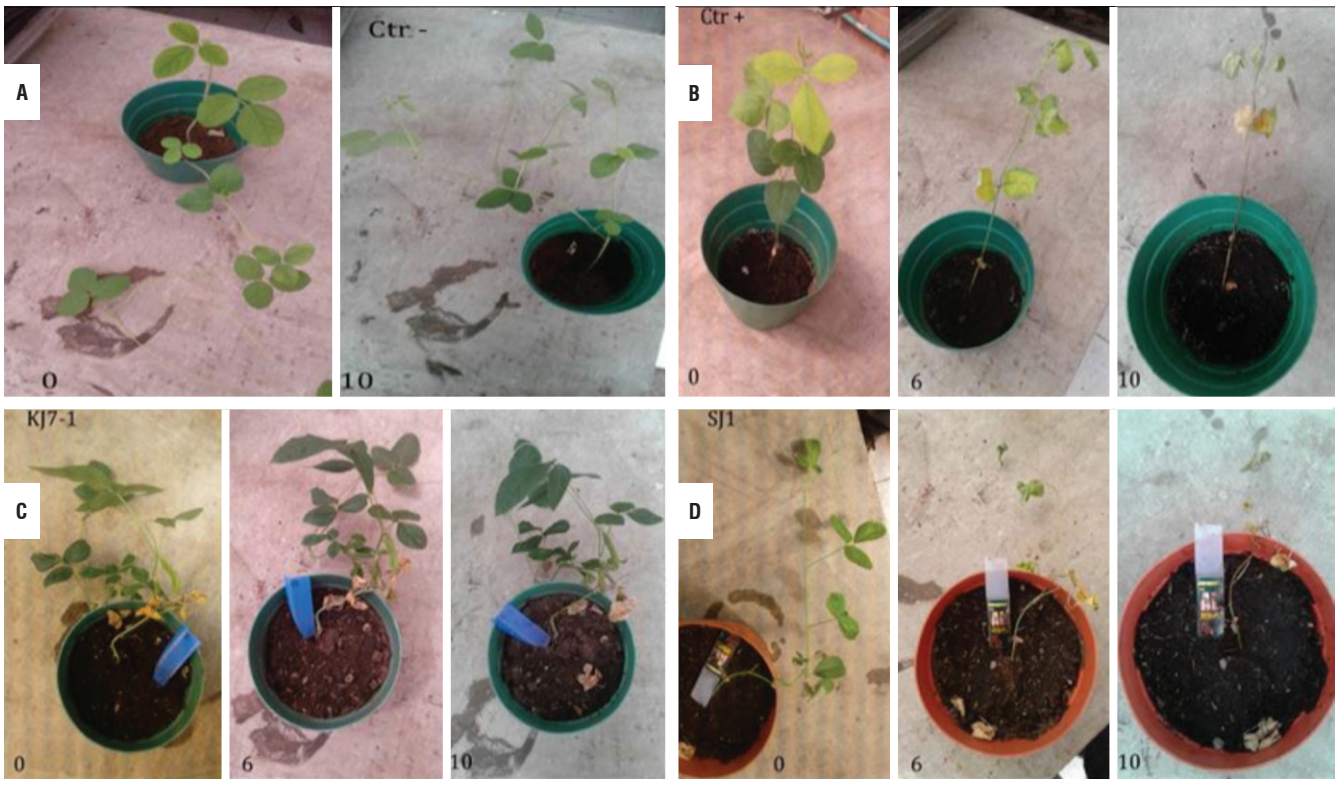


FIGURE 5. Greenhouse test for Glyphosate tolerance. A. Negative control, corresponding to a non-transformed plant painted with the herbicide solvent (tap water). B. Positive control, corresponding to a non-transformed plant painted with 0.5% commercial Glyphosate. C. Tolerant line, corresponding to KJ7 line. D. Susceptible line, corresponding to SJ1 line. C and D were painted with 0.5% commercial Glyphosate. Labels regarding the name of the lines are located at the top of each picture. The next day after the application event is located at the bottom of each picture.

However, SK7 compensates its low regeneration percentage with a high rate of shoot production per explant, with 17.7 shoots on average, being the highest value among the evaluated genotypes. In general, regeneration of the three plant varieties was good with the tested system, considering that percentage values of explants with shoots and number of shoots per explant are adequate to take as baseline on a process of genetic transformation.

Regeneration differences between genotypes could be related to the balance of growth regulators present in SIM and its interaction with tissues and endogenous factors of each variety. Concentration of growth regulators must be optimized for each variety separately (Paz *et al*., 2006; Soto *et al*., 2013; Li *et al*., 2017).

It is important to include a selector agent in SIM to avoid the generation of chimerisms, or escapes from the transformation system. Most soybean transformation trials follow an in-vitro and greenhouse selection using glufosinate ammonium (Zhang *et al*., 1999; Paz *et al*., 2004; Zeng *et al*., 2004; Paz *et al*., 2006; Xue *et al*., 2006; Song *et al*., 2013; Jia *et al*., 2015; Yang *et al*., 2016; Li *et al*., 2017), Kanamycin (Liu *et al*., 2004) or Hygromycin (Olhoft *et al*., 2003; Arun *et al*., 2015; Kuma *et al*., 2015). There are fewer reports on Glyphosate selection (Clemente *et al*., 2000; Soto *et al*., 2016). In this report, a selection scheme was used to produce adequate plants further individualized and propagated as primary transformants. It is important to emphasize that Glyphosate selection is based on phenotypical criteria (shoot height and appearance) instead of the categorical criteria used for other selector agents as live/dead shoots.

The maximum transformation frequencies obtained in other reports, with different soybean varieties, are generally ranging between 4 and 15.8%, with an average of 8.5% (Olhoft *et al*., 2003; Liu *et al*., 2004; Paz *et al*., 2004; Zeng *et al*., 2004; Paz *et al*., 2006; Yukawa *et al*., 2008; Jia *et al*., 2015; Yang *et al*., 2016; Li *et al*., 2017). In this report, a maximum transformation frequency on SK7 variety of 10.8% was obtained, which is above the average, reported for other varieties. P29 variety was also transformed with a frequency of 6.1%. This indicates that there are Colombian soybean varieties with potential to be genetically transformed, in this case, with special success on SK7 variety which had the highest regeneration capacity and also the highest transformation frequency.

Differences between varieties regarding their capacity to be transformed can be associated with defensive responses of plant tissues against bacterial infection and the capacity of cellular division, which varies between plant genotypes (Jia *et al*., 2015). There was a slight difference between both bacterial strains in relation to their infective capacity, showing AGL0 a higher infective ability than EHA105 on SK7. Both strains are derivatives from EHA101 strain, which in turn is derived from hypervirulent A281 strain, with a C58 chromosomic background (a nopaline type strain) (Lazo *et al*., 1991; Hood *et al*., 1993). So, considering that both strains have the same chromosomic background and vir helper vector, similar results are expected.

Recently, genome editing has been developed by addition, removal, or alteration on specific bases in the plant genome. A relevant technology of genome edition is CRISPR-Cas9 (clustered regularly interspaced short palindromic repeats associated to protein Cas9). The CRISPR-Cas9 technology has their origin in a bacterial defense system from viruses using RNA segments to target viral DNA, so as a result, Cas9 protein cut the parasite DNA. Basically, CRISPR-Cas9 is based on a guide RNA segment that binds to a specific sequence of DNA, using a Cas9 enzyme. This enzyme cuts the target DNA and repairs the cell machinery to add or remove the desired sequences. CRISPR-Cas9 system leaves no traces on the genome, which has been postulated as a form of genome modification different to transgenesis (Hussain *et al*., 2018). This affair was assessed by the European Union on July 25, 2018 claiming that genome edition should be subject to the same regulations as the conventional GM crops. CRISPR-Cas9 system has been applied in soybean by stress tolerance (edition of Sucrose non-fermenting related protein kinases), multiple loci edition, flowering delay (by edition of GmFT2a gene in a specific base or short deletion), and promoter edition with relevance in expression, among others (Du *et al*., 2016; Cai *et al*., 2018; Li *et al*., 2018; Kanazashi *et al*., 2018).

This work is a preliminary approach to establish a stable transformation platform for crops of economic relevance such as soybean in Colombia. It was possible to produce entire plants with evident Glyphosate tolerance in greenhouse conditions, but the process should be subjected to further optimization to get a totally refined platform. This is an important step to initiate the production of generic transgenics in this country, specifically designed to develop Glyphosate tolerance as a transgenic trait in soybean varieties, while the patents protecting this technology expired in recent years (Jefferson *et al*., 2015).

Acknowledgments

The authors would like to thank S. Leal, Y. Rodríguez and J. Mora for their technical assistance and to Kamerun and Panorama seed companies for their collaboration

as seed donors. This work was funded by the Colombian National Federation of Cereals and Legumes Farmers (Fenalce - Research and Transfer Program of National Fund for Soybean). The authors express their gratitude to Fenalce for its commitment to develop science and technology in our country. Rojas A.C. express her gratefulness to the National Department of Science and Technology (Colciencias, Call for Scholarships No. 528/2011) for her Ph.D. fellowship.

Literature cited

Arun, M., K. Subramanyam, T.S. Mariashibu, J. Theboral, G. Shivanandhan, M. Manickavasagam, and A. Ganapathi. 2015. Application of sonication in combination with vacuum infiltration enhances the *Agrobacterium*-mediated genetic transformation in Indian soybean cultivars. Appl. Biochem. Biotechnol. 175, 2266-2287. Doi: 10.1007/s12010-014-1360-x

Atif, R.M., E.M. Patat-Ochatt, L. Svabova, V. Ondrej, H. Klenoticova, L. Jacas, M. Griga, and S.J. Ochatt. 2013. Gene transfer in legumes. pp. 37-100. In: Lüttge, U., W. Beyschlag, D. Francis, and J. Cushman (eds.). Progress in Botany 74. Springer-Verlag, Berlin Heidelberg. Doi: 10.1007/978-3-642-30967-0_2

Bonny, S. 2008. Genetically modified Glyphosate-tolerant soybean in the USA: adoption factors, impacts and prospects. A review. Agron. Sustain. Dev. 28, 21-32. Doi: 10.1051/agro:2007044

Bourras, S., T. Rouxel, and M. Meyer. 2015. *Agrobacterium tumefaciens* gene transfer: how a plant pathogen hacks the nuclei of plant and nonplant organisms. Phytopathol. 105, 1288-1301. Doi: 10.1094/PHYTO-12-14-0380-RVW

Cai, Y., L. Chen, X. Liu, C. Guo, S. Sun, C. Wu, B. Jiang, T. Han, and W. Hou. 2018. CRISPR/Cas9-mediated targeted mutagenesis of *GmFT2a* delays flowering time in soya bean. Plant Biotechnol. J. 16, 176-185. Doi: 10.1111/pbi.12758

Clemente, T.E., B.J. LaVallee, A.R. Howe, D. Conner-Ward, R.J. Rozman, P.E. Hunter, D.L. Broyles, D.S. Kasten, and M.A. Hinchee. 2000. Progeny analysis of Glyphosate selected transgenic soybeans derived from *Agrobacterium*-mediated transformation. Crop Sci. 40, 797-803. Doi: 10.2135/cropsci2000.403797x

Doyle, J. 1991. DNA protocols for plants. pp. 283-293. In: Hewitt G.M., A.W.B. Johnston, and J.P.W. Young (eds.). Molecular techniques in taxonomy. Vol 57. Springer, Berlin, Heidelberg. Doi: 10.1007/978-3-642-83962-7_18

Du, H., X. Zeng, M. Zhao, X. Cui, Q. Wang, H. Yang, H. Cheng, and D. Yu. 2016. Efficient targeted mutagenesis in soybean by TALENs and CRISPR/Cas9. J. Biotechnol. 217, 90-97. Doi: 10.1016/j.jbiotec.2015.11.005

Duke, S.O. and A.L. Cerdeira. 2010. Transgenic crops for herbicide resistance. pp. 133-166. In: Kole C., C.H. Michler, A.G. Abbott, and T.C. Hall (eds.). Transgenic crop plants volume 2: Utilization and biosafety. Springer, Heidelberg, Germany. Doi: 10.1007/978-3-642-04812-8_3

Duke, S.O. and S.B. Powles. 2008. Glyphosate: a once-in-a-century herbicide. Pest Manag. Sci. 64, 319-325. Doi: 10.1002/ps.1518

Eisel, D., O. Seth, S. Grünewald-Janho, B. Kruchen, and B. Rüger (eds.). 2008. DIG Application Manual for Filter Hybridization. Editorial Roche Diagnostics GmbH.

Fenalce - Federación Nacional de Cultivadores de Cereales y Leguminosas. 2017. Situación del sector soya. Informe del Departamento de Información Económica y Estadística. URL: http://www.fenalce.org/nueva/plantillas/arch_web/Situacion_del_Sector_II_Trimestre_2017_-_Soya.pdf (accessed August 2017).

Gamborg, O.L., R.A. Miller, and K. Ojima. 1968. Nutrient requirements of suspension cultures of soybean root cells. Exp. Cell Res. 50, 151-158. Doi: 10.1016/0014-4827(68)90403-5

Hood, E.E., S.B. Gelvin, L.S. Melchers, and A. Hoekema. 1993. New *Agrobacterium* helper plasmids for gene transfer to plants. Transgenic Res. 2, 208-218. Doi: 10.1007/BF01977351

Hollander, M., D.A. Wolfe, and E. Chicken. 2014. Nonparametric statistical methods. John Wiley & Sons, Hoboken, New Jersey, USA. Doi: 10.1002/9781119196037

Hussain, B., S.J. Lucas, and H. Budak. 2018. CRISPR/Cas9 in plants: at play in the genome and at work for crop improvement. Brief. Funct. Genomics 1-10. Doi: 10.1093/bfgp/ely016

ICA - Instituto Colombiano Agropecuario. 2010. Resolución No 2404 del 19 de Julio de 2010, Por la cual se autoriza siembras comerciales de soya Roundup Ready® (MON-Ø4Ø32-6) para la subregión natural de la Orinoquía.

ILSI Research Foundation. 2017. GM crop database. ILSI Research Foundation, Washington D.C. URL: http://cera-gmc.org/gmcropdatabase (accessed August 2017).

ISAAA. 2016. Global status of commercialized Biotech/GM crops: 2016. ISAAA Brief No. 52. Ithaca, NY, USA.

Jefferson, D.J., G.D. Graff, C.L. Chi-Ham, and A.B. Bennett. 2015. The emergence of agbiogenerics. Nature Biotechnol. 33, 819-823. Doi: 10.1038/nbt.3306

Jia, Y., X. Yao, M. Zhao, Q. Zhao, Y. Du, C. Yu, and F. Xie. 2015. Comparison of soybean transformation efficiency and plant factors affecting transformation during the *Agrobacterium* infection process. Int. J. Mol. Sci. 16, 18522-18543. Doi: 10.3390/ijms160818522

Jiménez, J.P. 2014. Diseño de genes semi-sintéticos que confieran tolerancia a herbicidas en soya. MSc. thesis. Universidad Nacional de Colombia, Bogota.

Kanazashi, Y., A. Hirose, I. Takahashi, M. Mikami, M. Endo, S. Hirose, S. Toki, A. Kaga, K. Naito, M. Ishimoto, J. Abe, and T. Yamada. 2018. Simultaneous site-directed mutagenesis of duplicated loci in soybean using a single guide RNA. Plant Cell Rep. 37, 553-563. Doi: 10.1007/s00299-018-2251-3

Klümper, W. and M. Qaim. 2014. A meta-analysis of the impacts of genetically modified crops. PLOS One. 9, e111629. Doi: 10.1371/journal.pone.0111629

Kuma, K.M., V.S. Lopes-Caitar, C.C.T. Romero, S.M.H. Silva, M.K. Kuwahara, M.C.C.G. Carvalho, R.V. Abdelnoor, W.P. Dias, and F.C. Marcelino-Guimarães. 2015. A high efficient protocol for soybean root transformation by *Agrobacterium rhizogenes* and most stable reference genes for RT-qPCR analysis. Plant Cell Rep. 34, 1987-2000. Doi: 10.1007/s00299-015-1845-2

Lazo, G.R., P.A. Stein, and R.A. Ludwig. 1991. A DNA Transformation-competent *Arabidopsis* genomic library in *Agrobacterium*. Nature Biotechnol. 9, 963-967. Doi: 10.1038/nbt1091-963

Li, H.Q., C. Chen, R.R. Chen, X.W. Song, J.N. Li, Y.M. Zhu, and X.D. Ding. 2018. Preliminary analysis of the role of GmSnRK1.1 and GmSnRK1.2 in the ABA and alkaline stress response of the

soybean using the CRISPR/Cas9-based gene double-knockout system. Yi Chuan 40, 496-507. Doi: 10.16288/j.yczz.17-424

Li, S., Y. Cong, Y. Liu, T. Wang, Q. Shuai, N. Chen, J. Gai, and Y. Li. 2017. Optimization of *Agrobacterium*-mediated transformation in soybean. Front. Plant Sci. 8, 246. Doi: 10.3389/fpls.2017.00246

Liu, Z., H. Xiong, H. Xie, Y. Tao Qin, Z. Ran, and B. Guo. 2013. A technique for *Agrobacterium*-mediated transformation via germinating seeds of soybean. Adv. Mater. Res. 749, 413-417. Doi: 10.4028/www.scientific.net/AMR.749.413

Liu, H.K., C. Yang, and Z.M. Wei, 2004. Efficient *Agrobacterium tumefaciens*-mediated transformation of soybeans using an embryonic tip regeneration system. Planta 219, 1042-1049. Doi: 10.1007/s00425-004-1310-x

Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiol. Plant. 15, 473-497. Doi: 10.1111/j.1399-3054.1962.tb08052.x

Olhoft, P.M., L.E. Flagel, C.M. Donovan, and D.A. Somers. 2003. Efficient soybean transformation using hygromycin B selection in the cotyledonary-node method. Planta 216, 723-735.

Paz, M.M., H. Shou, Z. Guo, Z. Zhang, A.K. Banerjee, and K. Wang. 2004. Assessment of conditions affecting *Agrobacterium*-mediated soybean transformation using the cotyledonary node explant. Euphytica 136, 167-179. Doi: 10.1023/B:EUPH.0000030669.75809.dc

Paz, M.M., J.C. Martinez, A.B. Kalvig, T.M. Fonger, and K. Wang. 2006. Improved cotyledonary node method using an alternative explant derived from mature seed for efficient *Agrobacterium*-mediated soybean transformation. Plant Cell Rep. 25, 206-213. Doi: 10.1007/s00299-005-0048-7

R Development Core Team. 2008. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. URL: http://www.R-project.org

Song, Z., J. Tian, W. Fu, L. Li, L. Lu, L. Zhou, Z. Shan, G. Tang, and H. Shou. 2013. Screening Chinese soybean genotypes for *Agrobacterium*-mediated genetic transformation suitability. J. Zhejiang Univ. Sci. B 14, 289-298. Doi: 10.1631/jzus.B1200278

Soto, N., C. Delgado, Y. Hernández, Y. Rosabal, A. Ferreira, M. Pujol, F. Aragão, and G.A. Enríquez. 2016. Efficient particle bombardment-mediated transformation of Cuban soybean (INCA-Soy-36) using Glyphosate as a selective agent. Plant Cell Tissue Organ Cult. 128, 187-196. Doi: 10.1007/s11240-016-1099-x

Soto, N., A. Ferreira, C. Delgado, and G.A. Enríquez. 2013. *In vitro* regeneration of soybean plants of the Cuban Incasoy-36 variety. Biotecnol. Aplicada 30, 34-38.

Tillmann, M. and S. West. 2004. Identification of genetically modified soybean seeds resistant to Glyphosate. Sci. Agric. 61, 336-341. Doi: 10.1590/S0103-90162004000300017

USDA - United States Department of Agriculture. 2017. Oilseeds: world markets and trade. Informative circular from the foreign agricultural service. URL: https://apps.fas.usda.gov/psdonline/circulars/oilseeds.pdf (accessed August 2017).

Valencia, R.A. and G.A. Ligarreto. 2010. Mejoramiento genético de la soya (*Glycinemax* [L.] Merril) para su cultivo en la altillanura colombiana: una visión conceptual prospectiva. Agron. Colomb. 28, 155-163.

Widholm, J.M., J.J. Finer, L.O. Vodkin, H.N. Trick, P. LaFayette, J. Li, and W. Parrott. 2010. Soybean. pp. 473-498. In: Kempken F. and C. Jung (eds.). Genetic modification of plants, biotechnology in agriculture and forestry. vol. 64, Springer-Verlag, Berlin, Heidelberg. Doi: 10.1007/978-3-642-02391-0_24

Xue, R.G., H.F. Xie, and B. Zhang. 2006. A multi-needle-assisted transformation of soybean cotyledonary node cells. Biotechnol. Lett. 28, 1551-1557. Doi: 10.1007/s10529-006-9123-6

Yang, X.F., X.Q. Yu, Z. Zhou, W.J. Ma, and G.X. Tang. 2016. A high-efficiency *Agrobacterium tumefaciens* mediated transformation system using cotyledonary node as explants in soybean (*Glycine max* L.). Acta Physiol. Plant. 38, 60. Doi: 10.1007/s11738-016-2081-2

Yukawa, K., H. Kaku, H. Tanaka, Y. Koga-Ban, and M. Fukuda. 2008. Enhanced soybean infection by the legume "Super-Virulent" *Agrobacterium tumefaciens* strain KAT23. Biosci. Biotechnol. Biochem. 72, 1809-1816. Doi: 10.1271/bbb.80080

Zeng, P., D.A. Vadnais, Z. Zhang, and J.C. Polacco. 2004. Refined glufosinate selection in *Agrobacterium*-mediated transformation of soybean [*Glycine max* (L.) Merrill]. Plant Cell Rep. 22, 478-482. Doi: 10.1007/s00299-003-0712-8

Zhang, Z., A. Xing, P. Staswick, and T.E. Clemente. 1999. The use of glufosinate as a selective agent in *Agrobacterium*-mediated transformation of soybean. Plant Cell Tissue Organ Cult. 56, 37-46. Doi: 10.1023/A:1006298622969

Interaction between timing of foliar fertilizer application and different Metribuzin doses in carrot

Interacción entre la época de aplicación de un fertilizante foliar y diferentes dosis de Metribuzin en zanahoria

Gustavo Soares da Silva[1]*, Gabriella Daier Oliveira Pessoa Carneiro[2], Valesca Pinheiro Miranda[3], Christiane Augusta Diniz Melo[4], Marcelo Rodrigues dos Reis[5], and Antonio Alberto da Silva[6]

ABSTRACT

The objective of this research was to evaluate the potential of the foliar fertilizer FertiG as an attenuator of Metribuzin toxicity in the carrot crop. Experiments were carried out in the 2014 and 2015 crops cycles. The treatments were arranged in a 4 x 3 factorial scheme, four times of foliar fertilizer application and three doses of Metribuzin (0, 288 and 576 g ha^{-1}). In crop 1, the foliar fertilizer with the herbicide (288 g ha^{-1}) increased the commercial yield 5 d before the Metribuzin application. The use of FertiG associated to Metribuzin reduced the production of forked carrots 5 d before application and without the foliar fertilizer. The tank mixture decreased the total discard as well as the total yield (576 g ha^{-1}). In crop 2, the commercial yield increased 5 d before (576 g ha^{-1}) and 5 d after (288 g ha^{-1}) the application of Metribuzin when the leaf fertilizer was associated with the herbicide. The yield of the forked carrots decreased 5 d after the application of Metribuzin (288 g ha^{-1}), as well as the total discard. Thus, when associated with Metribuzin (288 g ha^{-1}), FertiG has the potential to attenuate the herbicide toxicity in carrots.

Key words: forked carrots, *Daucus carota*, herbicide, phytotoxicity, commercial yield.

RESUMEN

El objetivo fue evaluar el potencial del fertilizante foliar FertiG como atenuador de la toxicidad de Metribuzin en el cultivo de zanahoria. Se realizaron experimentos en las cosechas 2014 y 2015. Los tratamientos fueron arreglados en un esquema factorial 4 x 3, realizando cuatro aplicaciones del fertilizante foliar en diferentes épocas y tres dosis de Metribuzin (0, 288 y 576 g ha^{-1}). En la cosecha 1, el fertilizante foliar con el herbicida (288 g ha^{-1}) aumentó la productividad comercial cinco d antes de la aplicación del Metribuzin. El uso de FertiG asociado al Metribuzin redujo la producción de zanahorias bifurcadas cinco d antes de la aplicación y sin el fertilizante foliar. La mezcla en tanque disminuyó el descarte total, así como la productividad total (576 g ha^{-1}). En la cosecha 2, la productividad comercial aumentó cinco d antes (576 g ha^{-1}) y cinco d después (288 g ha^{-1}) de la aplicación del Metribuzin cuando se asoció el fertilizante foliar al herbicida. Las zanahorias bifurcadas disminuyeron cinco d después de la aplicación del Metribuzin (288 g ha^{-1}), así como el descarte total. Así, FertiG tiene potencial para atenuar la intoxicación en zanahoria bifurcada (288 g ha^{-1}) cuando se asocia al Metribuzin.

Palabras clave: zanahoria bifurcada, *Daucus carota*, herbicida, fitotoxicidad, productividad comercial.

Introduction

Carrot *(Daucus carota* L.) is native to the Mediterranean Sea region in the area where Afghanistan is located nowadays and transported to South America in the 16th century. It is a crop of great relevance in the Brazilian horticultural sector, occupying the fifth position among the vegetables with the largest participation in the production of the country (Carvalho *et al*., 2013). Known as the largest producer of vegetables in Brazil, the Alto Paranaiba region at Minas Gerais state, accounts for approximately 50% of the national carrot production (Anuário Brasileiro de Hortaliças, 2016), which is notorious for the city of São Gotardo. The carrot is a species of the family Apiaceae with presence of tuberous axial roots. It has a high content of beta-carotene and requires typical crop labors to avoid planting intolerance, control high water rate and to allow the proper soils conditions for the good development of the root.

Received for publication: 18 October, 2017. Accepted for publication: 21 February, 2018 Doi: 10.15446/agron.colomb.v36n1.68388

[1] University of São Paulo, Superior College of Agriculture "Luiz de Queiroz", Piracicaba (Brazil).
[2] Federal Rural University of Semi-árido, Mossoró (Brazil).
[3] Paulista State University "Júlio de Mesquita Filho", Botucatu (Brazil).
[4] Federal University of Triângulo Mineiro, Iturama (Brazil).
[5] Federal University of Viçosa – campus Rio Paranaíba, Rio Paranaíba (Brazil).
[6] Federal University of Viçosa, Viçosa (Brazil).
* Corresponding author: gustavusoares@gmail.com

CC BY NC ND

Weed interference has been one of the main biotic factors causing reduced yield and increased carrot production costs. The application of herbicides has been the most used method for weed control because it is the most efficient and the least labor demanding (Mascarenhas, 1984; Silva *et al*., 2017). The herbicides Clethodim, Linuron, Fenoxaprop, Fluazifop and Trifluralin (Agrofit, 2018) are registered for use in carrot cultivation. This low number of registered herbicides has hindered weed management by farmers, who often use herbicides recommended for other crops.

Metribuzin is an herbicide of the chemical group of triazinones, which acts as an inhibitor of photosystem II (Rodrigues and Almeida, 2011), and although it is not registered for the carrot crop in the country, it has already been used in post emergence by producers in the Alto Paranaiba. However, some research indicate that the use of Metribuzin may cause plant toxicity, which is usually observed due to the damage to the shoot organs of the plant (Bellinder *et al*., 1997; Jensen *et al*., 2004). These damages can cause a reduction of the green area of the leaves and consequently the decrease in the photosynthetic rate, the conversion of photoassimilates and the development of the plant, reducing the production and causing the marketable fraction to lose market value (Zobiole *et al*., 2010).

Substances known as safeners, which can be foliar fertilizers, plant growth regulators, amino acids, among others, can avoid problems caused by herbicides. These compounds have the ability to regulate adaptive responses of the plant to detoxify superoxide anions, which modifies the plant metabolism (Ananievaa *et al*., 2004). It has been observed that in some crops biostimulants are effective in minimizing the toxic effects of herbicides (Zobiole *et al*., 2011). Metribuzin has, as detoxification mechanism in the plant, the conjugation of the herbicidal molecule with the UDP-glucose through the enzyme glycosyl transferase (GT), or with the GSH by the enzyme glutathione S-transferase (GST) (Anzalone, 2010).

The safeners on the market are mainly applied to grain crops. These crops are cultivated and highly consumed around the world, thus high yields are constantly required (Galon *et al*., 2011). Due to the economic importance of these crops, researches have been focused on understanding the relationship between herbicides and grain crops systems, so information of this nature in the vegetables area is scarce.

Vegetable growers have used selective herbicides applied in post emergence, because they control weeds with morphological features similar to those plants that infest. However, these crops are not a target for the development and registration of specific products; thus, biostimulants associated with herbicides are used in post emergence in order to reduce phytotoxicity. In this research, the foliar fertilizer was used in order to assist the defense systems of the plant to metabolize the toxic compounds and protecting the plant from the effects of the herbicide. The main objective of this research was to evaluate the potential use of a foliar fertilizer (FertiG) associated with Metribuzin as attenuator of phytotoxicity in the carrot crop.

Materials and methods

The experiments were carried out in an experimental area located in the city of Rio Paranaiba, Minas Gerais (MG) state (19°14'59.6" S and 46°13'14.4" W), with elevation of 1,073 m a.s.l. The first experiment was performed during the months of August to December 2014 (crop 1) and the second experiment from October 2014 to February 2015 (crop 2). The temperature averages for the first and second crop were 22.72°C and 23.36°C and rainfall of 157.6 mm and 244.6 mm, respectively (Fig. 1). Irrigations were performed during the two crop seasons (crop 1 and 2), with a total irrigation volume of 400 mm, divided into approximately 4 mm per day.

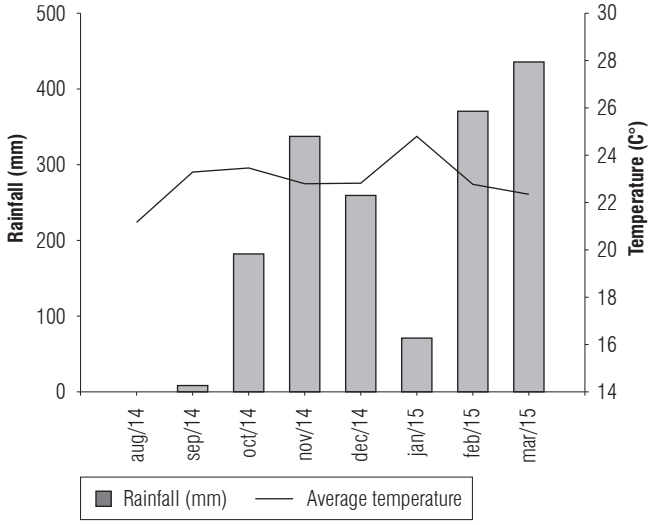


FIGURE 1. Rainfall (mm) and average temperature (°C) from August 2014 to March 2015. Rio Paranaiba – MG.

The soil of the experimental area was classified as a red-yellow Latosol, of clay texture. Based on the chemical and physical analysis of the soil (Tab. 1), fertilization was carried out with 80 kg N ha^{-1}, 600 kg P_2O_5 ha^{-1} and 200 kg K_2O ha^{-1}. The cover fertilization was divided in two applications; on the first one 28.5 kg N ha^{-1}, 6 kg P_2O_5 ha^{-1} and

TABLE 1. Physical and chemical characteristics of the soil of the experimental area. Rio Paranaiba – MG.

pH	P	K	Ca^{2+}	Mg^{2+}	Al^{3+}	H+Al	t	T	MO	P-rem
	mg dm^{-3}		---------- $cmol_c$ dm^{-3} ----------						dag kg^{-1}	mg L^{-1}
6.30	8.20	58.00	4.70	1.00	0.00	2.60	5.85	8.45	2.40	15.60

Sand	Silt	Clay
---------- % ----------		
21	17	62

Extractors: pH - H_2O; P and K - Mehlich 1; Ca, Mg, Al - KCl 1 mol L^{-1}; H+Al - $Ca(OAc)_2$ 0.5 mol L^{-1}; t – CTC effective; T – CTC to pH 7.0; MO – organic matter; P-rem – Remaining soil phosphorus content.

28.5 kg K_2O ha^{-1} were applied, the second event consisted on same composition of the first plus 78 kg K_2O ha^{-1}. The carrot cultivars used were Nayarit (crop 1), which belongs to the Nantes group and is adapted to mild temperatures, and Verano (crop 2), from the Alvorada group, adapted to higher temperatures.

The two experiments were carried out in a randomized complete block design, with four replicates. The treatments were arranged in a 4×3 factorial scheme, the first factor corresponding to the modalities of application of the foliar fertilizer (application 5 d before the herbicide (5 DBA), mixing in the tank with the herbicide (0), application 5 d after the use of the herbicide (5 DAA) and no FertiG application). The second factor was the herbicide Metribuzin doses (0, 288 and 576 g ha^{-1}). The fertilizer used was Fertiactyl® GZ (Timac AGRO, Spain), denominated in this research as FertiG, which has a composition of 13.0% N, 5.0% K_2O and 5.0% organic carbon.

The experimental plots were settled as four crop lines (5 m length) arranged in a double stand each. Plants were spaced at 0.20 m between lines and 0.07 m between double stands. Total research area was equal to 5 m^2; and the evaluation working area only covered 3 m^2. The plant population was 550,000 plants per ha, obtained after manual thinning performed at 20 d after crop emergence. In the total area, 675 g ha^{-1} of Linuron were applied in pre-emergence, avoiding any weed competition area during the crop cycle; manual weeding was performed when needed.

The treatments were applied when the carrot plants had three completely expanded leaves. The application of FertiG at a dose of 1 L ha^{-1} and of the herbicide (associated or not), was performed with a CO_2 pressurized sprayer at 200 kPa. Two spray nozzles 11002 spaced 50 cm apart were used, and the treatments were applied at approximately 50 cm to the target. The volume of application was equivalent to 200 L ha^{-1}.

At 30 and 60 d after application of the herbicide, 10 plants were randomly collected in each experimental plot to determine the shoot dry matter. The samples were conditioned in paper bags and dried in a forced air circulation oven at an average temperature of 72°C until reaching a constant mass, and then weighed in an analytical balance.

At 110 d after planting, the plants contained in the useful area of each plot were harvested and root classification was performed (Ceagesp, 2015). The roots were classified into commercial class (between 10 and 26 cm), forked and total discard (forked + discard). The sum of all classes was subsequently calculated, resulting in total yield.

The data were submitted to analysis of variance by the F test and the average results were compared by the Tukey's test ($P \leq 0.05$).

Results and discussion

Crop 1

Assessing the dry matter of the carrot, the associated use of FertiG and Metribuzin did not provide increments at 30 d. The dry matter of the carrot under the application of Metribuzin increased at 30 d in the absence of FertiG (Tab. 2). At 5 DBA, the use of FertiG associated with Metribuzin (288 and 576 g ha^{-1}) decreased the shoot dry matter of the carrot, and the isolated FertiG did not change. At the other times, the isolated application of FertiG or along with Metribuzin did not change the shoot dry matter.

FertiG increased the shoot dry matter of the carrot 5 DBA and 5 DAA in the absence of the herbicide at 60 d. The use of Metribuzin at a dose of 288 g ha^{-1} associated with FertiG increased dry matter by 23.5% at 60 d, compared to treatment without FertiG application, regardless of the application time at 60 d. The association of the herbicide (576 g ha^{-1}) with FertiG at the time of application (time 0) showed an increase in dry matter in relation to the other treatments (Tab. 2). In the present research, it was observed that the response to FertiG is closely related to the time of application and dose of the herbicide. At 60 d, in the presence of FertiG, the plant recovered the shoot dry matter

TABLE 2. Shoot dry matter of carrot (g) collected at 30 and 60 d after the application of different doses of herbicide Metribuzin associated or not with FertiG* at three different times – Crop 1. Rio Paranaiba – MG.

	Metribuzin (g ha^{-1})			CV (%)
	0	288	576	
	30 d			
5 DBA[1]	1.305 Aa[3]	0.905 Bb	1.004 Bb	14.17
0	1.214 Aab	1.035 Ab	0.962 Ab	
5 DAA[2]	0.931 Ab	0.846 Ab	0.902 Ab	
No application	1.255 Aa	1.482 Aa	1.479 Aa	
	60 d			
5 DBA	5.850 Aa	6.024 Aa	5.294 Bb	5.35
0	4.820 Bb	5.846 Aab	5.991 Aa	
5 DAA	5.842 Aa	5.365 ABb	5.336 Bb	
No application	4.536 Bb	4.392 Bc	5.161 Ab	

[1] DBA: days before application; [2]DAA: days after application; [3]Averages followed by the same capital letter in the row and averages followed by the same lowercase letter in the column do not differ according to the Tukey's test ($P \leq 0.05$); *FertiG - 13.0% N, 5.0% K_2O and 5.0% organic carbon.

due to the effect of FertiG, especially the dose of 288 g ha^{-1}. When evaluating the effect of biostimulant doses on sweet potatoes (*Ipomoea batatas*), Rós *et al.* (2015) also observed significant differences at 28, 37 and 46 d after planting, obtaining satisfactory results at 46 d; it was stated that the increase of the biostimulant dose caused an increase of shoot dry matter.

The use of FertiG as a single solution (dose 0) increased the commercial yield of carrots at 5 DBA, while FertiG associated with Metribuzin (288 g ha^{-1}) also increased commercial yield. However, at the dose 576 g ha^{-1} the yield rate was higher in the absence of FertiG (Tab. 3). In a study by Reghin *et al.* (2000), 10 mL L^{-1}, doses of the growth regulator Stimulate Mo® were tested in Peruvian carrot (*Arracacia xanthorrhiza*) further verifying that the number of roots per plant presented a linear trend response, directly correlated to the increase in the dose of the growth regulator. Dobrei *et al.* (2010) reported that Fertiactyl GZ® presented higher yield and higher sugar content after evaluating five grape fertilizers in grape cultivars for wine production characteristics.

The application of FertiG in the absence of Metribuzin decreased the yield of forked carrots, except at 5 DBA (Tab. 3). FertiG associated with Metribuzin increased the incidence of forked roots at doses 288 g ha^{-1} and 576 g ha^{-1} (time 0

TABLE 3. Commercial and forked carrot root yield (t ha^{-1}) in different application times of FertiG* associated and not associated with Metribuzin – Crop 1. Rio Paranaiba – MG.

	Commercial			Forked		
	Metribuzin (g ha^{-1})			Metribuzin (g ha^{-1})		
Time	0	288	576	0	288	576
5 DBA[1]	43.9 Aa[3]	44.3 Aa	30.3 Bb	4.2 Aa	1.6 Bc	1.5 Bc
0	31.9 Bb	40.1 Ab	29.0 Bb	2.8 Ad	3.6 Ab	2.8 Ba
5 DAA[2]	32.2 ABb	33.5 Ac	29.4 Bb	3.5 Bb	3.8 Aa	2.4 Cb
No application	34.9 Bb	33.6 Bc	39.6 Aa	3.4 Ac	2.6 Bc	2.0 Bc
CV (%)		4.66			5.10	

[1] DBA: days before application; [2]DAA: days after application; [3]Averages followed by the same capital letter in the row and averages followed by the same lowercase letter in the column do not differ according to the Tukey's test ($P \leq 0.05$); *FertiG– 13.0% N, 5.0% K_2O and 5.0% organic carbon.

TABLE 4. Total yield and total discard (forked + discard) of carrot roots (t ha^{-1}) in different application times of FertiG* associated or not with Metribuzin – Crop 1. Rio Paranaiba – MG.

	Total discard			Total yield		
	Metribuzin (g ha^{-1})			Metribuzin (g ha^{-1})		
Time	0	288	576	0	288	576
5 DBA[1]	15.7 Bc[3]	20.1 Aa	21.1 Aa	59.6 Ba	64.4 Aa	51.4 Cb
0	22.3 Aa	13.9 Bb	15.5 Bc	54.2 Ab	54.0 Ab	44.5 Bc
5 DAA[2]	18.2 Abc	18.2 Aa	17.9 Abc	50.4 ABb	51.7 Ab	47.2 Bbc
No application	18.9 Ab	20.0 Aa	19.2 Aab	53.8 Bb	53.5 Bb	58.8 Aa
CV (%)		8.59			4.66	

[1] DBA: days before application; [2]DAA: days after application; [3]Averages followed by the same capital letter in the row and averages followed by the same lowercase letter in the column do not differ according to the Tukey's test ($P \leq 0.05$); *FertiG – 13.0% N, 5.0% K_2O and 5.0% organic carbon.

and 5 DAA). Thus, the absence of FertiG as well as its application before the herbicide had the most representative effect (Tab. 3). At a dose of 576 g ha^{-1}, there was a constant reduction of forked carrots due to FertiG application. In general, the application of Metribuzin did not affect and even decrease the yield of forked carrots, contrary to the suspicions and the reports of producers regarding the action of the herbicide on the formation of carrots of this class.

At dose 0, FertiG isolated decreased the yield of discard carrots, except at time 0 (Tab. 4). In contrast, when the herbicide (288 g ha^{-1} and 576 g ha^{-1}) is associated with FertiG at the time of application, there is a decrease in total discard. The use of FertiG decreases the total discard only in the tank mix (time 0) (Tab. 4).

The application of FertiG in the absence of the herbicide (dose 0) and the association of Metribuzin with the leaf fertilizer (288 g ha^{-1}) increased the total yield by 11% and 17%, respectively, exclusively to the treatment 5 DBA. These results were corroborated with commercial yield data. At the dose 576 g ha^{-1}, a higher total yield was verified in the absence of FertiG application. Metribuzin provided an increase in total yield at doses of 288 g ha^{-1} 5 DBA and 576 g ha^{-1} without FertiG application.

When FertiG was applied along with the herbicide (576 g ha^{-1}) at the time of application, there was a decrease in the carrot classes, except in the commercial class. The tank mix may have an antagonism effect, causing the product formulation to interfere with its efficiency. Therefore, the safener should be applied separately when it causes damage to the crop; antagonism or competition scenarios may occur when the same site of action is shared with the herbicide (Roman and Pinto, 2003). Across all the experiment, when the dose of Metribuzin is increased, at a dose of 576 g ha^{-1} there was a yield decrease in all crop classes except for the total discard. This shows a possible phytotoxicity

TABLE 5. Shoot dry matter of carrot (g) collected at 30 and 60 d after application of different doses of FertiG* associated or not with the herbicide Metribuzin at different times – Crop 2. Rio Paranaiba – MG.

	Metribuzin (g ha^{-1})			CV (%)
	0	288	576	
		30 d		
5 DBA[1]	3.347 Bb[3]	3.273 Bb	4.346 Aa	13.10
0	3.412 Bb	4.315 Aa	4.303 ABa	
5 DAA[2]	3.600 Aab	3.765 Aab	3.720 Aa	
No application	4.536 Aa	4.388 Aa	4.466 Aa	
		60 d		
5 DBA	6.660 Aa	7.242 Aab	5.942 Ab	13.98
0	7.550 Aa	7.652 Aa	6.147 Ab	
5 DAA	6.122 Aa	5.707 Ab	6.102 Ab	
No application	7.320 Ba	3.070 Cc	10.850 Aa	

[1]DBA: days before application; [2]DAA: days after application; [3]Averages followed by the same capital letter in the row and averages followed by the same lowercase letter in the column do not differ according to the Tukey's test ($P \leq 0.05$); *FertiG – 13.0% N, 5.0% K_2O and 5.0% organic carbon.

TABLE 6. Commercial and forked carrot root yield (t ha^{-1}) at different application times of FertiG* associated or not with Metribuzin– Crop 2. Rio Paranaiba – MG.

	Commercial			Forked		
	Metribuzin (g ha^{-1})			Metribuzin (g ha^{-1})		
Time	**0**	**288**	**576**	**0**	**288**	**576**
5 DBA[1]	29.0 Ba[3]	25.8 Bb	34.7 Aa	5.2 Ba	6.2 Bb	11.1 Aa
0	29.3 Aa	30.1 Aa	25.6 Bb	5.0 Ba	10.5 Aa	6.7 Bb
5 DAA[2]	26.7 Bab	30.6 Aa	23.8 Bb	5.2 Aa	4.5 Ab	6.1 Ab
No application	24.5 Bb	28.6 Aab	26.1 ABb	8.1 Ba	11.6 Aa	9.1 ABab
CV (%)		7.20			25.33	

[1]DBA: days before application; [2]DAA: days after application; [3]Averages followed by the same capital letter in the row and averages followed by the same lowercase letter in the column do not differ according to the Tukey's test ($P \leq 0.05$); *FertiG – 13.0% N, 5.0% K_2O and 5.0% organic carbon.

TABLE 7. Total yield and total discard (forked + discard) of carrot roots (t ha^{-1}) at different application times of FertiG* associated or not with Metribuzin – Crop 2. Rio Paranaiba – MG.

	Total discard			Total yield		
	Dose (g ha^{-1})			Dose (g ha^{-1})		
Time	**0**	**288**	**576**	**0**	**288**	**576**
5 DBA[1]	20.2 Ba[3]	21.0 Bbc	25.4 Aa	49.2 Ba	46.9 Bb	60.1 Aa
0	18.2 Ba	24.4 Aab	19.3 Bb	48.2 Bab	54.4 Aa	44.8 Cc
5 DAA[2]	18.9 Aa	17.3 Ac	19.5 Ab	45.5 ABb	47.9 Ab	43.3 Bc
No application	22.2 Ba	27.7 Aa	23.1 Bab	46.7 Bab	56.3 Aa	49.2 Bb
CV (%)		10.57			3.54	

[1]DBA: days before application; [2]DAA: days after application; [3]Averages followed by the same capital letter in the row and averages followed by the same lowercase letter in the column do not differ according to the Tukey's test ($P \leq 0.05$); *FertiG – 13.0% N, 5.0% K_2O and 5.0% organic carbon.

at higher doses even in the presence of FertiG, and the use of the correct dose of the herbicide provides yield gains.

The interaction between a safener and a herbicide is designed to create a synergism towards the weed control (Hatzios and Burgos, 2004). In crop 1, the effect of FertiG use was desirable as long as the application of FertiG before the herbicide (288 g ha^{-1}) caused a preventive effect. Thus, FertiG possibly acted improving the plant's defense system, so that the herbicide was applied at a dose that the plant was able to metabolize, maintaining a greater total and commercial yield and also decreasing the yield of forked carrots.

Crop 2

FertiG applied in association with Metribuzin (576 g ha^{-1}) 5 DBA increased the shoot dry matter of the carrot in 23.8%, compared to the other doses at 30 d (Tab. 5). At 60 d, the application of FertiG associated with Metribuzin (288 g ha^{-1}) increased the dry matter more than 55%, regardless the time of application. However, at 576 g ha^{-1} there was a constant reduction correlated to the absence of FertiG (no application) (Tab. 5). In Canada, Jensen *et al.* (2004) reported that at the third to fifth leaf stages, carrot culture was tolerant to 280 g Metribuzin ha^{-1}. Bellinder *et al.* (1997) reported that the application of up to 280 g Metribuzin ha^{-1}caused mild injury to carrot plants (<18%); the authors further stated that tolerance to Metribuzin in carrot increased along with the leaf development stage.

The FertiG at 5 DBA (576 g ha^{-1}) increased the commercial carrot yield by 27.5% (Tab. 6). The commercial yield increased with the use of FertiG at 5 DAA (288 g ha^{-1}); at a dose of 576 g ha^{-1} the previous herbicide application strategy was considered as the most accurate FertiG application. When evaluating the development of lettuce under the effect of two foliar fertilizers (Fertiactyl GZ® and Ruter AA®), Bezerra *et al.* (2007) observed that with increasing the concentration, there was an increment of 26.2% in root length with Fertiactyl GZ®. For the same biostimulants, Costa *et al.* (2008) reported higher root length in watermelon seedlings.

The combined use of FertiG with the herbicide (288 g ha^{-1}) reduced forked carrots at 5 DBA and 5 DAA. In the presence of Metribuzin at a dose of 288 g ha^{-1}, FertiG in tank mix is considered as the most accurate application. When the dose increased to 576 g ha^{-1}, the best application time is at 5 DBA (Tab. 6). The application of FertiG before and after the herbicide (288 g ha^{-1}) decreased the total discard compared to the control. When FertiG was applied along with Metribuzin at the initial time 0 (576 g ha^{-1}) and 5 DAA (288 g ha^{-1} and 576 g ha^{-1}), it reduced total discard (Tab. 7).

The total yield at a dose of 288 g ha^{-1} increased whether the Metribuzin was applied along with FertiG and alone. At a dose of 576 g ha^{-1}, the previous herbicide application increased the yield compared to the other application events (Tab. 7). The results of the two crops show that the Metribuzin was not toxic to the carrot crop, with total yield being raised at a dose of 576 g ha^{-1} in crop 1 and 288 g ha^{-1} in crop 2, without increasing the total discard rate.

In the climatic conditions of Brazil in high altitude regions, carrots can be cultivated throughout the year according to the average temperature, precipitation and adapted cultivars (Pessoa Carneiro *et al.*, 2017). In this study, each carrot genotype (Nayarit and Verano) was selected for the most appropriate planting season. Jensen *et al.* (2004) reported that there was no reduction in carrot yield when treated with 560 g ha^{-1} of Metribuzin, and differential tolerance to Metribuzin could occur on each of the genotypes grown according to the edaphoclimatic conditions.

Conclusions

Applications of only Metribuzin in the carrot crop did not cause phytotoxicity. However, when Metribuzin was applied at a dose of 288 g ha^{-1} associated with FertiG before or after the application of the herbicide, there was an increase in commercial and total root yields in crop 1 and a reduction of forked carrots in crop 2. Therefore, when FertiG is associated with Metribuzin has the potential to mitigate the phytotoxicity caused to the carrots.

Acknowledgments

The authors wish to thank the National Counsel of Technological and Scientific Development – CNPq and the Agricultural Cooperative of Alto Paranaiba – COOPADAP.

Literature cited

Agrofit - Sistema de Agrotóxicos Fitossanitário. In: Ministério da Agricultura, Pecuária e Abastecimento do Brasil. URL: http://extranet.agricultura.gov.br/agrofit_cons/principal_agrofit_cons (accessed 19 January 2018).

Ananievaa, E.A., K.N. Christova, and L.P. Popova. 2004. Exogenous treatment with Salicylic acid leads to increased antioxidant capacity in leaves of barley plants exposed to Paraquat. J. Plant Physiol. 161(3), 319-328. Doi: 10.1078/0176-1617-01022

Anzalone, A. 2010. Detoxificación de herbicidas en plantas. pp. 13-24. In: Congreso de la Sociedad Venezolana para el Combate de Malezas. Barquisimeto, Venezuela.

Bellinder, R.R., J.J. Kirkyland, and R.W. Wallace. 1997. Carrot (*Daucus carota*) and weed response to Linuron and Metribuzin applied at different crop stages. Weed Technol. 11(2), 235-240. Doi: 10.1017/S0890037X00042895

Bezerra, P.S.G., L.C. Grangeiro, M.Z. Negreiros, and J.F. Medeiros. 2007. Utilização de bioestimulante na produção de mudas de alface. Científica 35(1), 46-50.

Carvalho, A.D.F., F. Resende, J. Pinheiro, R. Pereira, and G.O. Silva. 2013. Avaliação de genótipos de cenoura em sistemas convencional e orgânico de produção nas condições edafoclimáticas do Distrito Federal. URL: https://www.infoteca.cnptia.embrapa.br/bitstream/doc/960913/1/bpd94.pdf (accessed 15 June 2017).

Carvalho, C., B.B. Kist, and M. Treichel. 2016. Cenoura – O que é que há velhinho? Anuário Brasileiro de Hortaliças. 2016. URL: http://www.editoragazeta.com.br/flip/anuario-hortalicas-2016/files/assets/basic-html/index.html#4 (acessed 17 July 2017).

Ceagesp - Companhia de Entrepostos e Armazéns Gerais de São Paulo. 2015. Normas de Classificação – Cenoura. URL: http://www.ceagesp.gov.br/wp-content/uploads/2015/07/cenoura.pdf (accessed 12 May 2017).

Costa, C.L.L., Z.V.B. Costa, C.O. Costa Júnior, R. Andrade, and J.G.R. Santos. 2008. Utilização de bioestimulante na produção de mudas de melancia. Rev. Verde Agroecol. Des. Sust. 3(3), 110-115.

Dobrei, A., F. Sala, A. Ghiţă, and M. Mălăescu. 2010. The usage of small volume fertilizers and biostimulators for increasing the bioproductivity of the vine growing system. J. Agric. Sci., 42(3), 1-908.

Galon, L., C. Maciel, D. Agostinetto, G. Concenço, and P.V.D. Moraes. 2011. Seletividade de herbicidas às culturas pelo uso de protetores químicos. Rev. Bras. Herbic. 10(3), 291-304. Doi: 10.7824/rbh.v10i3.167

Hatzios, K.K. and N. Burgos. 2004. Metabolism-based herbicide resistance: regulation by safeners. Weed Sci. 52(3), 454-467. Doi: 10.1614/P2002-168C

Jensen, K.I.N., D.J. Doohan, and E.G. Specht. 2004. Response of processing carrot to Metribuzin on mineral soils in Nova Scotia. Can. J. Plant Sci. 84(2), 669-676. Doi: 10.4141/P03-083

Mascarenhas, M.H.T. 1984. Controle de plantas daninhas em cenoura e mandioquinha-salsa. Informe Agropecuário 10(120), 36-40.

Pessoa Carneiro, G.D.O., G.S. Silva, A.R. Barbosa, D.V. Silva, and M.R. Reis. 2017. Selectivity of Metribuzin in postemergence of culture of carrot. Planta Daninha 35. Doi: 10.1590/s0100-83582017350100036

Reghin, M.Y., R.F. Otto, and J.D. Silva. 2000. "Stimulate Mo" e proteção com "Tecido não Tecido" no pré-enraizamento de mudas de mandioquinha-salsa. Hortic. Bras. 18(1),53-56.

Rodrigues, B.N. and F.S. Almeida. 2011. Guia de herbicidas. Ediçao dos Autores, Londrina, PR, Brazil.

Roman, E.S. and J.J.O. Pinto. 2003. Antídoto no herbicida. Revista Cultivar. URL: http://www.grupocultivar.com.br/acervo/300 (accessed 27 September 2017).

Rós, A.B., N. Narita, and H.S. Araújo. 2015. Uso de regulador de crescimento na cultura da batata-doce. Científica. 43(2), 135-142. Doi: 10.15361/1984-5529.2015v43n2p135-142

Silva, G.S., G.D.O. Pessoa, D.V. Silva, E.R.D. Campos, M.R. Reis, and A.A. Silva. 2017. Use of Metribuzin, associated with different foliar fertilizers, on carrot crops. Rev. Colomb. Cienc. Hortic. 11(2), 351-358. Doi: 10.17584/rcch.2017v11i2.7355

Zobiole, L.H.S., R.S. Oliveira Jr., J. Constantin, and D.F. Biffe. 2011. Prevenção de injúrias causadas por glyphosate em soja RR por meio do uso de aminoácidos. Planta Daninha 29(1), 195-205. Doi: 10.1590/S0100-83582011000100022

Zobiole, L.H.S., R.S. Oliveira Jr., J. Constantin, D.F. Biffe, and R.J. Kremer. 2010. Uso de aminoácido exógeno na prevenção de injúrias causadas por glyphosate na soja RR. Planta Daninha. 28(3), 643-653. Doi: 10.1590/S0100-83582010000300022

Preferences of *Scirtothrips dorsalis* Hood 1919 (Thysanoptera: Thripidae) for different structures of cotton (*Gossypium hirsutum* L.) plants in the Magdalena warm valley of Colombia

Preferencias de *Scirtothrips dorsalis* Hood 1919 (Thysanoptera: Thripidae) por diferentes estructuras de la planta del algodón (*Gossypium hirsutum* L.) en el valle cálido del Magdalena

Everth Ebratt[1]*, Andrés Rodríguez[2], Buenaventura Monje[2], Edgar Varón[2], Helena Brochero[3], and Arturo Goldarazena[4]

ABSTRACT

Thrips samples were collected from cotton crops in the Andean region of the Magdalena warm valley, an area represented by the Colombian departments of Tolima, Huila and Cundinamarca. Ten cotton plants were randomly selected per hectare in each plot. Five young leaves, five floral buds, five opened flowers and five bolls or fruits were inspected. Immature stages were separated from the adults and a first classification was made according to the present thrips morphotypes, separating the adults of possible *S. dorsalis* specimens from the others. T-Student and Kruskal-Wallis tests were performed in order to find statistical differences between the different evaluated variables. The selectivity of *S. dorsalis* for each plant structure was determined by Z tests, Spearman correlation analysis and the Bray-Curtis similarity index. *Scirtothrips dorsalis* was found in 77% (n = 46) of the inspected sites. The species exhibited greater affinity to the boll, followed by young leaves and buds. Opened flowers constituted a resource not frequented by the pest with a similarity range of I = 0.8 (<1). It is suggested that cotton plants are hosts to the thrips species; it means that the pest life cycle is highly associated to the cotton production in the Andean region. The importance of the results related to the cotton production and other crops associated to the insect species in the region is discussed.

Key words: thrips, bolls, host, selectivity.

RESUMEN

En la región Andina que comprende el valle cálido del alto Magdalena representado por los departamentos de Tolima, Huila y Cundinamarca en Colombia se recolectaron muestras de trips en cultivos de algodón. En cada predio se seleccionaron diez plantas de algodón al azar por hectárea en las cuales se inspeccionaron cinco hojas jóvenes o terminales foliares, cinco botones florales, cinco flores abiertas y cinco cápsulas o frutos. Los estados inmaduros se separaron de los adultos y se hizo una primera clasificación de acuerdo a los morfotipos de trips presentes, separando los adultos de posibles especímenes de *S. dorsalis* de los demás. Se llevaron a cabo pruebas de T y Krustall-Wallis con el fin de encontrar diferencias estadísticas entre las variables evaluadas. La selectividad de *S. dorsalis* por cada estructura de planta fue determinada por medio de pruebas de Z, análisis de correlación de Spearman y el índice de similitud de Bray-Curtis. Se encontró *Scirtothrips dorsalis* en el 77% (n = 46) de los predios inspeccionados. La especie presentó mayor afinidad por las cápsulas, seguido de las hojas jóvenes y botones florales. Las flores abiertas constituyeron un recurso no frecuentado por la plaga con un rango de similitud de I = 0.8 (<1). Se sugiere que las plantas de algodón constituyen hospedantes para la especie, lo que implica que el ciclo de vida de la plaga está altamente asociado a la producción de algodón en la región andina y se discute la importancia de los resultados en términos de la producción de algodón en esta región y otros cultivos que se han encontrado asociados a la especie de insecto.

Palabras clave: trips, cápsulas, hospedero, selectividad.

Introduction

Cotton (*Gossypium hirsutum* L.) is an important crop highly related to the economic, social and agro-industrial development of many communities. In Colombia there are approximately 10,284 ha planted with cotton, mainly distributed in the Caribbean, Orinoquia and Andean regions. The Andean region represents 7,000 ha in the Magdalena warm valley which corresponds to the departments of Tolima, Huila and Cundinamarca. The national average yield is 1,971 kg of fiber ha^{-1} (Conalgodón, 2018).

Received for publication: 31 July, 2017. Accepted for publication: 16 February, 2018

Doi: 10.15446/agron.colomb.v36n1.66714

[1] Instituto Colombiano Agropecuario ICA, Bogota (Colombia).
[2] Corporación Colombiana de Investigaciones Agropecuarias AGROSAVIA, C.I. Nataima, Colombia.
[3] Facultad de Ciencias Agrarias, Universidad Nacional de Colombia, Bogota (Colombia).
[4] Earth and Life Institute – ELIB, Université Catholique de Louvain, Belgium.
* Corresponding author: everth.ebratt@ica.gov.co

CC BY NC ND

The cotton industry generates 10,945 direct jobs with a production cost of $4.5 million pesos/ha (Conalgodón, 2018). As a strategy for pest management, the Instituto Colombiano Agropecuario (ICA) implemented the plan for the exclusion, suppression and eradication of cotton pests (ICA, 2000) in all producing regions. In this context, there are two annual planting seasons, the first one in the first half of the year in the Andean region, and the second one at the end of the second semester in the Orinoquia and Caribbean regions (ICA, 2000).

The Thripidae family comprises important pests of cotton because they are associated with direct damages such as leaf distortion, defoliation, bud and boll abortion, premature boll opening, and indirect damage such as viruses transmission that can affect yield and fiber quality (Wilson and Bauer, 1993; Leigh, 1995; Cermeli *et al*., 2009; EFSA, 2014; ThripsWiki, 2017). In the global context of cotton production, the genera *Caliothrips* Daniel, *Retithrips* Marchal, *Sericothrips* Haliday, *Thrips* Linneaeus, *Frankliniella* Karny and *Scirtothrips* Shull have been registered (Wilson and Bauer, 1993; Leigh, 1995; Mailhot *et al*., 2007; Kumar *et al*., 2013).

The insect *S. dorsalis* Hood, 1919 is a polyphagous species of tropical Asian origin (Ananthakrishnan, 1993) that feeds on young leaves of more than 200 species of dicotyledonous plants in about 40 different botanical families (Hoodle and Mound, 2003; Hooddle *et al*., 2008). *S. dorsalis* feeds preferentially from the epidermis and, sometimes, from palisade tissues and tissues of the apex of young fruits, especially when they are still hidden under the calyx. In many hosts, it can feed on the upper surface when infestation levels are high. Larvae and adults are often located in the midrib or near the damaged leaf tissue area. Pupae can be found in leaf litter, leaf axils, and deformed leaves or under the calyx (Kumar *et al*., 2013). The associated damage in plants infested by *S. dorsalis* is characterized by a silvering on the surface of the infested leaf, feeding scars, linear thickening of the leaf blade, and brown spots on leaves and fruits. A concentric ring often appears in healed tissue around the apex. Poor fruit formation and early leaf senescence take place as well (Hodges *et al*., 2005; Kumar *et al*., 2013).

Thrips *S. dorsalis* are small insects (<1 mm in length), with thigmotactic behavior and similar morphology features as other thrips species (Kumar *et al*., 2011). It is a species of great economic importance in the world, which was registered for the first time in Colombia in 2010 (ICA, 2012). Due to this, it is necessary to evaluate the potential and actual phytosanitary impact of the species at regional and national levels. The objective of the present study was to determine the infestation and preferences of *S. dorsalis* for the structures of young leafs, floral buds, flowers and bolls (fruits) in cotton plants from the Magdalena warm valley of Colombia.

Materials and methods

Study area

The geographical context of this study corresponded to the Andean region, the Magdalena warm valley in the departments of Huila, Tolima and Cundinamarca. The research area presents an altitudinal range below 600 m a.s.l., characterized by tropical dry forest vegetation (bs-T) (Holdridge, 1967; IAvH, 2014). Farms were inspected in the municipalities of Villavieja, Campoalegre (Huila); Venadillo, Natagaima, Ambalema, Lerida, Armero, Espinal (Tolima) and Ricaurte (Cundinamarca) (Fig. 1).

Sampling

The sampling included 60 farms with productive cotton crops (60 to 90 d after sowing) with floral buds, opened flowers and bolls (fruits). All cotton crops corresponded to genetically modified or transgenic varieties. Ten cotton plants were randomly selected per hectare in each plot. Five young leaves or leaf terminals, five floral buds, five opened flowers and five bolls or fruits were randomly inspected in the lower, middle or upper strata of each plant. The thrips were collected using brushes previously moistened with ethanol and deposited in 1.5 mL plastic vials with 70% ethanol. The sampling unit was represented by 20 vials per inspected plant. In each plot, a code that represented the geographical location, the number assigned to the plant and the plant structures B (buds), C (boll), F (flower), H (leaf), P (farms) were assigned (Pedigo, 1996). The samples were packed in plastic bags according to the farm and municipality, and were transported to the Laboratories of Entomology of the Nataima research center of the Corporación Colombiana de Investigación Agropecuaria AGROSAVIA (Espinal-Tolima) and BIOQUALITYAGRO in the municipality of Mosquera, Cundinamarca, with the purpose of preparing the specimens and carrying out the taxonomic identification. The geographical coordinates for some localities were obtained and/or corrected using Google Earth (www.googleearth.com) and the map was made in Arcmap (ArcGis) software (Pulido *et al*., 2015).

Sample treatment

Immature stages were separated from the adults and a first classification was made according to the present thrips morphotypes, separating the adults of possible *S. dorsalis* specimens from the others. The number of adults was recorded and the specimens were rinsed using 5-10% KOH.

Then the specimens were washed with distilled water and dehydrated in ethanol for semi-permanent mounting in Hoyer's solution on slides (Mound and Marullo, 1996). The taxonomic identification was made based on the morphological characters and according to the available keys (Hoddle and Mound, 2003; Moritz *et al.*, 2007; Kumar *et al.*, 2013), and with the aid of a Leica® ZOOM 2000 stereoscope (Wetzlar, Germany) and a Nikon® Type-119YS2-T microscope (Tokyo, Japan) and photographic camera Canon® Type SX530-16MP (Tokyo, Japan).

Data analysis

The abundance of *S. dorsalis* adults in each plant structure (young leaf, floral buds, opened flower and boll) per plant, per plot, in each municipality and department evaluated was determined. Considering this information, the percentages of infestation per plot and the preference of *S. dorsalis* for certain plant structures in cotton plants were estimated. T-Student and Kruskal-Wallis tests were performed in order to find statistical differences between the different evaluated variables (Ebratt *et al.*, 2004). The selectivity of *S. dorsalis* for each plant structure was determined by Z tests ($P \leq 0.05$) with paired comparisons per structure, and Spearman correlation analysis between infested plant structures (STATISTICA v10; InfoStat, 2016). Percentage records of the calculations to obtain the Bray-Curtis similarity index* were transformed on a logarithmic scale in base 10, in order to define the preference for the resource as follows: a) positive preference (I>1), b) neutral or accidental preference (I = 1), and c) non-preference (I<1). This is a statistic used to quantify the compositional dissimilarity between two different sites, based on counts at each site:

$$* \text{BCij} = 1 - \left(\frac{2\,Cij}{Si + Sj}\right)$$

where C_{ij} *Cij* is the sum of the lesser values for only those species in common between both sites, while S_i and S_j *Si* and *Sj* are the total number of specimens counted at both sites (Silveira-Neto *et al.*, 1976; Johnson, 1980; Ramirez, 2006; Zamar, 2011; Yara and Reinoso, 2012).

Results

Presence and distribution

A total of 12,000 plant structure samples were obtained. These samples corresponded to 30 farms in the department of Tolima (10 in the northern zone, 10 in the central zone and 10 in the southern zone), 20 farms in the department of Huila (10 in the northern zone and 10 in the central zone) and 10 farms in the southwestern zone of Cundinamarca.

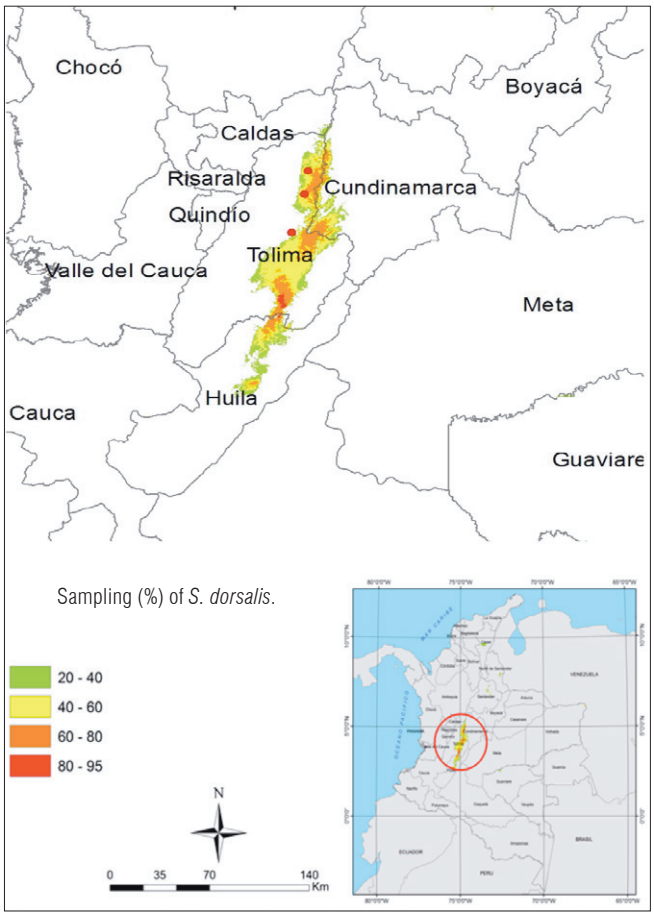


FIGURE 1. Sampling sites of *S. dorsalis* in cotton crops in the Magdalena warm valley.

The thrips *S. dorsalis* was found in 46 farms (77%) with infestation rates between 20 and 100% (Tab. 1). The species was not recorded in 14 farms (23%) located in the municipalities of Armero (5 farms), Ambalema (1 farm), Venadillo (1 farm), El Espinal (5 farms), Natagaima (2 farms), all located in the department of Tolima. The infestation in bolls reached 61.6% (n = 60 ± 6.33), followed by young leaves with 55% (n = 60 ± 6.47), floral buds with 41.66% (n = 60 ± 6.41) and opened flowers with 28.33% (n = 60 ± 5.86). In all cases, *S. dorsalis* was found to be associated with young leaves and bolls (n = 60; $R = 0.4592$; $P<0.001$) whereas flowers and floral buds showed low abundance (C = 0.85; gl = 3; H = 19.65; $P<0.0001$) (Fig. 2). The south of Cundinamarca and south of Tolima presented the highest abundances in young leaves, bolls and floral buds. The same preference was found in the central and northern zones of Huila and in the central area of Tolima, but with significantly low abundances. In the northern region of Tolima, *S. dorsalis* abundances were significantly lower in young leaves, flowers, floral buds and bolls (C = 0.94; gl = 5; H = 19.89; $P<0.0008$) (Fig. 3).

TABLE 1. Sampling and abundance location of *S. dorsalis* Hood (Thysanoptera: Thripidae) in cotton crops from the Magdalena warm valley, Colombia.

Geographic coordinates			Geografic Location				Abundance of *S. dorsalis* in structures			
Number	N	W	Altitude (m a.s.l.)	Departament	Municipality	Life Zone	Leaf	Flower	Floral buds	Boll
1	05° 00' 51,4"	074° 54' 18,4"	312			bs-T	0	0	1	4
2	04° 59' 29,1"	074° 54' 19,3"	312			bs-T	0	0	0	0
3	05° 00' 22,9"	074° 54' 21,4"	296	TOLIMA	Armero	bs-T	0	0	0	0
4	04° 59' 29,1"	074° 54' 19,3"	312			bs-T	0	0	0	0
5	05° 00' 30,1"	074° 54' 22,4"	296			bs-T	0	0	0	0
6	04° 40' 50,2"	074° 54' 31,0"	368	TOLIMA	Ambalema	bs-T	0	0	0	0
7	04° 54' 54,8"	074° 52' 29,6'	275	TOLIMA	Venadillo	bs-T	0	0	0	0
8	04° 40' 40,1"	074° 54' 18,8"	360			bs-T	0	0	0	2
9	05° 06' 24,7"	074° 53' 23,2"	368	TOLIMA	Armero	bs-T	0	1	0	5
10	05° 05' 11,8"	074° 52' 44,8"	324			bs-T	0	0	0	0
11	04° 09' 4,74"	074° 51' 9,6"	318			bs-T	46	8	10	10
12	04° 09' 7,26"	074° 51' 38,2"	330			bs-T	0	0	0	0
13	04° 09' 58,6"	074° 55' 21,8"	356			bs-T	0	3	0	0
14	04° 11' 38,3"	074° 57' 57.7"	391			bs-T	0	0	0	0
15	04° 08' 13,8"	074° 51' 34,6"	321	TOLIMA	El Espinal	bs-T	0	0	0	0
16	04° 07' 38,1"	074° 50' 29,7"	319			bs-T	16	11	0	21
17	04° 09' 24,9"	074° 56' 55.2"	342			bs-T	0	0	2	0
18	04° 09' 24,9"	074° 56' 55,1"	355			bs-T	2	0	0	0
19	04° 07' 37,8"	074° 50' 30,1"	388			bs-T	0	0	0	0
20	04° 11' 21,2"	074° 57' 19,4"	394			bs-T	0	0	0	0
21	03° 29' 30,7"	075° 07' 47,5"	348			bs-T	9	1	15	8
22	03° 29' 41,9"	075° 07' 52,9"	347			bs-T	15	2	4	1
23	03° 29' 48,8"	075° 07' 40,1"	348			bs-T	11	0	6	0
24	03° 29' 3,9"	075° 07' 53,2"	348			bs-T	0	0	0	0
25	03° 29' 2,6"	075° 07' 48,5"	344	TOLIMA	Natagaima	bs-T	5	0	5	0
26	03° 28' 12,4"	075° 07' 43,2"	356			bs-T	0	0	0	0
27	03° 28' 51,1"	075° 07' 32,8"	347			bs-T	5	0	4	0
28	03° 40' 37,9"	075° 06' 9,78"	333			bs-T	4	0	8	6
29	03° 40' 15,7"	075° 05' 50,5"	333			bs-T	27	2	6	19
30	03° 39' 57,3"	075° 05' 40,3"	329			bs-T	5	1	9	2
31	03° 22' 32,8"	075° 09' 45,8"	374			bs-T	0	0	0	3
32	03° 22' 29,5"	075° 09' 43,9"	370			bs-T	0	0	0	5
33	03° 21' 54,9"	075° 09' 46,9"	373			bs-T	3	0	2	1
34	03° 11' 46,2"	075° 13' 45,6"	387			bs-T	0	0	0	1
35	03° 12' 32,4"	075° 13' 37,9"	402	HUILA	Villavieja	bs-T	0	0	2	5
36	03° 12' 35,4"	075° 13' 31,0"	387			bs-T	0	0	0	9
37	03° 12' 40,6"	075° 13' 28,8"	383			bs-T	2	0	3	4
38	03° 12' 29,5"	075° 13' 24,2"	386			bs-T	23	0	0	6
39	03° 20' 46,0"	075° 11' 33,4"	376			bs-T	7	0	3	5
40	03° 20' 48,7"	075° 13' 29,7"	377			bs-T	3	0	1	8
41	02° 41' 34,4"	075° 21' 04,6"	519	HUILA	Campoalegre	bs-T	5	9	5	6
42	02° 41' 35,7"	075° 21' 11,3"	518			bs-T	5	1	1	0

Continue

Geographic coordinates			Geografic Location				Abundance of *S. dorsalis* in structures			
Number	N	W	Altitude (m a.s.l.)	Departament	Municipality	Life Zone	Leaf	Flower	Floral buds	Boll
43	02° 41' 43,6"	075° 21' 13,9"	509	HUILA	Campoalegre	bs-T	1	0	0	3
44	02° 42' 11,1"	075° 21' 01,9"	498			bs-T	5	0	2	12
45	02° 42' 23,5"	075° 20' 54,4"	507			bs-T	0	8	0	10
46	02° 43' 12,2"	075° 19' 01"	547			bs-T	5	5	2	5
47	02° 40' 46,4"	075° 21' 23,6"	526			bs-T	11	5	6	10
48	02° 40' 54,7"	075° 21' 25,9"	527			bs-T	6	6	1	4
49	02° 40' 55,7"	075° 21' 21,8"	525			bs-T	2	9	0	4
50	02° 40' 39.0"	075° 21' 36,2"	532			bs-T	5	0	2	0
51	04° 17' 5,8"	074° 44' 22,0"	302	CUNDINAMARCA	Ricaurte	bs-T	23	0	0	15
52	04° 17' 03,5"	074° 44' 19,4"	301			bs-T	18	0	0	23
53	04° 17' 01,4"	074° 44' 16,3"	301			bs-T	8	0	0	20
54	04° 17' 18,9"	074° 44' 07,4"	305			bs-T	17	0	0	16
55	04° 17' 19,7"	074° 44' 01,5"	311			bs-T	10	0	0	6
56	04° 17' 19,7"	074° 44' 05,2"	345			bs-T	0	0	0	18
57	04° 17' 18,3"	074° 44' 11,0"	283			bs-T	17	3	4	12
58	04° 17' 52,7"	074° 44' 31,7"	291			bs-T	37	0	8	3
59	04° 17' 43,7"	074° 44' 23,8"	297			bs-T	0	1	0	14
60	04° 16' 30,6"	074° 44' 1,9"	290			bs-T	4	0	0	0

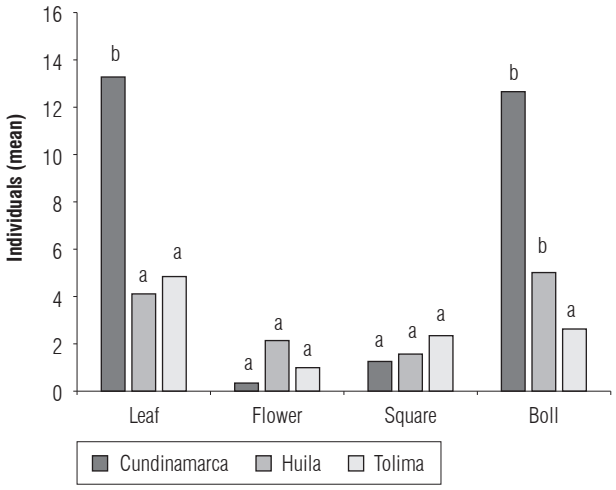


FIGURE 2. Abundance of *S. dorsalis* on plant structures of the cotton crop per department (C = 0.85; gl = 3; H = 19.65; $P<0.0001$).

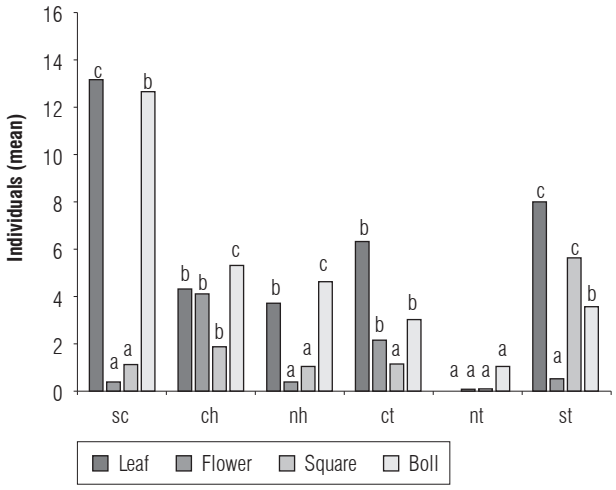


FIGURE 3. Mean abundance of *S. dorsalis* in structures per sampled geographical area (sc. South of Cundinamarca; ch. Central Huila; nh. North of Huila; ct. Central Tolima; nt. North of Tolima; st. South of Tolima; l. Leaf; f. Flower; s. Floral buds; b. Boll) (C = 0.94; gl = 5; H = 19.89; $P<0.0008$).

Preference for structures

According to the similarity indices (Is) applied, significant differences ($P<0.05$) were found in the abundances of *S. dorsalis* between structures in the cotton plant: leaf-flower (Is = 0.40), leaf-bud (Is = 0.71), leaf-boll (Is = 0.52), flower-bud (Is = 0.24), flower-boll (Is = 0.16) and bud-boll (Is = 0.78). The Bray-Curtis similarity-affinity index showed significant differences between the paired structures flower-boll (Rs = 0.6742; $P = 0.01$), flower-leaf (Rs = 0.5900; $P = 0.006$) and boll-leaf (Rs = 0.5277; $P = 0.017$). However, no significant differences were observed between the paired structures flower-bud (Rs = 0.4244; $P = 0.062$) and boll-bud (Rs = 0.4037; $P = 0.077$). The preference analysis based on the abundance of *S. dorsalis* in the evaluated structures revealed that the species had a higher affinity for the bolls (I = 1.7>1), followed by young leaves (I = 1.3>1) and buds (I = 1.2>1). Opened flowers were not a frequented resource for the pest with a similarity range of I = 0.8 (<1) (N = 60;

cophenetic correlation = 0.927; read cases = 60; omitted cases = 0; $P<0.05$). Paired comparisons using the Z test ($P = 0.05$) revealed that there were significant differences between boll-flower and young leaf-flower (Fig. 4).

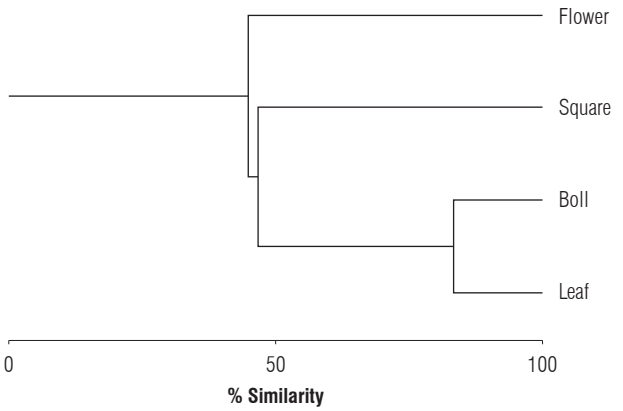


FIGURE 4. Bray-Curtis clustering analysis for *S. dorsalis* in structures of the cotton crop in the Magdalena warm valley in Colombia.

Discussion

This study indicates that *S. dorsalis* was found to be associated with cotton crop plants of the Magdalena warm valley in Colombia, with a preference for young leaves, floral buds and bolls. It is clear that these microhabitats have sufficient food and reproductive resources to complete the life cycle and maintain the populations of the species (Mound and Stiller, 2011), which poses a high risk for crop yield and profitability (Mound and Stiller, 2011; Mannion *et al*., 2013). It has been reported that leaves and other non-flowering structures may be more stable food sources for the development of immature stages (Funderburk *et al*., 2002), contrasting the information described to the genus *Frankliniella* Karny, strongly associated with flowers due to the fact that pollen constitutes an essential nutritional contribution to increase egg production. In general, it is understood that young leaves have a higher nutritional content, but also more secondary metabolites, so insect populations must develop efficient strategies to regulate their intake (Schoonhoven *et al*., 2005), as observed in *S. dorsalis*, which prefers to feed around the main veins of the leaves or in areas bordering damaged zones (EFSA, 2014).

Due to the fact that immature stages of *S. dorsalis* were found during the sampling performed in the present study, it is suggested that cotton plants constitute true hosts where the species can develop and complete its life cycle (Mound and Marullo, 1996; Marullo, 2004; Marullo, 2009; Vierbergen *et al*., 2010; Alves-Silva *et al*., 2013). This result is relevant because there is scarce knowledge about host plants and feeding strategies of the genus *Scirtothrips*, which has been a barrier for the definition of surveillance and control strategies (Zwölfer, 1983; Lacasa *et al*., 1996; Mound and Marullo, 1996; Hall *et al*., 1997; Pérez, 1999; Hernandez, 2005; Alves-Silva, 2013; Kumar *et al*., 2013; Burckhardt *et al*., 2014). The specialization in insects corresponds to the general rule and not to the exception (Pérez, 1999; Schoonhoven *et al*., 2005), so that the recognition of preferences could offer important ethological elements in the differentiation of the species and could also give clarity on their economic importance (Thorsteinson, 1960; Stern, 1973; Zwölfer, 1983; Bernays and Chapman, 1994; Sha *et al*., 1998; Zamar and Neder, 2012; Cook *et al*., 2013). In the present study, symptoms such as leaf distortion (leaf curl), leaf wilting, leaf fall, and discoloration of boll and floral buds (bronzing), were attributed to the insect presence in the cotton plants (Fig. 5).

The insect *S. dorsalis* is an introduced species in Colombia, with a type "r" reproductive strategy and records of polyphagia (Morse and Hoddle, 2006; Rodríguez, 2006; Liebhold and Tobin, 2008). All the above, in conjunction with the distribution recorded in the present study, can generate negative effects on biodiversity, uncultivated native flora and other crops of economic interest such as mango, citrus, avocado, tomato, chili and aromatic herbs, which are also established in the Magdalena warm valley in the Andean region of Colombia (Mound and Palmer, 1981; Hoodle and Mound, 2003; Liebhold and Tobin, 2008; Kumar *et al*., 2011; Kumar *et al*., 2013; Wegier and Piñero, 2013). It is clear that the introduction and establishment of non-native species affect trophic networks and can alter processes of population dynamics that allow species regulation (Lewis, 1997; Schoonhoven *et al*., 2005; Gutiérrez, 2006). This fact must be considered as an important risk if the cultivated area is a cotton monoculture.

Considering the percentages of infestation and insect preferences found for cotton crop in the Andean region of Colombia, it is clear that *S. dorsalis* could become a major phytosanitary problem that should be carefully monitored because: a) it could adversely affect the expected yields and the quality of the fiber, b) it produces direct damages that cause the fall of leaves, floral buds and bolls, c) it favors the distortion in leaf blades in the shape of rosettes, d) it contributes to the foliar malformation, e) it can generate dwarfism and death of the plant (Fig. 6). Several researches have established that *S. dorsalis* causes indirect damage related to the transmission of viral particles such as *Groundnut chlorotic fan-spot virus*, *Groundnut yellow spot virus* and *Tobacco streak virus*, as recorded in cotton crops in Australia and the United States of America with a reduction of 77% in seed production, 25% in yield and 67% in

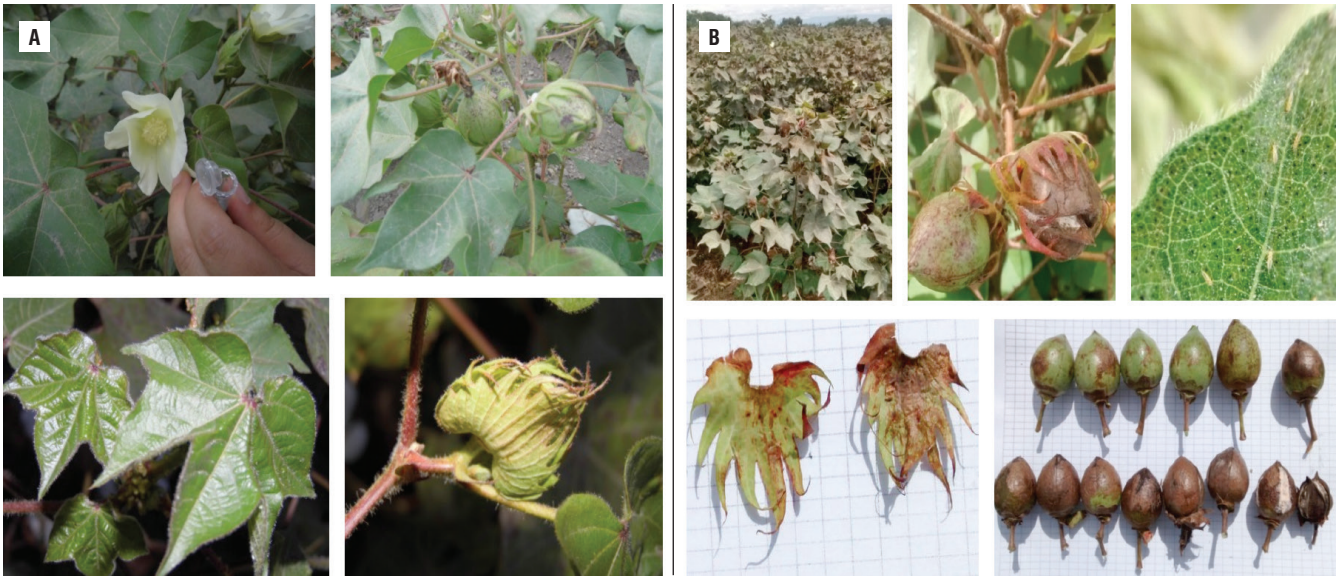


FIGURE 5. Cotton crop. **A.** Structures without damage. **B.** Structures affected by *S. dorsalis*.

fiber quality, (Funderburk, 2002; Jones, 2005; Shukla *et al*., 2005; Riley *et al*., 2011; EFSA, 2014). It has been found that *Frankliniella tritici* may be associated with cotton flowers (10%) causing detriments due to its feeding, but also as a carrier of the fungus *Fusarium verticillioides*, causal agent of the so-called Hard Lock syndrome (Mailhot *et al*., 2007), which coupled with the damage caused by *S. dorsalis* to cotton plants, constitute important risks for the cotton production chain. However, it is interesting that the insect was not present in 14 of the total farms evaluated (23%), which could be explained by agronomic management practices based on the regular use of insecticidal molecules of chemical synthesis, or due to abiotic factors that do not favor the establishment of the species. The entomological surveillance of the species should be continued, not only in cotton crops but in other plants that can serve as hosts, which can be established through models of distribution and ecological niche of the species (Pulido *et al*., 2015).

Conclusions

The importance of the association of *S. dorsalis* in cultivated cotton plants described in the present study leads to opportunities to perform projects oriented towards biology, ethology and the recognition of natural enemies of this pest species. Those projects may allow the definition of strategies of integrated pest management and the evaluation of the impact on the cotton production chain for the country.

The tropical dry forests correspond to a life zone where agriculture has grown with many extensive crops such as chili, mango, rice and cotton, which have been reported as host plants for *S. dorsalis*. This species has been dispersed and established in this region because it is considered as a favorable ecological niche in this area.

The introduction and establishment of species such as *S. dorsalis* could affect the trophic networks present, altering processes of population dynamics that allow the regulation of the species. This, for the present study, would constitute an important risk to the evaluated areas, if these areas have appropriate environmental conditions as ecological niches for the establishment of the invasive species.

Acknowledgments

The authors would like to thank the different associations of farmers of the cotton sector in the departments of Tolima, Huila and Cundinamarca for the support received in the accomplishment of this work. We thank the officials of the Colombian Agricultural Institute in the different departmental sections, the Faculty of Agricultural Sciences, Bogota at the National University of Colombia, Bogota headquarters, the company BIOQUALITY AGRO and agricultural research center AGROSAVIA-Nataima.

Literature cited

Alves-Silva, E., P.K. Maruyama, A. Cavalleri, and K. Del-Claro. 2013. Flower stage and host plant preference by floral herbivore thrips (Insecta: Thysanoptera: *Frankliniella*) in a Brazilian savanna. Stud. Neotrop. Fauna Environ. 48(1), 25-31. Doi: 10.1080/01650521.2012.751785

Ananthakrishnan, T.N. 1993. Bionomics of thrips. Annu. Rev. Entomol. 38(1), 71-92. Doi: 10.1146/annurev.en.38.010193.000443

Bernays, E.A. and R.F. Chapman. 1994. host-plant selection by phytophagous insects. Chapman and Hall, New York. Doi: 10.1007/b102508

Burckhardt, D., D. Ouvrard, D. Queiroz, and D. Percy. 2014. Psyllid host-plants (Hemiptera: Psylloidea): resolving a semantic problem. Fla. Entomol. 97(1), 242-246. Doi: 10.1653/024.097.0132

Cermeli M., J. Perozo, and L. Piñango. 2009. *Scirtothrips dorsalis* Hood (Thysanoptera, Thripidae) nuevo insecto plaga del algodonero (*Gossypium hirsutum* L.) en Venezuela. Entomotropica 24(2), 85-88.

Conalgodon, 2018. Estadísticas. URL: http://www.conalgodon.com/node/56 (accessed August 2016).

Cook, D.R., B.R. Leonard, E. Burris, and J. Gore. 2013. Impact of thrips infesting cotton seedlings on cotton yield distribution and maturity. J. Cotton Sci. 17, 23-33.

Ebratt E.E., R. Martínez, and A. Acosta, 2004. Construcción de la base de conocimientos de un sistema experto para el manejo integrado de poblaciones de insectos plaga en el cultivo de algodón en Colombia. MSc Thesis. Universidad Nacional de Colombia, Bogota.

EFSA. 2014. Scientific opinion on the pest categorisation of *Scirtothrips dorsalis*. EFSA panel on plant health (PLH). EFSA J. 12, 3915. Doi: 10.2903/j.efsa.2014.3915

Funderburk, J. 2002. Ecology of thrips. pp. 121-128. In: Mound, L.A. and R. Marullo (eds.). Thrips and Tospoviruses: Proceedings of the 7th International Symposium on Thysanoptera, Università degli studi mediterranea di Reggio Calabria. Facolta di Agraria. Dipartimento di Agrochimica ed Agrobiologia. Australian National Insect Collection CSIRO. Canberra, Australia.

Gutiérrez, F. 2006. Estado de conocimiento de especies invasoras. Propuesta de lineamientos para el control de los impactos. Instituto de Investigación de Recursos Biológicos Alexander von Humboldt, Bogota.

Hall, L.S., P.R. Krausman, and M.L. Morrison. 1997. The habitat concept and a plea for standard terminology. Wildl. Soc. Bull. 25(1), 173-182.

Hernández, O.V. 2005. Plantas hospederas y estrategias de alimentación en moscas de la fruta. pp. 25-32. In: Díaz, F., J. Rivera, S. Zepeda, and E. Hernández (eds.). XVI Curso Internacional sobre moscas de la fruta, Centro Internacional de Capacitación en moscas de la fruta, Programa Moscamed-Moscafrut SAGARPA-IICA. Metapa de Domínguez, Chiapas, Mexico.

Hoddle, M.S. and L.A. Mound. 2003. The genus *Scirtothrips* in Australia (Insecta, Thysanoptera, Thripidae) Zootaxa. 268(1), 1-40. Doi: 10.11646/zootaxa.268.1.1

Hoddle, M.S., J.M. Heraty, P.F. Rugman-Jones, L.A. Mound, and R. Stouthamer. 2008. Relationships among species of *Scirtothrips* (Thysanoptera: Thripidae, Thripinae) using molecular and morphological data. Ann. Entomol. Soc. Amer. 101(3), 491-500. Doi: 10.1603/0013-8746(2008)101[491:RASOST]2.0.CO;2

Hodges, G., G.B. Edwards, and W. Dixon. 2005. Chilli thrips *Scirtothrips dorsalis* Hood (Thysanoptera: Thripidae) A new pest thrips for Florida. In: Florida Department of Agriculture & Consumer Services, Division of Plant Industry. URL: http://www.freshfromflorida.com/pi/enpp/ento/chillithrips.html (accessed October 2017).

Holdridge, L.R. 1967. Life zone ecology. Tropical Science Center, San José, Costa Rica. Instituto Geográfico Agustin Codazzi. URL: http://www.igac.gov.co/igac (accessed January 2017).

IAvH. 2014. El bosque seco tropical en Colombia. In: Pizano, C., and H. García (eds.), Bogota.

ICA. 2000. Plan nacional de exclusión, supresión y erradicación económica del picudo del algodonero. Doc. 21641. URL: https://www.ica.gov.co/getdoc/b8b6617f-bd3f-4bb8-b337-16d918712423/planpicudo_v1.aspx (accessed April 2017).

ICA. 2012. Situación actual del trips amarillo del ají *Scirtothrips dorsalis* (Hood) (Thripidae:Thripinae) en Colombia. Boletin epidemiológico. ICA. Bogota. URL: https://www.ica.gov.co/Areas/Agricola/Servicios/Epidemiologia-agricola/BOLETINES/Nacionales/2012/B_N_SDORSALIS_DIC_2012.aspx (accessed April 2017).

Johnson, D.H. 1980. The comparison of usage and availability measurements for evaluating resource preference. Ecology 61(1), 65-71. Doi: 10.2307/1937156

Jones, D.R. 2005. Plant viruses transmitted by thrips. Eur. J. Plant. Pathol. 113(2), 119-157. Doi: 10.1007/s10658-005-2334-1

Kumar, V., D.R. Seal, D.J. Schuster, C. Mckensie, L.S. Osborne, J. Maruniak, and S. Zhang. 2011. *Scirtothrips dorsalis* (Thysanoptera: Thripidae): scanning electron micrographs of key taxonomic traits and a preliminary morphometric analysis of the general morphology of populations of different continents. Fla. Entomol. 94(4), 941-955. Doi: 10.1653/024.094.0431

Kumar, V., G. Kakkar, C. Mckenzie, D.R. Seal, and L.S. Osborne. 2013. An overview of chilli thrips, *Scirtothrips dorsalis* (Thysanoptera: Thripidae) Biology, Distribution and Management. pp. 53-77. In: Soloneski, S. and M. Larramendy (eds.). Weed and Pest Control - Conventional and New Challenges, Intech, Rijeka, Croatia. Doi: 10.5772/55045

Lacasa, A., J.M. Llorente, and J.A. Sanchez. 1996. Un *Scirtothrips* (Thysanoptera: Thripinae), causa daños en los cítricos en España. Bol. Sanid. Veg. Plagas 22(1), 79-95.

Leigh, T.F. 1995. Bionomics of cotton thrips: A review. pp. 61-70. In: Parker, B. L., M. Skinner, and T. Lewis (eds.). Thrips Biology and Management. Springer Science & Business Media. Doi: 10.1007/978-1-4899-1409-5_5

Lewis, T. 1997. Thrips as crop pests. CABI, New York, USA.

Liebhold, A.M. and P.C. Tobin. 2008. Population ecology of insect invasions and their management. Annu. Rev. Entomol. 53(1), 387-408. Doi: 10.1146/annurev.ento.52.110405.091401

Mailhot, D.J., J.J. Marois, and D.L. Wright. 2007. Influence of flower thrips on *Fusarium* hardlock severity. Plant Dis. 91(11), 1423-1429. Doi: 10.1094/PDIS-91-11-1423

Mannion, C.M., A.I. Derksen, D.R. Seal, L.S. Osborne, and C.G. Martin. 2013. Effects of rose cultivars and fertilization rates on populations of *Scirtothrips dorsalis* (Thysanoptera: Thripidae) in Southern Florida. Fla. Entomol. 96(2), 403-411.

Marullo, R. 2004. Host-plant range and relationships in the Italian thrips fauna. Acta Phytopathol. Entomol. 39(1-3), 243-254. Doi: 10.1556/APhyt.39.2004.1-3.23

Marullo, R. 2009. Host-plant ranges and pest potential: habits of some thrips species in areas of southern Italy. Bull. Insectology 62(2), 253-255.

Moritz, G.B., C. O'Donnell, and M.P. Parrella. 2007. Pest thrips of North America associated with domestic and imported crops. CBIT Publishing, Queensland, Australia.

Morse, J.G. and M.S. Hoddle. 2006. Invasion biology of thrips. Annu. Rev. Entomol. 51(1), 67-89. Doi: 10.1146/annurev.ento.51.110104.151044

Mound, L.A. and R. Marullo. 1996. The thrips of Central and South America: an introduction. Memoirs on Entomology International, Gainesville, FL.

Mound, L.A. and J.M. Palmer. 1981. Phylogenetic relationships between some genera of Thripidae (Thysanoptera). Entomol. Scand. 15(1), 153-17.

Mound, L.A. and M. Stiller. 2011. Species of the genus *Scirtothrips* from Africa (Thysanoptera, Thripidae). Zootaxa 2786(1), 51-61.

Pedigo, L.P. 1996. Entomology and pest management. Prentice-Hall Pub., Englewood Cliffs, NJ.

Pérez, C.T. 1999. La especialización en los insectos fitófagos: Una regla más que una excepción. Boletín S.E.A. URL: http://sea-entomologia.org/PDF/BOLETIN_26/B26-053-759.pdf (accessed April 2017).

Pulido, H.L., A.A. Rudas, J.A. Betancourt. W.E. Grant, and S.J. Vilchez. 2015. Distribución inusual y potencial de la garrapata común del ganado, *Rhipicephalus* (Boophilus) *microplus*, en zonas tropicales de alta montaña de los Andes colombianos. Biota Colomb. 16(2), 75-95.

Ramirez, G.A. 2006. Ecología: Métodos de muestreo y análisis de poblaciones y comunidades. Editorial Pontificia Universidad Javeriana, Bogota.

Riley, D.G., V.J. Shimat, R. Srinivasan, and S. Diffie. 2011. Thrips vectors of tospoviruses. J. Integr. Pest Manag. 2(1), 1-10. Doi: 10.1603/IPM10020

Rodríguez, L.F. 2006. Can invasive species facilitate native species? Evidence of how, when, and why these impacts occur. Biol. Invasions 8(4), 927-939. Doi: 10.1007/s10530-005-5103-3

Schoonhoven, L.M., J. Van Loon, and M. Dicke. 2005. Insect plant biology. Oxford University Press, UK.

Sha, M., D. Danforth, N. Tugwell, and M.J. Cochran. 1998. Plant-based economic injury level for assessing economic thresholds in early-season cotton. J. Cotton Sci. 2(1), 35-52.

Shukla, S., G. Kalyani, N. Kulkarni, F. Waliyar, and S.N. Nigam. 2005. Mechanism of transmission of tobacco streak virus by *Scirtothrips dorsalis*, *Frankliniella schultzei* and *Megalurothrips usitatus* in groundnut, *Arachis hypogaea* L. J. Oilseeds Res. 22(1), 215-217.

Silveira-Neto, S., O. Nakano, and N.A. Villa Nova. 1976. Manual de ecologia dos insetos. Agronômica Ceres, Piracicaba, SP, Brazil.

Stern, V.M. 1973. Economic thresholds. Annu. Rev. Entomol. 18(1), 259-280. Doi: 10.1146/annurev.en.18.010173.001355

Thorsteinson, A.J. 1960. Host selection in phytophagous insects. Annu. Rev. Entomol. 5(1), 193-218. Doi: 10.1146/annurev.en.05.010160.001205

ThripsWiki. 2017. Thripidae. URL: http://thrips.info/w/index.php?title=Thripidae&oldid=33251 (accessed April 2017).

Vierbergen, G., H. Kucharczyk, and D.J. Kirk. 2010. A key to the second instar larvae of the Thripidae of the Western Palaearctic region (Thysanoptera). Tijdschr. Entomol. 153(1), 99-160. Doi: 10.1163/22119434-900000294

Wegier, B.A. and D.D. Piñero. 2013. Diversidad genética y conservación de *Gossypium hirsutum* silvestre y cultivado en México. PhD thesis. Universidad Nacional Autónoma de México, Mexico D.F.

Wilson, L.J. and L.R. Bauer. 1993. Species composition and seasonal abundance of thrips (Thysanoptera) on cotton in the Namoi valley. Aust. J. Entomol. 32(2), 187-192. Doi: 10.1111/j.1440-6055.1993.tb00569.x

Yara, C. and G. Reinoso. 2012. Hormigas cazadoras (Ectatomminae y Ponerinae) en fragmentos de bosque seco y sus matrices (Tolima, Colombia). Rev. Colomb. Entomol. 38(2), 329-337.

Zamar, M.I. 2011. La diversidad de thrips del Cono Sur. El caso de las zonas áridas en Jujuy, Argentina. Metod. Ecol. Systemat. 6(3), 71-88.

Zamar, M.I. and L.E. Neder. 2012. Asociación Thysanoptera(Insecta)-Vicia faba (Fabaceae) en la prepuna y puna de Jujuy, Argentina. Rev. Biol. Trop. 60(1), 119-128. Doi: 10.15517/rbt.v60i1.2639

Zwölfer, H. 1983. Life systems and strategies of resource exploitation in tephritids. pp. 16-30. In: R. Cavalloro (ed.). Fruit flies of economic importance. Balkema, Rotterdam.

Boron-zinc interaction in the absorption of micronutrients by cotton

Interacción boro-zinc en la absorción de micronutrientes por el cultivo del algodón

Érica de Oliveira Araújo[1]*, Elcio Ferreira Dos Santos[2], and Marcos Antonio Camacho[3]

ABSTRACT

B-Zn interaction modifies the nutritional dynamics of copper (Cu), iron (Fe) and manganese (Mn) in cotton. The main objective of this research was to evaluate the effect of B and Zn concentrations on the absorption of Cu, Fe and Mn in cotton plants grown in a nutrient solution. A completely randomized experimental design with three replicates was performed, in a 4×5 factorial scheme, corresponding to four concentrations of B (0, 20, 40 and 80 μM L^{-1}) and five concentrations of Zn (0, 1, 2, 4 and 8 μM L^{-1}). At 115 days after emergence, the plants were collected, divided into roots, shoots and fruits, and chemically analyzed. The results allowed to conclude that the Cu content and total Cu in the fruit, total Cu in the roots, Cu efficiency, Fe content in the roots, Fe absorption efficiency, Mn content in the fruit, and Mn absorption efficiency of cotton are influenced by the concentrations of B in the solution. The interaction between B and Zn affected the total Fe in the roots, Fe content and total Fe content in the fruit, Fe transport efficiency, total Mn in the shoots and Mn transport efficiency. In addition, Zn acts differently according to the supply of B and vice versa.

Key words: *Gossypium hirsutum* L., copper, iron, manganese, nutritional efficiency.

RESUMEN

La interacción boro-zinc (B-Zn) modifica la dinámica nutricional del cobre (Cu), hierro (Fe) y manganeso (Mn) en el cultivo del algodón. El objetivo del presente trabajo fue evaluar el efecto de concentraciones de B y Zn sobre la absorción de Cu, Fe y Mn por plantas de algodón creciendo en solución nutritiva. Se utilizó un diseño completamente al azar con tres repeticiones en un esquema factorial 4×5, siendo cuatro las concentraciones de B (0, 20, 40 y 80 μM L^{-1}) y cinco las concentraciones de Zn (0, 1, 2, 4 y 8 μM L^{-1}). A los 115 días después de emergencia las plantas fueron recolectadas, divididas en raíz, parte aérea y frutos, y sometidas a análisis químicos. Los resultados permitieron concluir que el contenido y el total de Cu en el fruto, el contenido de Cu en la raíz, la eficiencia de utilización de Cu, el total de Fe en la raíz, la eficiencia de absorción de Fe, el total de Mn en el fruto y la eficiencia de absorción de Mn son influenciadas por las concentraciones de B en la solución. La interacción B-Zn afectó el contenido de Fe en la raíz, el contenido y el total de Fe en el fruto, eficiencia de transporte de Fe, el total de Mn en la parte aérea y la eficiencia del transporte de Mn, Adicionalmente Zn actúa de manera diferente de acuerdo al suministro de B y viceversa.

Palabras clave: *Gossypium hirsutum* L., cobre, hierro, manganeso, eficiencia nutricional.

Introduction

Cotton farming is one of the main activities of the Brazilian agribusiness. The increasing use of more productive varieties worldwide, with a higher nutrient demand, requires a better knowledge on the nutritional relationships in cotton (Rochester and Constable, 2015). In this context, a better knowledge of the nutritional dynamics is important for the establishment of an efficient fertilization program.

Gossypium hirsutum L. – species of greatest use for cotton production in Brazil – is responsive to the application of micronutrients, especially in low natural fertility areas. Among the micronutrients, boron (B) and zinc (Zn) are the most limiting elements to achieve better yields (Araújo *et al*., 2012; Araújo *et al*., 2013). B is directly related to the metabolism of the ribonucleic acid (RNA), different functions of the plasmatic membrane and constitutes the structure of the cell wall and pectic substances associated with it, especially the middle lamella (Wimmer and Eichert, 2013). Zn is required in many active sites of different proteins, such as carbonic anhydrase and superoxide dismutase. The deficiency of this nutrient reduces the photosynthetic rate through the modification of the activity of enzymes involved in the process of carbon fixation (Broadley *et al*., 2007; Assunção *et al*., 2013).

B-Zn interaction influences different metabolic processes of the plant, interfering with the mineral composition through the stimulation or inhibition of the absorption

Received for publication: 22 July, 2017. Accepted for publication: 16 February, 2017 Doi: 10.15446/agron.colomb.v36n1.66539

[1] Doutorado em Agronomia (Produção Vegetal), Universidade Federal da Grande Dourados (Brasil)
[2] Doutorando em Ciências. Centro de Energia Nuclear na Agricultura – CENA/USP, Programa de Pós-graduação em Ciências, Piracicaba, SP (Brasil).
[3] Doutorado em Ciencia do Solo. Universidade Estadual do Mato Grosso do Sul (UEMS) Aquidauana - MS.
* Corresponding author: ericabb25@hotmail.com

of other nutrients (Baxter, 2009). These relationships are highly variable and may occur inside the cells or in the rhizosphere (Morgan and Connolly, 2013). The B-Zn interactions in cotton are correlated (Araújo *et al*., 2012) and the reduction in B absorption as a function of Zn is common. Besides cotton (Araújo *et al*., 2012), this result has already been observed for lemon (Rajaie *et al*., 2009), olive tree (Jasrotia *et al*., 2014) and wheat (Nasim *et al*., 2015). Thus, the effect of the B-Zn interaction on the absorption of these nutrients is evident. However, there is still limited information on the effect of B-Zn interaction in the absorption dynamics of other micronutrients, such as Cu, Fe and Mn.

Many nutritional relationships have been observed between micronutrients (Baxter, 2009; Milner *et al*., 2013; Aibara and Miwa, 2014). These nutritional interactions have been cataloged as complex, with initial modification at a molecular level, resulting in a variation of the absorption and mineral composition of the plants. Thus, the observed nutritional interaction depends on the availability of nutrients and on the analyzed plant species (Araújo *et al*., 2012; Araújo *et al*., 2013). Therefore, the need for studies that address the classic nutritional interaction between B and Zn, evaluating the absorption of Cu, Fe and Mn is evident. Understanding the micronutrient interaction would enhance the use of fertilizers, reducing production costs. The hypothesis of this study is that the combinations of B and Zn in nutrient solution modify the total content and the efficiency of absorption, use and transport of Cu, Fe and Mn in cotton. Given the above, the main objective of this research was to evaluate the effect of B and Zn concentrations on the absorption of Cu, Fe and Mn in cotton cultivated in nutrient solution.

Materials and methods

The experiment was conducted in an agricultural greenhouse of the Plant Production Sector of the State University of Mato Grosso do Sul (UEMS), in Aquidauana-MS, at the geographic coordinates 20°28' S and 55°48' W with an altitude of 174 m a.s.l. The experimental design was completely randomized with three replicates, in a 4×5 factorial scheme, corresponding to four concentrations of B (0, 20, 40 and 80 μM L^{-1}), applied in the form of boric acid, and five concentrations of Zn (0, 1, 2, 4 and 8 μM L^{-1}), applied in the form of zinc sulfate.

The experimental units consisted of plastic pots with 3 L capacity, filled with washed and sterilized quartz sand. Cotton seeds, cv. FiberMax 910, were placed to germinate on trays with moistened sand. Five days after emergence, when cotyledon leaves developed, three seedlings were transplanted to each experimental unit, where they received a complete and diluted (1/5) nutrient solution (Epstein and Bloom, 2006). At 28 days after emergence, thinning was performed leaving only one plant in each experimental unit. The nutrient solution application started according to each treatment, irrigating three times a day with deionized water. In the treatment solutions lacking a specific nutrient, the concentrations were identical to those of the complete solution, except for the absent nutrient.

The nutrient solution showed the following composition: 6.0 mL of 1 mol L^{-1} KNO_3; 4.0 mL of 1 mol L^{-1} $Ca(NO_3)_4{\cdot}H_2O$; 2.0 mL of 1 mol L^{-1} $NH_4H_2PO_4$; 1.0 mL of 1 mol L^{-1} $MgSO_4{\cdot}7H_2O$; 1.0 mL of 0.2 mol L^{-1} Fe-EDTA; 1.0 mL of 0.05 mol L^{-1} KCl; 1.0 mL of 0.02 mol L^{-1} H_3BO_3; 1.0 mL of 0.002 mol L^{-1} $MnSO_4{\cdot}H_2O$; 1.0 mL of 0.002 mol L^{-1} $ZnSO_4{\cdot}7H_2O$; 1.0 mL of 0.0005 mol L^{-1} $CuSO_4{\cdot}5H_2O$; 1.0 mL of 0.0005 mol L^{-1} H_2MoO_4 (85% MoO_3).

At 115 days after emergence, the plants were collected and divided into roots, shoots (stem and leaves) and fruits (boll). All the collected plant material was rinsed in a detergent solution at 3 mL L^{-1}, running water, 0.1 mol L^{-1} HCl solution and deionized water, respectively. The samples were placed in paper bags and then dried in a forced-air oven at 65°C for 72 h. After drying, the plant material was ground in a Wiley-type mill (Thomas Scientific, USA). The samples were subjected to nitric-perchloric digestion for the determination of Cu, Fe and Mn contents in the different plant parts (Malavolta *et al*., 1997).

The determination of Cu, Fe and Mn was used to calculate the absorption efficiency (mg g^{-1}) = [total content of nutrient in the plant (mg)/root dry matter (g)] (Swiader *et al*., 1994); transport efficiency (%) = [(content of nutrient in the shoots (mg)/total content of nutrient in the plant (mg)) x 100] (Li *et al*., 1991); and use efficiency (g^2 mg) = [(total dry matter produced (g))^2/total content of nutrients in the plant (mg)] (Siddiqi and Glass, 1981).

The results were statistically assessed with an analysis of response surface, using the statistical package SAS and adopting a 0.05 significance level. Initially, an analysis of variance was performed and, according to the significance of the F test, the study of polynomial regression was conducted (for cases with significant interaction), through the RSREG procedure.

Results and discussion

The interaction between the B and Zn treatments influenced the Fe content in the boll, total Fe in the roots and in the boll, total Mn in the shoots and transport efficiency of Fe and Mn (Tab. 1). There was a significant response (P<0.05) of B concentrations for Cu content in the boll, total Cu in the roots and in the boll, Fe content and total Fe in the roots, Mn content in the boll, Cu use efficiency and the efficiencies of absorption of Fe and Mn (Tab. 1).

Zn doses in the solution did not affect individually any of the studied variables. Various studies indicate antagonistic relationship between Zn and cationic micronutrients, such as Cu, Fe and Mn. This interaction occurs due to the competition of the cations for the absorption sites (Broadley *et al.*, 2007; Baxter, 2009; Assunção *et al.*, 2013). This study did not find any antagonistic relationship between Zn and other cationic micronutrients (Fe, Mn and Cu), disagreeing with the results reported by Rajaie *et al.* (2009), Aref (2011) and Lima Neto and Natale (2014) in other plant species. Hence, only B doses and the combined doses of B and Zn influenced the absorption of Cu, Fe and Mn in cotton (Tab. 1).

Cu content and total Cu in the cotton fruit were influenced by the B concentrations in the solution. The increase in B concentrations in the solution until 40 μM L^{-1} promoted a reduction in the Cu content and total Cu in cotton bolls (Figs. 1A, 1B). B doses higher than 40 μM L^{-1} increased Cu content and total Cu in the bolls. Aref (2011) observed an increment in the concentration of Cu in maize leaves with the increase in B concentrations in the planting substrate. Ahmed *et al.* (2011) observed a positive effect of B doses on Cu content and total Cu in different parts of cotton plants. However, the total Cu in cotton roots linearly decreases with the increment in B concentrations in the solution (Fig. 1C), agreeing with the results found by Esringü *et al.* (2002) who observed a rgjeduction of Cu concentration in strawberry roots with the increase in the supply of B reporting an antagonistic relationship between the nutrients.

Cu use efficiency for the production of dry matter was positively influenced by the supply of B in the solution, so that the increase in B concentrations promoted linear increment in the Cu use efficiency of cotton plants (Fig. 1D). B is related to various biochemical processes in the plants; thus, its concentrations in the cultivation substrate may directly or indirectly affect the use of Cu by the plant (Malavolta, 2006; Wimmer and Eichert, 2013).

TABLE 1. Summary of the analysis of variance: coefficient of variation and *P*-value for the effect of B, Zn and the B-Zn interaction for content, total and efficiency of absorption, transport and use of Cu, Fe and Mn.

Parameters	CV (%)	*P*-values		
		B	Zn	B x Zn
Copper (Cu)				
Cu content in the shoots (g kg^{-1})	67.29	0.846	0.065	0.532
Cu content in the roots (g kg^{-1})	37.53	0.110	0.216	0.184
Cu content in the boll (g kg^{-1})	26.09	0.000*	0.468	0.119
Total Cu in the shoots (mg)	66.09	0.805	0.065	0.488
Total Cu in the roots (mg)	44.49	0.028*	0.476	0.053
Total Cu in the boll (mg)	32.30	0.000*	0.555	0.129
Absorption efficiency of Cu (mg g^{-1})	33.21	0.197	0.105	0.387
Transport efficiency of Cu (%)	42.92	0.560	0.159	0.173
Use efficiency of Cu (mg g^{-1})	32.17	0.013*	0.339	0.205
Iron (Fe)				
Fe content in the shoots (g kg^{-1})	28.92	0.209	0.364	0.173
Fe content in the roots (g kg^{-1})	32.57	0.003*	0.878	0.121
Fe content in the boll (g kg^{-1})	32.66	0.000*	0.078	0.000*
Total Fe in the shoots (mg)	31.16	0.220	0.531	0.207
Total Fe in the roots (mg)	32.06	0.000*	0.646	0.005*
Total Fe in the boll (mg)	41.74	0.000*	0.346	0.000*
Absorption efficiency of Fe (mg g^{-1})	27.72	0.005*	0.800	0.253
Transport efficiency of Fe (%)	37.44	0.000*	0.830	0.009*
Use efficiency of Fe (mg g^{-1})	31.32	0.054	0.578	0.186
Manganese (Mn)				
Mn content in the shoots (g kg^{-1})	14.73	0.060	0.103	0.053
Mn content in the roots (g kg^{-1})	28.83	0.057	0.491	0.469
Mn content in the boll (g kg^{-1})	19.53	0.000*	0.653	0.218
Total Mn in the shoots (mg)	18.69	0.000*	0.264	0.003*
Total Mn in the roots (mg)	36.62	0.059	0.867	0.055
Total Mn in the boll (mg)	24.89	0.063	0.089	0.456
Absorption efficiency of Mn (mg g^{-1})	17.72	0.000*	0.131	0.592
Transport efficiency of Mn (%)	21.8	0.104	0.777	0.035*
Use efficiency of Mn (mg g^{-1})	19.6	0.051	0.116	0.506

B: Boron; Zn: Zinc; CV: Coefficient of variation. *Significant by F test at $P \leq 0.05$.

B concentrations in the solution promoted a significant increment in Fe content and total Fe in cotton roots (Figs. 2A, 2B). Similar results were obtained by Esringü *et al.* (2012) in strawberry, in which the Fe content in the roots increased with the concentration of 10 μM L^{-1} of B, decreasing at the concentration of 20 μM L^{-1} of B. These results suggest that B has certain affinity for Fe and that there may be a synergetic relationship between the nutrients. Rajaie *et al.* (2009) also observed significant increase in the concentration of Fe with the increment in B levels in *Citrus aurantifolia*.

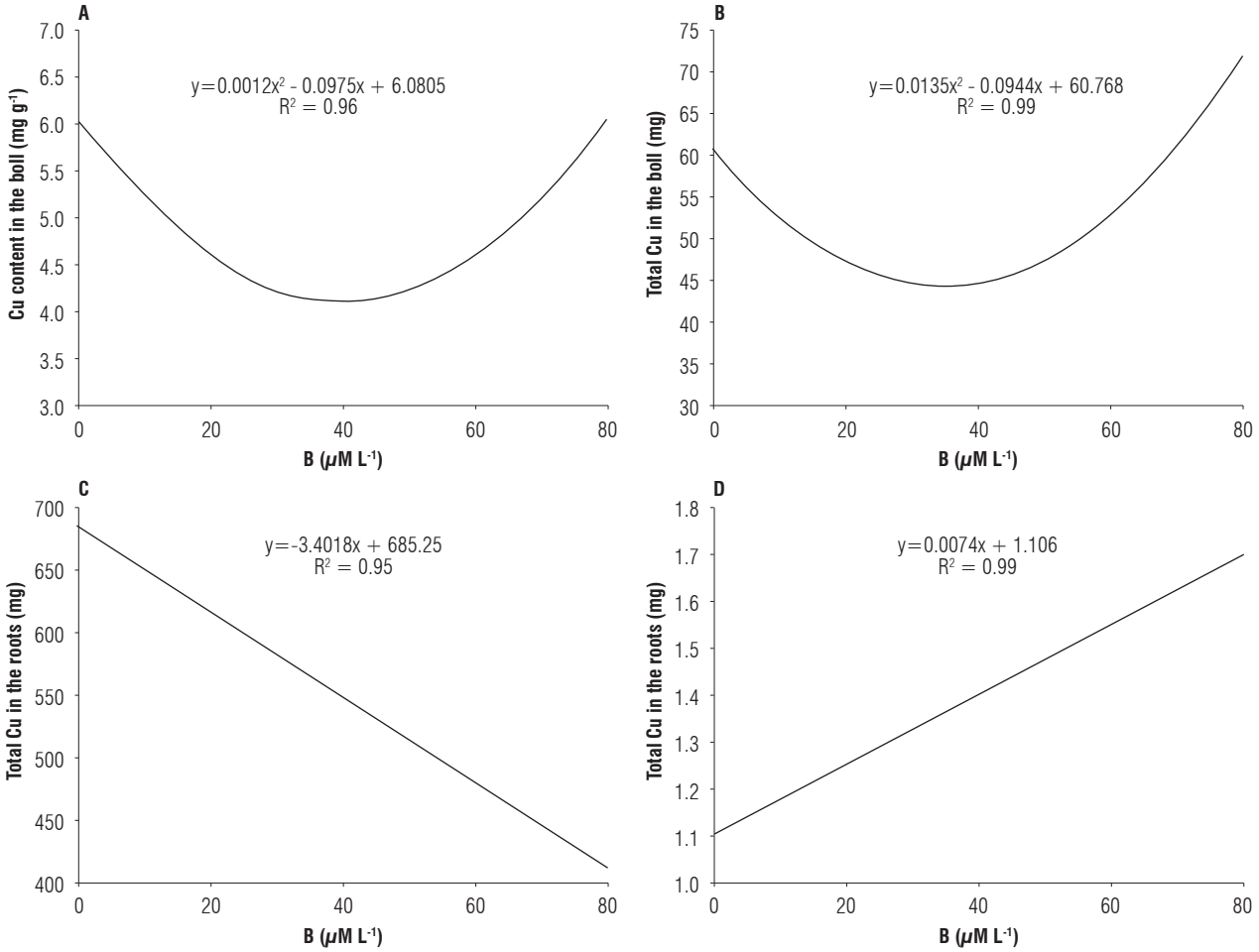


FIGURE 1. Content of Cu in the boll (A), total Cu in the boll (B), content of Cu in the roots (C) and Cu absorption efficiency (D) of cotton in response to different B concentrations in the nutrient solution.

Fe content and total Fe in the cotton fruit were significantly affected by the interaction between B and Zn in the solution. The increase in B concentrations in the solution promoted the reduction in Fe contents and total Fe in the fruit with the increment in Zn concentrations (Figs. 2C, 2D). Ahmed *et al.* (2011) reported that the Fe content, in different parts of the cotton crop, significantly increased with the increment in the applied levels of B. The increase in Fe concentration in the fruit with the increasing levels of B has also been reported in guava (Salvador *et al.*, 2003) and in *Citrus aurantifolia* (Rajaie *et al.*, 2009), disagreeing with the results obtained in the present study. There is a lack of available information about the influence of B on the absorption of other micronutrients, and the results vary according to the analyzed plant species.

Fe absorption efficiency positively responded to the B supply in the solution, so that the increase in B concentrations promoted increment in the Fe absorption efficiency of cotton (Fig. 3E). Fe transport efficiency was affected by the interaction between B and Zn in the solution, demonstrating an antagonistic relationship between B and Zn at the highest concentrations of B, and a synergetic relationship at the highest concentrations of Zn (Fig. 3F). It is possible to observe an increment of 25% in the transport of Fe by the roots with the increase in Zn concentrations at 0 μM L^{-1} of B.

The Mn content in the fruit decreased with the increment in B concentrations, until 40 μM L^{-1} of B, with an increase at the highest concentrations of the nutrient (80 μM L^{-1}), the same behavior observed for Cu content and total Cu in the cotton fruit (Fig. 3A). Aref (2011), working with maize plants, observed that the Mn content was affected by B fertilization, with a reduction in leaf Mn content under these conditions. On the other hand, Esringü *et al.* (2012) observed an increment in leaf Mn content with the increase in B concentrations, until 10 μM L^{-1}, with reduction at the highest B concentration (30 μM L^{-1}). In contrast, the data of the present study indicate that Mn positively responded to B only at the highest concentration of the nutrient.

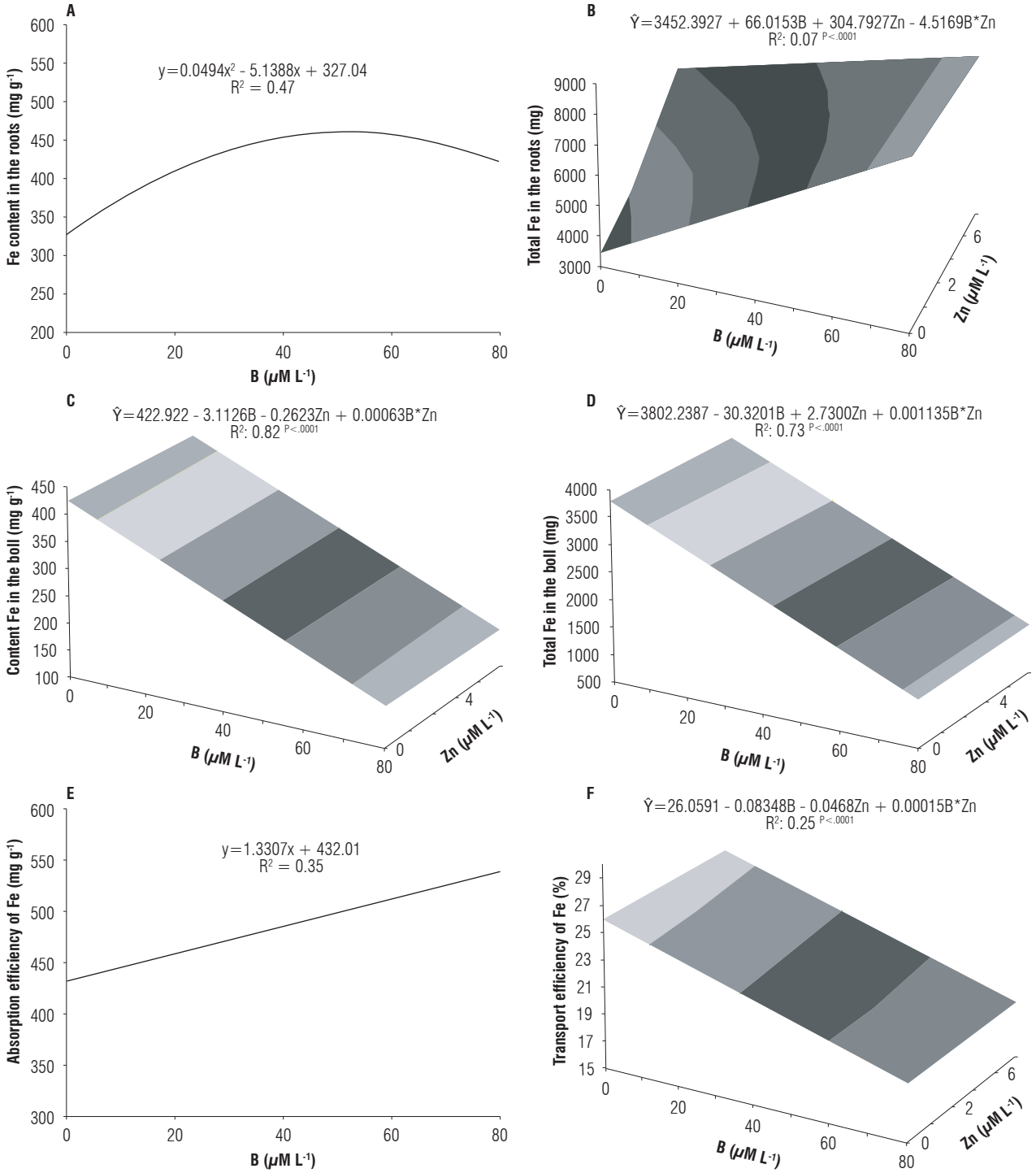


FIGURE 2. Content of Fe in the roots (A), total Fe in the roots (B), content of Fe in the boll (C), total Fe in the boll (D) and efficiency of absorption (E) and transport (F) of Fe of cotton in response to different B and Zn concentrations in the nutrient solution.

The total Mn in the shoots and Mn transport efficiency were negatively influenced by the increase in B concentration at 0 μM L^{-1} of Zn and positively influenced at 8 μM L^{-1} of Zn (Figs. 3B, 3D). It is possible to observe an increment of 49% in the transport of Mn by the roots with the increase in Zn concentrations at 0 μM L^{-1} of B. This reduction in Mn content and total Mn in cotton roots and shoots in response to the increase in B concentrations may be due to the effect of dilution or the antagonistic relationship between B and Mn. The source of B can influence, as a regulator or inhibitor, the accumulation and use of other nutrients (Aref, 2011). Mn absorption efficiency linearly increased with

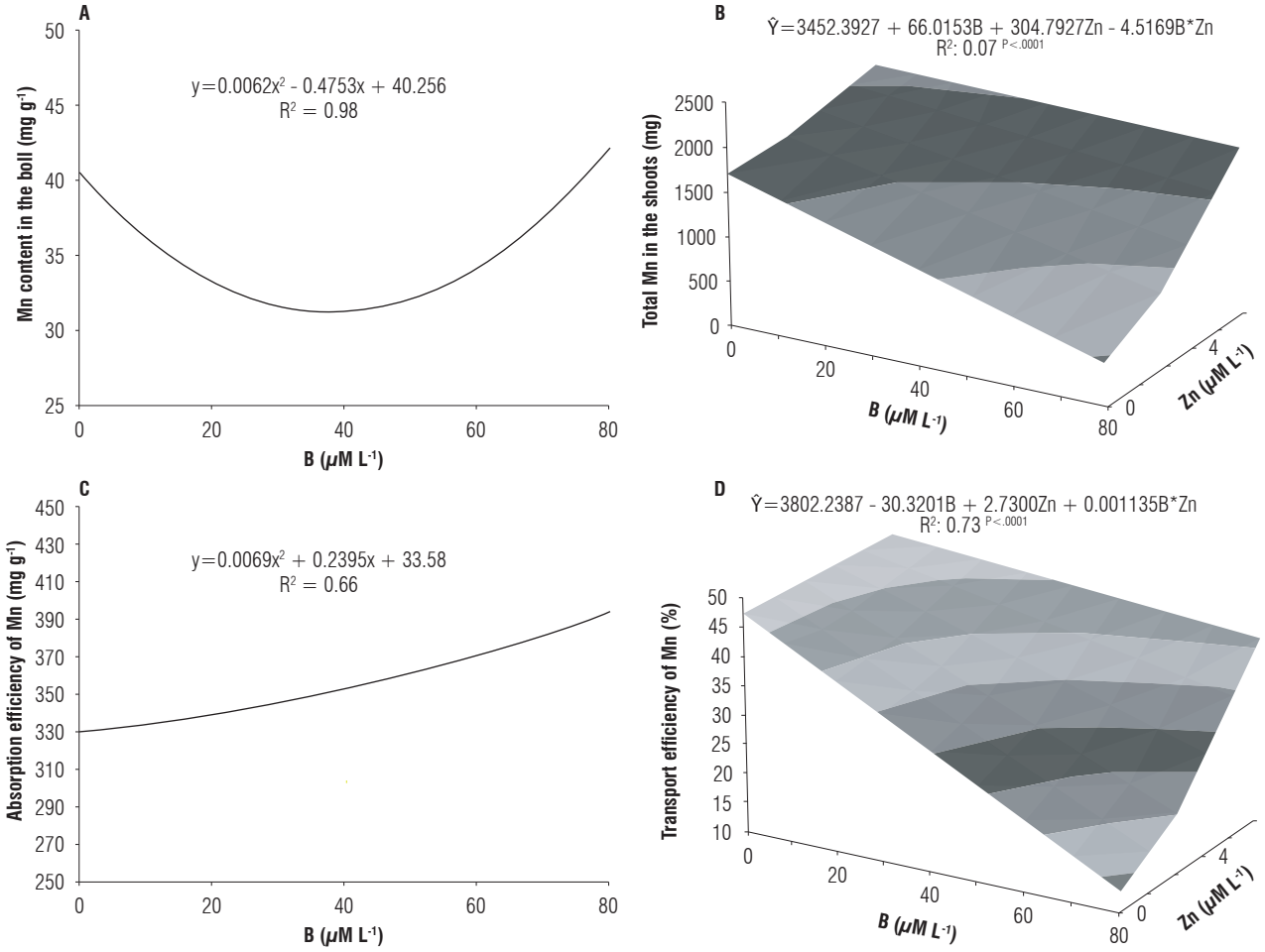


FIGURE 3. Content of Mn in the boll (A), total Mn in the shoots (B) and efficiency of absorption (C) and transport (D) of Mn of cotton in response to different B and Zn concentrations in the nutrient solution.

the increment in B concentrations (Fig. 3C). B is involved in physiological processes that control the absorption and transport of Cu, Fe, Mn and Zn (Malavolta, 2006; Wimmer and Eichert, 2013). Dursun *et al*. (2010) also observed increase in Cu, Fe and Mn contents in leaves of tomato and pepper as a function of B doses.

Conclusions

Cu content and total Cu in the fruit, total Cu in the roots, Cu use efficiency, Fe content in the roots, Fe absorption efficiency, Mn content in the fruit and Mn absorption efficiency of cotton are influenced by the concentrations of B in the solution.

The B-Zn interaction affected total Fe in the roots, Fe content and total Fe in the fruit, Fe transport efficiency, total Mn in the shoots and Mn transport efficiency; in addition, Zn acts differently according to the B supply and vice versa.

Literature cited

Ahmed, N., M. Abid, F. Ahmad, M.A. Ullah, Q. Javaid, and M.A. Ali. 2011. Impact of boron fertilization on dry matter production and mineral constitution of irrigated cotton. Pakistan J. Bot. 43(6), 2903-2910.

Aibara, I. and K. Miwa. 2014. Strategies for optimization of mineral nutrient transport in plants: multilevel regulation of nutrient-dependent dynamics of root architecture and transporter activity. Plant Cell Physiol. 55, 2027-2036. Doi: 10.1093/pcp/pcu156

Araújo, E.O., E.F. Santos, and M.A. Camacho. 2012. Interação boro e zinco no crescimento, desenvolvimento e nutrição do algodoeiro. Rev. Bras. Cienc. Agrar. 7(sup.), 720-727.

Araújo, E.O., E.F. Santos, and M.A. Camacho. 2013. Absorption of calcium and magnesium by cotton plant grown under different concentrations of boron and zinc. Agrária 8, 383-389.

Aref, F. 2011. Influence of zinc and boron nutrition on copper, manganese and iron concentrations in maize leaf. Aust. J. Basic Appl. Sci. 5(7), 52-62.

Assunção, A.G.L., D.P. Persson, S. Husted, J.K. Schjørring, R.D. Alexander, and M.G.M. Aarts. 2013. Model of how plants

sense zinc deficiency. Metallomics 5, 1110-1116. Doi: 10.1039/c3mt00070b

Baxter, I. 2009. Ionomics: studying the social network of mineral nutrients. Curr. Opin. Plant Biol. 12, 381-386. Doi: 10.1016/j.pbi.2009.05.002

Broadley, M.R., P.J. White, J.P. Hammond, I. Zelko, and A. Lux, A. 2007. Zinc in plants. New Phytol. 173, 677-702. Doi: 10.1111/j.1469-8137.2007.01996.x

Dursun, A., M. Turan, M. Ekinci, A. Gunes, N. Ataoglu, A. Esringu, and E. Yildirim. 2010. Effects of boron fertilizer on tomato, pepper and cucumber yields and chemical composition. Commun. Soil Sci. Plant Anal. 41(1), 1576-1593. Doi: 10.1080/00103624.2010.485238

Eptein, E. and A.J. Bloom. 2006. Mineral nutrition of plants: principles and perspectives. Editora Planta, Londrina, Brazil.

Esringu, A., M. Turan, A. Gunes, A. Esitken, and P. Sambo. 2011. Boron application improves on yield and chemical composition of strawberry. Acta Agric Scand. B. 8, 1651-1913. Doi: 10.1080/09064711003776867

Jasrotia, A., P. Bakshi, V.K. Wali, B. Bhushan, and D.J. Bhat. 2014. Influence of girdling and zinc and boron application on growth, quality and leaf nutrient status of olive cv. Frontio. Afr. J. Agr. Res. 9, 1354-1361.

Li, B., S.E. Mckeand, and H.L. Allen. 1991. Genetic variation in nitrogen use efficiency of loblolly pine seedlings. Forest Sci. 37(2), 613-626.

Lima Neto, A.J. de and W. Natale. 2014. Content, accumulation and nutritional efficiency of nutrients on rootstocks of caramboleira in composted substrate with zinc. Agrária 9, 236-243.

Malavolta, E. 2006. Manual of mineral nutrition of plants. Agronômica Ceres, São Paulo, Brazil.

Malavolta, E., C.G. Vitti, and S.A. Oliveira. 1997. Assessment of nutritional status of plants: principles and applications. Brazilian Association for research of phosphate and Potash, Piracicaba, Brazil.

Milner, M.J., J. Seamon, E. Craft, and L.V. Kochian. 2013. Transport properties of members of the ZIP family in plants and their role in Zn and Mn homeostasis. J. Exp. Bot. 64, 369-381. Doi: 10.1093/jxb/ers315

Morgan, J.B. and E.L. Connolly. 2013. Plant-Soil Interactions: Nutrient Uptake. Nat. Ed. Knowl. 4(8), 2.

Nasim, M., Z. Rengel, T. Aziz, B.D. Regmi, and M. Saqib. 2015. Boron toxicity alleviation by zinc application in two barley cultivars differing in tolerance boron toxicity. Pak. J. Agri. Sci. 52, 151-158.

Rajaie, M., A.K. Ejraie, H.R. Owliaie, and I. Tavakoli. 2009. Effect of zinc and boron interaction on growth and mineral composition of lemon seedlings in a calcareous soil. Int. J. Plant Prod. 3(1), 39-50.

Rochester, I.J. and G.A. Constable. 2015. Improvements in nutrient uptake and nutrient use-efficiency in cotton cultivars released between 1973 and 2006. Field Crops Res. 173, 14-21. Doi: 10.1016/j.fcr.2015.01.001

Salvador, J.O., A. Moreira, E. Malavolta, and C.P. Cabral. 2003. Influência do boro e do manganês no crescimento e na composição mineral de mudas de goiabeira. Ciênc. agrotec. 27(2), 325-331.

Siddiqi, M.Y. and A.D.M. Glass. 1981. Utilisation index: a modified approach to the estimation and comparison of nutrient utilization efficiency in plants. J. Plant Nutr. 4, 289-302.

Swiader, J.M., Y. Chyan, and F.G. Freiji. 1994. Genotypic differences in nitrate uptake and utilization efficiency in pumpkin hybrids. J. Plant Nutr. 17(10), 1687-1699.

Wimmer, M.A. and T. Eichert. 2013. Review: mechanisms for boron deficiency-mediated changes in plant water relations. Plant Sci. 203, 25-32. Doi: 10.1016/j.plantsci.2012.12.012

Effect of different sources of nitrogen on the vegetative growth of Andean blueberry (*Vaccinium meridionale* Swartz)

Efecto de diferentes fuentes de nitrógeno sobre el crecimiento vegetativo de agraz (*Vaccinium meridionale* Swartz)

Laura Katalina González[1], Laura Natalia Rugeles[1], and Stanislav Magnitskiy[1*]

ABSTRACT

The Andean blueberry (*Vaccinium meridionale* Swartz) is a small wild shrub that grows in Colombia in sub-paramo areas. The berry has a high export potential due to the high content of antioxidants in the fruits. The objective of the present research was to evaluate the effect of fertilization with different nitrogen sources on the vegetative growth of plants with four treatments: a control of 0% N, 50%NH_4^+-50%NO_3^-, 100% NO_3^-, and 100% NH_4^+. The dose of 70 mg L^{-1} N was used in the treatments with application of N. The plants with best growth were those fertilized with ammonia sources. The treatment with 100% NH_4^+ obtained the highest average number of shoots (22) and leaves (254) per plant, and the highest dry weight of plant organs at 148 d after planting of rooted cuttings followed by treatment with 50%NH_4^+-50%NO_3^-. Applications of 0% N and 100% NO_3^- resulted in higher rates of anthocyanin synthesis and lower contents of chlorophyll in leaves regarding to the N sources containing NH_4^+. The plants fertilized with 0% N and 100% NO_3^- presented lesser growth than those fertilized with 50%NH_4^+-50%$NO_3^-$and 100% NH_4^+. This study allows to conclude that the Andean blueberry requires a fertilization plan with ammonia sources.

Key words: macronutrients, cuttings, growth indexes, Ericaceae.

RESUMEN

El agraz *Vaccinium meridionale* Swartz es un arbusto silvestre pequeño, que crece en Colombia en zonas de subpáramo, con un alto potencial para posicionarse en el mercado internacional, gracias a su alto contenido de antioxidantes en los frutos. El presente trabajo tuvo como objetivo evaluar el efecto de la fertilización con diferentes fuentes de nitrógeno sobre el crecimiento de plantas de agraz en etapa vegetativa, con cuatro tratamientos: testigo sin aplicación del N, 50% NH_4^+-50%NO_3^-, 100% NO_3^- y 100% NH_4^+. Se utilizó la dosis de 70 mg L^{-1} del N en los tratamientos con la aplicación del nitrógeno. Las plantas fertilizadas con fuentes amoniacales tuvieron el mejor crecimiento, siendo el tratamiento de 100% NH_4^+ el que obtuvo el mayor número de brotes (22), mayor número de hojas (254) por planta y mayores pesos secos de los órganos a los 148 días después de la siembra de estacas enraizadas, seguido del tratamiento con 50%NH_4^+-50%NO_3^-. Los tratamientos con 0% N y 100% NO_3^- tuvieron una mayor síntesis de antocianinas y una menor cantidad de clorofilas en hojas con respecto a las plantas fertilizadas con fuentes de NH_4^+. Las plantas fertilizadas con 0% N y 100% NO_3^- presentaron un crecimiento menor que aquellas fertilizadas con 50% NH_4^+-50%NO_3^- y 100%NH_4^+. Este estudio permite afirmar que el agraz requiere planes de fertilización con fuentes amoniacales.

Palabras clave: macronutrientes, estacas, índices de crecimiento, Ericaceae.

Introduction

The production of exotic fruits in Colombia has been increasing in recent times due to the tropical conditions that allow a constant production and a permanent fruit supply over the year (Contreras *et al*., 2011). The Andean blueberry *Vaccinium meridionale* Swartz (Ericaceae), known locally as *agraz* or *mortiño*, has raised its importance in the country as one of the exotic fruit crops of high value for national and international markets due to its high nutraceutical and medicinal properties (Maldonado *et al*., 2014; González *et al*., 2017). The plant has many uses including fresh fruit consumption, juices, jams, wines, ice cream or pastry, as well as the industry of beverages and processed food (Zapata *et al*., 2016; López *et al*., 2017). According to Garzón *et al*. (2010), the antioxidant effect of fruits of Andean blueberry exceeds up to 3 times the raspberry fruits and other *Rubus* species, which is related to a high content of anthocyanins in its fruits (Montoya *et al*., 2012).

The genus *Vaccinium* comprises more than 450 species (Abreu *et al*., 2014); in the Andes several of these plants grow in open mountain slopes at altitudes between 2,300-3,500 m a.s.l., so they are considered as sub-paramo and

Received for publication: 7 December, 2017. Accepted for publication: 9 February, 2018 Doi: 10.15446/agron.colomb.v36n1.69304

[1] Faculty of Agricultural Sciences, Universidad Nacional de Colombia, Bogota.
* Corresponding author: svmagnitskiy@unal.edu.co

CC BY NC ND

paramo plants (Chamorro and Nates-Parra, 2015). In wild populations, the Andean blueberry is presented as an evergreen shrub of 1-4 m size, with globose berries from 5 to 10 mm in diameter, purple-dark color when mature, and an edible slightly acid pulp. Ligarreto *et al*. (2011) recognized the Andean blueberry as a promising species for cultivation in the country due to its high adaptability to different environments of the Andean highlands. However, despite the productive potential of the Andean blueberry, little research has been carried out on the mineral nutrition of this plant; this lack of information hinders a possible expansion and productive positioning of the Andean blueberry crop in the country.

In general, the plants of genus *Vaccinium*, such as most calcifuge plants, are limited to acid soils (pH 4.2 to 6.5) characterized by high content of organic matter, high availability of iron and manganese, and the presence of ammonium (NH_4^+) as the predominant form of N in the soil solution (Korcak, 1988; Bryla and Strik, 2015). According to Maqbool (2013), the nitrification activity, as a conversion of NH_4^+ to NO_3^-, is limited in soils where blueberries have evolved, and, consequently, most of the N available in soil is present as ammonium. Townsend (1967, 1969), Greidanus *et al*. (1972), and Peterson *et al*. (1988) agreed that fertilization of blueberries with NH_4^+ sources stimulates their growth, while supplying the plants with N as nitrate (NO_3^-) induces leaf chlorosis and decreases growth. These symptoms seem to be related to a lower rate of absorption and/or assimilation of NO_3^- compared with NH_4^+ (Merhaut and Darnell, 1995) considering that N is the element of the highest demand that *Vaccinium* plants require during the vegetative growth (Maqbool, 2013).

The plants absorbing NO_3^- require a nitrate reductase enzyme, which is referred to be inefficient in blueberries (Merhaut and Darnell, 1996) to reduce NO_3^- to NH_4^+ and metabolize it into amino acids that are used for protein synthesis (Salisbury and Ross, 2000). Studies conducted by Peterson *et al*. (1988), Sugiyama and Ishigaki (1994), Merhaut and Darnell (1995) indicated that the uptake of NO_3^- by roots of commercial *Vaccinium* sp. is limited compared to NH_4^+ due to the low activity of nitrate reductase (Poonnachit and Darnell, 2004). According to Alt *et al*. (2017), the supply of NO_3^- to the roots of *V. ashei* and *V. corymbosum* did not increase its transport in the xylem nor increased the nitrate reductase activity in leaves, which shows the presence of limitations in the absorption and translocation of NO_3^- to the shoot. These limitations result in the reduction in growth of blueberries fertilized with NO_3^-. Also, availability of carbohydrates at the time of nitrogen assimilation can have a marked effect on absorption and assimilation of N and blueberry growth (Merhaut and Darnell, 1996). In particular, N-deficient plants had increased carbon allocation towards formation of phenolic substances, such as anthocyanins (Bryant *et al*., 1983).

Currently, most studies on the Andean blueberry assess the use of the fruits, plant propagation, and ecology (Chamorro and Nates-Parra, 2015, and references therein). According to Ligarreto *et al*. (2011), in Colombia, a high genetic variability of the Andean blueberry exists in wild populations; however the variation in size of fruits is low, which can be useful to use this species as a commercial crop. At the same time, to our knowledge, no published reports are available on fertilization plans or determination of nutrient requirements of the Andean blueberry.

Considering that Andean blueberry as well as blueberries (*Vaccinium* sp.) are established as commercial crops by cuttings and the further lack of information about nitrogen fertilization in the Andean blueberry, this study was developed under the assumption that Andean blueberry present a preference for using ammonia sources of N compared to NO_3^- sources for its growth. The objective of the research was to evaluate the effect of mineral nutrition with different nitrogen sources (NH_4^+, NO_3^- and their combination) on the content of pigments and vegetative growth of the Andean blueberry.

Materials and methods

Plant establishment

Plant material was obtained from apical branches of adult Andean blueberry (*Vaccinium meridionale* Swartz) plants grown in the greenhouses of the Faculty of Agricultural Sciences of the Universidad Nacional de Colombia (Bogota, Colombia). The base of the cuttings was treated with rooting substance consisting of 0.5:4.5 w/w mixture of Hormonagro® (Colinagro, Bogota, Colombia) and kaolinite making a 400 mg kg^{-1} naphthalene acetic acid concentration in the rooting mixture. After the substrate mixing process, the cuttings were placed in plastic propagation trays of 8x8 cm alveoli using 3 cuttings per alveolus. The substrate used for rooting of cuttings consisted of a 1:1 w/w mixture of Klassman® (Klasmann-Deilmann, Geeste, Germany) peat without nutrients and quartz sand that was previously disinfected. The cuttings were placed on a greenhouse bench at low light conditions (70% black plastic mesh), where received mist irrigation twice a day for 5 min. During the rooting period the plants were not fertilized.

After 90 d, the rooted cuttings were collected with 7.95±0.19 cm length, 0.54±0.27 g fresh weight (FW), 7.14±2.91 leaves,

and 8.43±3.55 cm^2 leaf area (average ± standard deviation); the leaves were disposed directly over the cutting stem, so that the cuttings had no lateral shoots. The cuttings were transplanted into 15x20 cm black plastic bags, one cutting per bag, in the substrate consisting of a 5:1 v/v mixture of Klassman® peat without nutrients and quartz sand. The substrate was previously disinfected at a temperature of 105 °C for 6 h in an oven. The substrate had the following chemical characteristics before starting fertilization treatments: 1.3% total N, 0.02% total P, 0.61% total Ca, 0.02% total K, 0.04% total Mg, 67.6 meq 100 g^{-1} CEC, pH 6.0, and 0.73 dS m^{-1} EC (saturated paste) (IGAC, 2006). The 0.5 cm layer of rice husk was placed on the substrate surface around each plant to inhibit the growth of algae.

The transplanted cuttings were positioned in plastic covered greenhouse having average values of 23°C (day) and 15°C (night) air temperature, 63% relative air humidity (Kestrel® 4000 Weather Meter, Nielsen-Kellerman Co, Boothwyn, USA), and 1193.5 µmol m^{-2} s^{-1} light conditions (quantum censor Li-189, Li-Cor Inc., Lincoln, USA).

Fertilizer applications

Two days after transplanting into the bags, the application of nutrient solutions to cuttings via roots was initiated. Around 30 ml of the nutrient solution was applied 3 times per week and per plant to maintain the substrate humidity at a constant field capacity, according to the four treatments: 1) Control without N; 2) 50% NH_4^+ - 50% NO_3^-; 3) 100% NO_3^-; and 4) 100% NH_4^+.

During 148 d of fertilization the plants were treated with nutrient solution; no water was applied to the plants at any time. The concentration of N in the nutrient solution was 70 mg L^{-1}, except the control treatment with 0 mg L^{-1} N. The nutrient solution contained other mineral elements in doses: 16 mg L^{-1} P, 40 mg L^{-1} K, 30 mg L^{-1} Ca, 24 mg L^{-1} Mg, and 0.1 mg L^{-1} Fe. These concentrations of N and other mineral elements were adapted from chemical composition of nutrient solutions used for growth of rooted blueberry cuttings under hydroponics conditions (Poonnachit and Darnell, 2004; Tamada, 2004; Glonek and Komosa, 2013). The micronutrients, except Fe, were applied to the nutrient solution in quantity 1 ml L^{-1} according to the methodology followed Flórez and Cruz (2004). Distilled water was used to prepare the nutrient solutions.

As sources of mineral elements, pure salts of analytical grade were used: NH_4NO_3, $Ca(NO_3)_2$, $NaNO_3$, and Urea for nitrogen; $CaCl_2$ for calcium; K_2HPO_4 for potassium and phosphorus; $MgSO_4$ for magnesium and sulphur, $FeSO_4 \cdot 7H_2O$ for iron, and sources of other micronutrients according to Flórez and Cruz (2004). The sources of iron and other micronutrients were mixed with the rest of the nutrient solution immediately before applying to the plants.

A completely randomized design was carried out with 4 treatments; each treatment consisted of 35 plants at the start of the experiment. Five destructive samplings were carried out: 1st sampling: 2 d after transplanting (dat) (the moment when fertilizer application started); 2nd sampling: 42 dat; 3rd sampling: 77 dat; 4th sampling: 111 dat; 5th sampling: 148 dat. During each sampling 7 plants were randomly removed in each treatment to obtain direct and indirect growth variables.

The following direct variables were quantified on each 7 plants per treatment: number of shoots (primary branches originated at the cutting stem) and leaves; leaf area using leaf area meter LI 3100C® (LI-COR Inc., USA); dry weight (DW) of leaves, shoots, and adventitious roots obtained after drying samples at 65°C for 48 h; relative chlorophyll contents in leaves using SPAD® 502 (Minolta, Japan) on leaf limb, in 3 leaves of each cutting; contents of monomeric total anthocyanins in leaves (mg 100 g^{-1} FW) taking 3 leaves per each plant and using the differential pH method (extraction with 1% HCL in methanol, dilution in buffers pH 1.0 and pH 4.5 and spectrophotometry according to Lee *et al*. (2005)). The anthocyanin contents were measured using λ=530 nm (Spectrophotometer Spectronic® 501, Milton Roy, USA) and were calculated as cyanidin-3-glucoside equivalents (molecular weight of 449.2 g mol^{-1}).

Growth indexes as indirect variables of plant growth were calculated according to Hunt (2013): Leaf area index (LAI) = L_A/G_A; Specific leaf area (SLA, cm^2 g^{-1}) = L_A/L_W; Net assimilation rate (NAR, g m^{-2} d^{-1}) = $(1/L_A)x(dS_{DW}/dt)$, where L_A – leaf area (cm^2), G_A – area of soil surface; L_w – leaf DW (g), S_{DW} – DW of shoots + leaves; t – time (d).

Statistical analysis

The data reported at each sampling moment were average values of 7 replicates. To determine the significant differences between the treatments, a Tukey test ($P \leq 0.05$) was applied. The results were analyzed with the statistical program SAS® 9.2.1 (SAS Institute, Inc., USA).

Results and discussion

Contents of chlorophyll and anthocyanins in leaves

The relative chlorophyll contents in leaves, for treatments 100% NH_4^+ and 50%NH_4^+ - 50%NO_3^-, increased throughout the experiment and corresponded to 45.94 and 54.88 SPAD

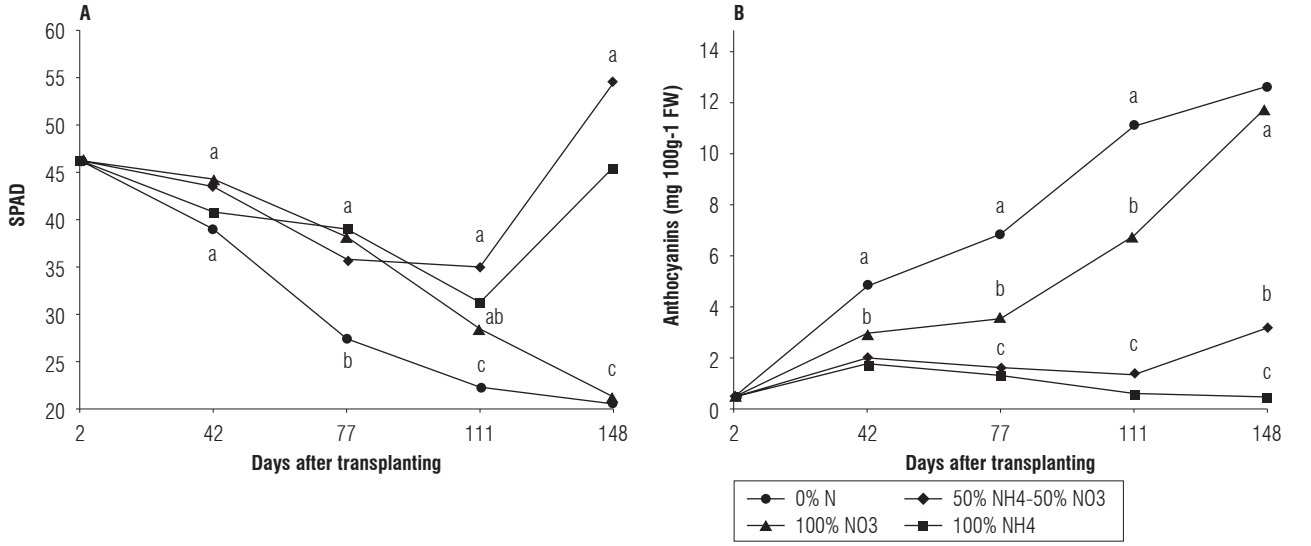


FIGURE 1. A) Relative chlorophyll contents (SPAD units) and B) Anthocyanin contents (mg 100 g^{-1} FW) in leaves of the Andean blueberry treated with different sources of N. Means followed by the different letters at each sampling date are significantly different according to Tukey test ($P \leq 0.05$).

units at 148 dat, respectively (Fig. 1A). The plants treated with 0% N and 100% NO_3^- at 148 dat had significantly lower chlorophyll levels in leaves of 20.44 and 21.40 SPAD units, respectively (Fig. 1A). A lower synthesis of chlorophyll in these treatments can be attributed to the preference of the Andean blueberry for ammonia forms of N and progressed N deficiency in the treatment of 0% N. For anthocyanin synthesis, the opposite trend was observed, where the treatments with 0% N and 100% NO_3^-accumulated significantly higher contents of anthocyanins in leaves at the end of the experiment, with 12.79 and 11.68 mg 100 g^{-1} FW, respectively (Figs. 1B and 2). This behavior could be an adaptive response of N-deficient plants to protect leaves with low levels of chlorophyll (Fig. 1A) from the excess of light (Salisbury and Ross, 2000).

Deficiencies of mineral nutrients can cause accumulation of anthocyanins in leaves of *Vaccinium* sp. (Routray and Orsat, 2011). According to Salisbury and Ross, (2000), N deficiency regulates routes of secondary metabolism in plants, such as synthesis of phenylpropanoids including flavonoids and anthocyanins. Bryla and Strik (2015) affirmed that plants of *V. corymbosum* deficient in N presented poor growth, with leaves that turned pale green or yellow and developed a reddish tinge. Leitzke *et al.* (2015) found that fertilization of blueberry with ammonium sulfate induced a higher synthesis of chlorophyll, and that high doses of the same source (more than 7.37 g per plant at reproductive stage), can negatively influence its growth and production. The same authors indicated increased contents of anthocyanins in plants subjected to abiotic or biotic stress; these effects were attributed to an excessive accumulation of N in leaves or a reduction of the soil pH and an increased availability of aluminum in soil when fertilized with ammonium sources of N (Leitzke *et al.*, 2015).

Accumulation of anthocyanins in blueberry fruits contribute to their high commercial attractiveness due to their nutraceutical effects (Li *et al.*, 2017); however, in the present study, the accumulation of anthocyanins in leaves was, evidently, an adaptive response to stress associated with a reduced production of chlorophyll. At the same time, blueberry leaves are used in food industry as sources of antioxidants due to their high content of anthocyanins (Ferlemi and Lamari, 2016). The leaves of Andean blueberry are used in beverage industry (Zapata *et al.*, 2015),

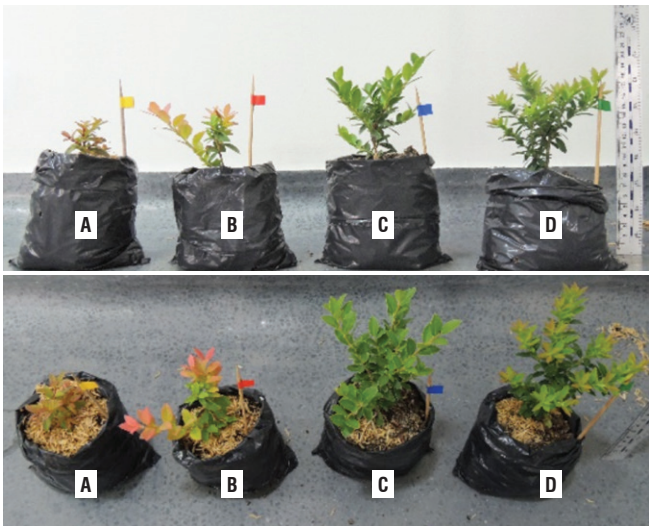


FIGURE 2. Plants of the Andean blueberry fertilized with A) 0% N, B) 100% NO_3^-, C) 50%NH_4^+-50%NO_3^-,or D) 100% NH_4^+at 148 dat.

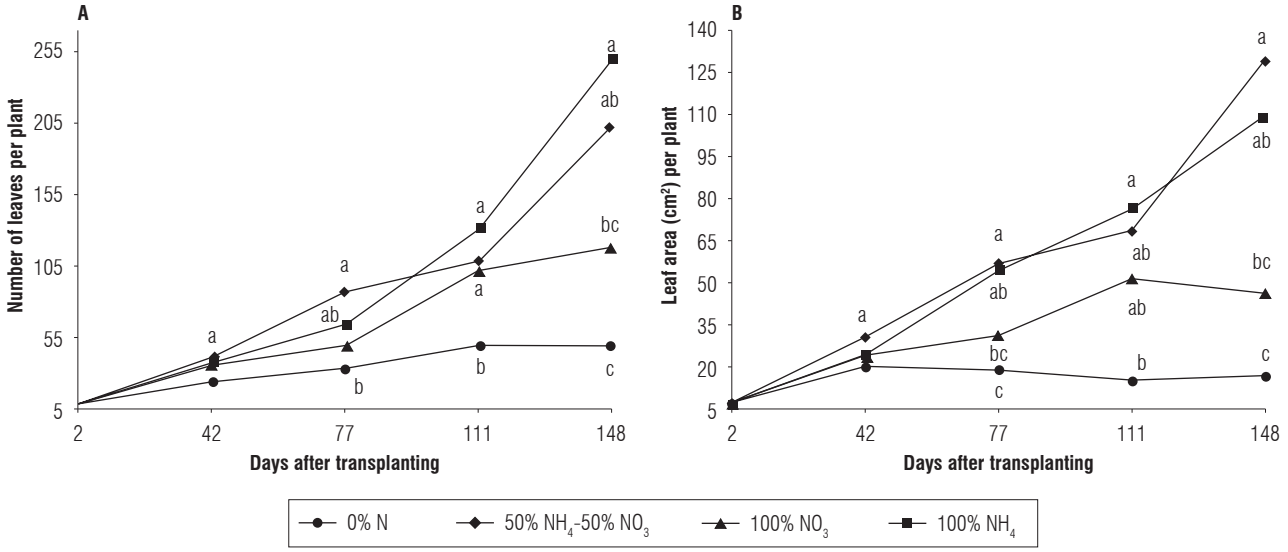


FIGURE 3. A)Number of leaves and B)Leaf area of the Andean blueberry treated with different sources of N. Means followed by the different letters at each sampling date are significantly different according to the Tukey test ($P \leq 0.05$).

therefore, an addition of NO_3^- to fertilizer formulas could be a practice aimed to increase anthocyanin contents in leaves for this purpose.

Number of leaves and leaf area

The number of leaves and leaf area progressively increased over time, where the treatment with 100% NH_4^+ reached a maximum of 254 leaves per plant (Fig. 3A) and the treatment of 50%NH_4^+ - 50%NO_3^- reached the maximum 130 cm^2 of leaf area (Fig. 3B) at 148 dat. Determination of leaf area is important to characterize crop growth, its potential yield, and efficiency in use of solar radiation, water, and mineral nutrients (Williams and Martinson, 2003). The growth rate of leaves is affected by expansion of young cells, which are produced by cell division in the meristematic tissues (Salisbury and Ross, 2000). For this reason, inadequate supply of N could diminish rates of leaf growth as it was evidenced in the treatments with only NO_3^- sources, where the plants of Andean blueberry generated fewer leaves and lesser leaf area (Fig. 3) due to the preference of various Ericaceae to NH_4^+ sources of N (Percival and Privé, 2002). The plants treated with 0% N and 100% NO_3^- obtained maximum 50 and 119 leaves at 148 dat, respectively. The plants of 0% N treatment had poor leaf emission throughout the experiment and presented leaf abscission at 148 dat due to the lack of N supply, apparently, exhausting N reserves of cutting stem.

Number of shoots

The plants of Andean blueberry fertilized with NH_4^+ sources responded with a higher production of shoots (i.e. primary branches) on the cutting stem compared to the plants of NO_3^- fertilization (Fig. 4). The number of shoots per cutting progressively increased over time, with the treatment of 100% NH_4^+ reaching maximum values of 22 shoots per plant followed by the treatment 50% NH_4^+ - 50% NO_3^-, with 20 shoots per plant. During the evaluation period, control treatment 0% N had always the lowest number of new shoots (Fig. 4) as compared with other treatments. According to Takamizo and Sugiyama (1991), higher concentrations of N were recorded in shoots of blueberry plants fertilized with NH_4^+ with respect to those fertilized with NO_3^-; the higher concentration of N, in turn, could contribute to formation of new shoots.

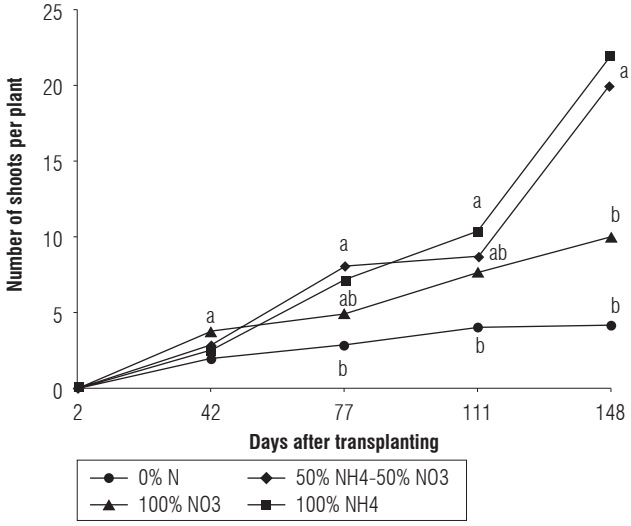


FIGURE 4. Number of shoots per plant of the Andean blueberry treated with different sources of N. Data means followed by the different letters at each sampling date are significantly different according to Tukey test ($P \leq 0.05$).

Dry weight of leaves, shoots, and roots

The highest leaf weight was registered in treatments 100% NH_4^+ and 50%NH_4^+ - 50%NO_3^- (1.85 and 1.37 g DW, respectively) at 148 dat, while the other treatments had less than 1 g DW of leaves per plant (Fig. 5A). The accumulation of dry matter in leaves is directly related to the number of shoots and new leaves and to photosynthetic capacity that allows producing photoassimilates and developing higher leaf area. The plants supplied with 100% NO_3^-, apparently, managed to assimilate some fraction of NO_3^- into the cell proteins and surpass the treatment with 0% N, which counted up mainly on N reserves of cutting stem/roots to emit few leaves. This agrees with the data reported for blueberry by Merhaut and Darnell (1996), who affirm that, although the plants of *Vaccinium* sp. can absorb both NH_4^+ and NO_3^- under the range of pH conditions optimal for growth, assimilation of NO_3^- by these plants is limited as compared to NH_4^+. It should be noted that, in our experiment, the differences among the treatments in plant growth might be, at least in part, influenced by the changes in substrate pH that depended on the source of N used for the application.

Root DW in the treatments 100% NH_4^+ and 50%NO_3^- - 50%NH_4^+ increased over the time of the experiment (Fig. 5C); these plants exhibited the highest values of 0.52 and 0.40 g root DW per plant, respectively, at 148 dat. This correlates with the superiority of the same treatments in emission of leaves and shoots (Figs. 4 and 5) and agrees with Merhaut and Darnell (1995), who affirmed the lower assimilation rate of NO_3^- in *Vaccinium* sp in comparison with that of NH_4^+. Birkhold and Darnell (1993) indicated that *V. ashei* during vegetative growth had a high concentration of N in both shoots and roots, since fast-growing organs strongly demand this element. The roots can serve as the storage site for N, which would be later translocated to new growing organs, even though the N concentration was adequate in the nutrient solution (Birkhold and Darnell, 1993). In another deciduous species as *V. corymbosum*, the highest accumulation of biomass during vegetative stage of growth corresponded to a period of maximum N absorption, with new emerging leaves being the organs of main demand for N (Fang *et al*., 2017).

Plant strategies to adapt for different types of stress frequently consist in decreasing leaf expansion, increasing root growth, or promoting the leaf abscission (Yepes and Buckeridge, 2011). The distribution of DW between the organs at 148 dat showed that plants of 0% N and 100% NO_3^- assigned a higher proportion of biomass to formation of roots as compared with the treatments with NH_4^+ (Tab. 1).

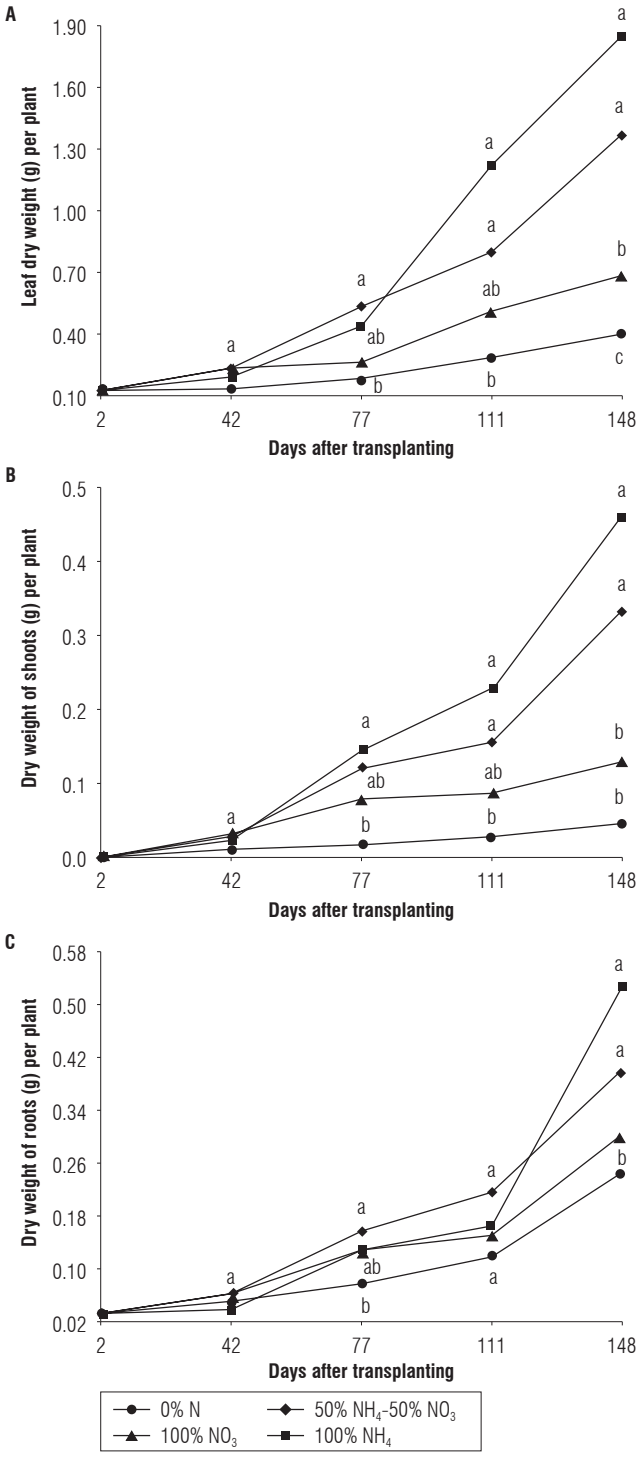


FIGURE 5. Dry weight of leaves (A), shoots (B) and roots (C) of the Andean blueberry treated with different sources of N. Data means followed by the different letters at each sampling date are significantly different according to the Tukey test ($P \leq 0.05$).

This could indicate that the plants searched for N sources to compensate N deficiency when growing without NH_4^+ supply. Additionally, the plants of Andean blueberry, in the

TABLE 1. Distribution of dry weight among organs in plants of the Andean blueberry treated with different sources of N, % total dry weight of the plant (stem+shoots+leaves+roots).

	2 dat	148 dat			
	Initial DW distribution	Control without N	100% NO_3^-	50%NH_4^+-50%NO_3^-	100%NH_4^+
Leaves	34.0	43.5	52.5	59.8	58.5
Shoots	0	4.8	9.8	15.0	14.2
Stem of cutting	58.6	24.9	17.1	8.2	10.2
Roots	7.4	26.8	20.6	17.0	17.1

absence of NH_4^+, expanded leaf area through emitting new leaves but invested much less resources in the formation of new shoots as compared to plants supplied with NH_4^+ (Tab. 1; Fig. 2).

Growth indexes

Leaf area index (LAI) expresses the leaf area over the surface area occupied by the plant (Hunt, 2013). The LAI of plants treated with N had an ascending behavior over the time (Fig. 6A) and at some point would reach a maximum value, at which the plants will have the maximum capacity to intercept solar energy (Hunt, 2013). The ascending behavior of LAI can be attributed to a high rate of leaf formation and foliar expansion, especially in treatments 100% NH_4^+ and 50%NH_4^+ - 50%NO_3^-. The maximum values of LAI were not observed (Fig. 6A) because the moment of evaluation was limited to 148 dat of vegetative growth; the highest LAI of 1.06 was recorded at 148 dat for the treatment 100% NH_4^+. In treatment with 0% N, LAI did not exceed 0.2. Therefore, in plants of 100% NO_3^- and, especially, 0% N, the behavior of LAI can be explained by the deficiency of N, with a consequent reduction in leaf area and a reduction in the proportion of dry mass accumulated in leaf tissues (Curtis and Läuchli, 1986). This deficiency might have progressively reduced photosynthetic rate in these plants due to a lower chlorophyll content accompanied by an increased production of anthocyanins (Fig. 1) and, in some cases, caused leaf senescence. According to behavior of LAI, the treatments with NH_4^+ could be considered as the best treatments. These plants produced the highest number of leaves (Fig. 3A) and shoots (Fig. 4) that allowed them a better interception of light that, consequently, would be reflected in higher production of photoassimilates and, therefore, allowed a greater accumulation of biomass in comparison with treatments of 100% NO_3^- and 0% N.

Specific leaf area (SLA) is defined as a ratio between the total leaf area of the plant and dry weight of leaves (Hunt, 2013). The tendency of SLA in all treatments was to increase between 42 and 77 dat (Fig. 6B), which can be explained by a higher increase in leaf area as compared to the increase in leaf DW. In this period, the increase of SLA could be explained by the expansion of leaf area in pre-existing leaves and new leaves, such as in 50%NH_4^+ - 50%NO_3^- treatment. Apparently, this period of growth between 42 and 77 dat was characterized by increasing leaf surface, while the leaves maintained a low amount of mesophyll layers. The SLA increase in 0%N treatment from 42 to 77 dat could be due to consumption of photoassimilates/starch available in leaves/stem of cutting, which, in turn, might have reduced leaf thickness. Also, the marked decrease in SLA in 0% N treatment starting from 77 dat until the end of the experiment (148 dat) is attributed to the poor formation of new leaves (Fig. 3A), because the plants were depleting their N reserves. In other treatments, the decrease in SLA between 77 and 148 dat can be explained by a lower expansion of leaf area and increased transport of photoassimilates and/or N towards the leaves, which implied an increase in weight/thickness of leaves. This behavior is common in deciduous blueberries, where an adequate supply of N results in N storage and subsequent remobilization for growth of new leaves and shoots (Birkhold and Darnell, 1993).

The net assimilation rate (NAR) is defined as the increase in plant dry weight per unit of leaf area and time (Hunt, 2013) and indicates average photosynthetic efficiency of plants. In plants treated with 100% NH_4^+ or 50%NH_4^+ - 50%NO_3^-, NAR reached the highest values starting from 77 dat because, in this period, the plants were emitting new leaves (Fig. 3A). The treatments with NH_4^+sources presented the highest values of NAR because, as shown by LAI, these plants had the maximum leaf growth allowing the highest photosynthetic efficiency as well as the largest accumulation of dry matter.

In plants treated with 100% NO_3^-or 0% N, the decrease in NAR between 42 and 77 dat (Fig. 6C) indicated a reduced photosynthetic efficiency in this period, which can be attributed to the reduction in activity of Rubisco and/or other proteins associated with the Calvin cycle (Salisbury and Ross, 2000). It can be hypothesized that an atypical increase in NAR between 77 and 111 dat for treatments 0% N and

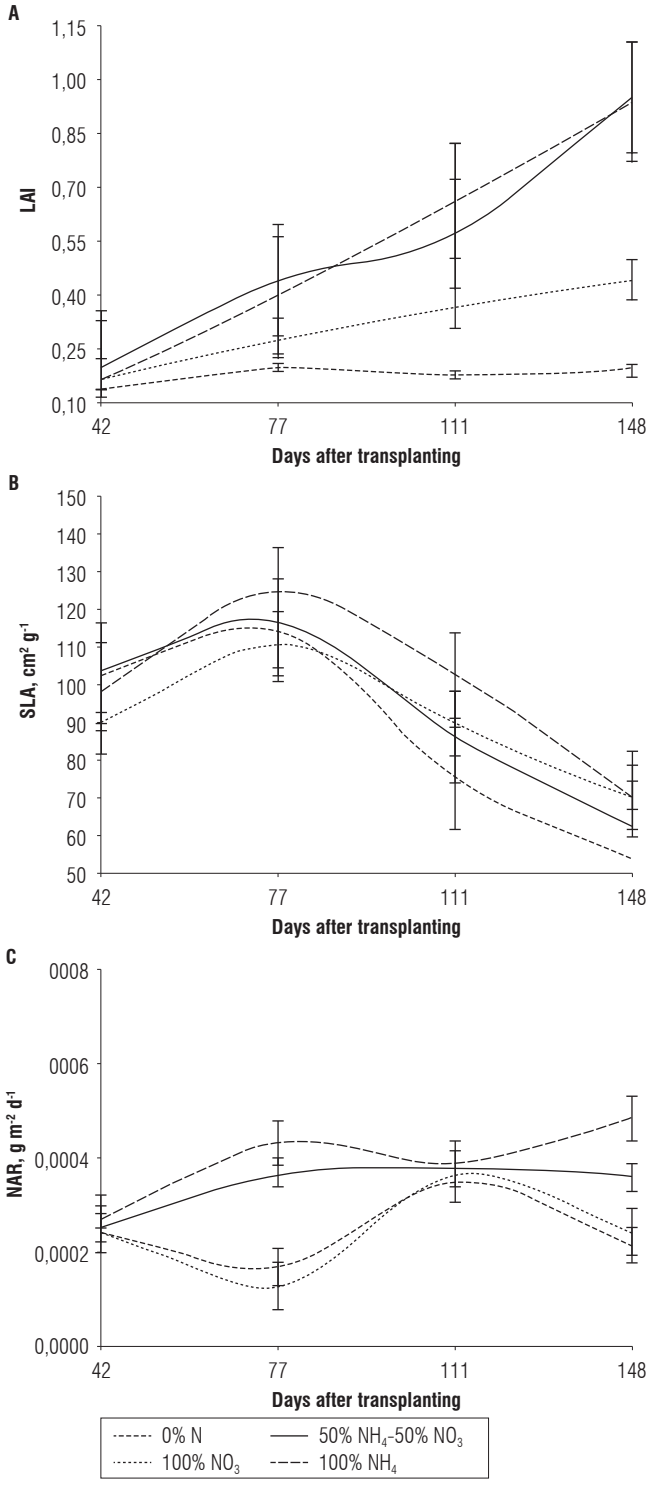


FIGURE 6. A) Leaf area index (LAI), B)Specific leaf area (SLA), and C)Net assimilation rate (NAR) of Andean blueberry plants treated with different sources of N. Vertical bars indicate standard deviations.

100% NO_3^- was due to re-traslocation of N reserves preserved in the stem of cuttings towards the leaves. Between 111 and 148 dat, in treatments 0% N and 100% NO_3^-, NAR decreased due to the senescence and abscission of leaves indicating N deficiency. In general, this study indicated that the Andean blueberry during vegetative growth, similarly to commercial blueberries *Vaccinium* sp, requires fertilizers with ammonia sources of N.

Conclusions

The effect of the nitric and ammonia fertilization on the growth of Andean blueberry was demonstrated showing that at vegetative stage of growth the plants had a higher accumulation of dry matter, higher number of shoots and leaves with application of NH_4^+ as N source. The plants subjected to NO_3^- fertilization presented an increased production of anthocyanins in leaves due to the stress caused by nitrogen deficiency and associated with low synthesis of chlorophyll.

Acknowledgments

The authors express their gratitude to the Faculty of Agricultural Sciences of the National University of Colombia, Bogota. The authors greatly acknowledge the help of Dr. Gustavo Ligarreto for the donation of Andean blueberry cuttings for this study, Dr. Liz Patricia Moreno for the review of the manuscript and critical suggestions, and Dr. Hector Lancheros (El Bosque University, Bogota) and Ing. Mariam Vásquez for their contributions to the experiment establishment.

Literature cited

Abreu, O.A., G. Barreto, and S. Prieto. 2014. *Vaccinium* (Ericaceae): Ethnobotany and pharmacological potentials. Emirat. J. Food Agric. 26(7), 577-591. Doi: 10.9755/ejfa.v26i7.16404

Alt, D.S., J.W. Doyle, and A. Malladi. 2017. Nitrogen-source preference in blueberry (*Vaccinium* sp.): Enhanced shoot nitrogen assimilation in response to direct supply of nitrate. J. Plant Physiol. 216, 79-87. Doi: 10.1016/j.jplph.2017.05.014

Birkhold, K.T. and R.L. Darnell. 1993. Contribution of storage and currently assimilated nitrogen to vegetative and reproductive growth of rabbiteye blueberry. J. Amer. Soc. Hort. Sci. 118(1), 101-108.

Bryant, J.P., F.S. Chapin, and D.R. Klein. 1983. Carbon/nutrient balance of boreal plants in relation to vertebrate herbivory. Oikos 40, 357-368.

Bryla, D.R. and B.C. Strik. 2015. Nutrient requirements, leaf tissue standards, and new options for fertigation of northern highbush blueberry. HortTech. 25(4), 464-470.

Chamorro, F.J. and G. Nates-Parra. 2015. Floral and reproductive biology of *Vaccinium meridionale* (Ericaceae) in the Eastern Andes of Colombia. Rev. Biol. Trop. 63(4), 1197-1212.

Contreras, J., L. Calderón-Jaimes, E. Guerra-Hernández, and B. García-Villanova. 2011. Antioxidant capacity, phenolic content and vitamin C in pulp, peel and seed from 24 exotic fruits from Colombia. Food Res. Int. 44(7), 2047-2053. Doi: 10.1016/j.foodres.2010.11.003

Curtis, P.S. and A. Läuchli. 1986. The role of leaf area development and photosynthetic capacity in determining growth of kenat under moderate salt stress. Aust. J. Plant Physiol. 18, 553-565.

Fang, Y., J. Williamson, R. Darnell, Y. Li, and G. Liu. 2017. Nitrogen uptake and allocation at different growth stages of young southern highbush blueberry plants. HortSci. 52(6), 905-909. Doi: 10.21273/HORTSCI11723-17

Ferlemi, A.V. and F.N. Lamari. 2016. Berry leaves: an alternative source of bioactive natural products of nutritional and medicinal value. Antioxidants 5(2), 1-20. Doi: 10.3390/antiox5020017

Flórez, V. and R. Cruz. 2004. Guías de Laboratorio de Fisiología Vegetal. Unibiblos, Universidad Nacional de Colombia, Bogota.

Glonek, J. and A. Komosa. 2013. Fertigation of highbush blueberry (*Vaccinium corymbosum* L.). Part I. The effect on growth and yield. Acta Sci. Pol., Hortorum Cultus 12(3), 47-57.

Garzón, G.A., C.E. Narváez, K.M. Riedl, and S.J. Schwartz. 2010. Chemical composition, anthocyanins, non-anthocyanin phenolics and antioxidant activity of wild bilberry (*Vaccinium meridionale* Swartz) from Colombia. Food Chem. 122, 980-986. Doi: 10.1016/j.foodchem.2010.03.01

González, M., I. Samudio, L.G. Sequeda-Castañeda, C. Celis, J. Iglesias, and L. Morales. 2017. Cytotoxic and antioxidant capacity of extracts from *Vaccinium meridionale* Swartz (Ericaceae) in transformed leukemic cell lines. J. Appl. Pharm. Sci. 7(3), 24-30. Doi: 10.7324/JAPS.2017.70305

Greidanus, T., L.A. Peterson, L.E. Schrader, and M.N. Dana. 1972. Essentiality of ammonium for cranberry nutrition. J. Amer. Soc. Hort. Sci. 97, 272-277.

Hunt, R. 2013. Basic growth analysis: Plant growth analysis for beginners. Unwin Hyman Ltd., London.

IGAC. 2006. Métodos analíticos del laboratorio de suelos. 6th ed.Instituto Geográfico Agustín Codazzi, Bogota.

Korcak, R.F. 1988. Nutrition of blueberry and other calcifuges. Hort. Rev. 10, 183-227.

Lee, J., R.W. Durst, and R.E. Wrolstad. 2005. Determination of total monomeric anthocyanin pigment contentof fruit juices, beverages, natural colorants, and wines by the pH differential method: Collaborative study. J. AOAC Int. 88(5), 1269-1278.

Leitzke, L., L. Picolotto, I. Pereira, G. Vignolo, J. Schmitz, M. Vizzotto, and L. Antunes. 2015. Nitrogen fertilizer affects the chemical composition of the substrate, the foliar nutrient content, the vegetative growth, the production and fruit quality of blueberry. Científica, 43(4), 316-324.

Li, D., B. Li, Y. Ma, X. Sun, Y. Lin, and X. Meng. 2017. Polyphenols, anthocyanins, and flavonoids contents and the antioxidant capacity of various cultivars of highbush and half-high blueberries. J. Food Composit. Anal. 62, 84-93. Doi: 10.1016/j.jfca.2017.03.006

Ligarreto, G.A., M.D.P. Patiño, and S.V. Magnitskiy. 2011. Phenotypic plasticity of *Vaccinium meridionale* (Ericaceae) in wild populations of mountain forests in Colombia. Rev. Biol. Trop. 59(2), 569-583.

López-Padilla, A., D. Martín, D. Villanueva, L. Jaime, A. Ruiz-Rodríguez, C.E. Restrepo, D.M. Rivero, and T. Fornari. 2017. *Vaccinium meridionale* Swartz extracts and their addition in beef burgers as antioxidant ingredient. J. Sci. Food Agric. 97, 1097-1110. Doi: 10.1002/jsfa.8483

Maldonado, M.E., S.S. Arango-Varela, and B.A. Rojano. 2014. Free radical scavenging capacity and cytotoxic and antiproliferative effects of *Vaccinium meridionale* Sw. against colon cancer cell lines. Rev. Cubana Plant Med. 19(2), 172-184.

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

Merhaut, D. and R. Darnell. 1995. Ammonium and nitrate accumulation in containerized southern highbush blueberry plants. HortSci. 30, 1378-1381.

Merhaut, D. and R. Darnell. 1996. Vegetative growth and nitrogen/carbon partitioning in blueberry as influenced by nitrogen fertilization. HortSci. 121(5), 875-879.

Montoya, C.G., J.D.H. Arredondo, M.L. Arias, C.I.M. Cano, and B.A. Rojano 2012. Cambios en la actividad antioxidante en frutos de mortiño (*Vaccinium meridionale* Sw.) durante su desarrollo y maduración. Rev. Fac. Nal. Agr. Medellín 65(1), 6487-6495. Doi: 10.15446/rfnam

Percival, D.C. and J.P. Privé. 2002. Nitrogen formulation influences plant nutrition and yield components of lowbush blueberry (*Vaccinium angustifolium* Ait). Acta Hort. 574, 347-351. Doi: 10.17660/ActaHortic.2002.574.52

Peterson, L.A., E.J. Stang, and M.N. Dana. 1988. Blueberry response to NH_4^+-N and NO_3^--N. J. Amer. Soc. Hort. Sci. 113, 9-12.

Poonnachit, U. and R. Darnell. 2004. Effect of ammonium and nitrate on ferric chelate reductase and nitrate reductase in *Vaccinium* species. Annals Bot. 93, 399-405. Doi: 10.1093/aob/mch053

Routray, W. and V. Orsat. 2011. Blueberries and their anthocyanins: factors affecting biosynthesis and properties. Compr. Rev. Food Sci. Food Safety 10(6), 303-320. Doi: 10.1111/j.1541-4337.2011.00164.x

Salisbury, F.B. and C.W. Ross. 2000. Fisiología de las Plantas. Desarrollo de las Plantas y Fisiología Ambiental. Editorial Paraninfo, Madrid.

Sugiyama, N. and K. Ishigaki. 1994. Uptake of nitrate-nitrogen by blueberry plants. J. Plant Nutr. 17(11), 1975-1982. Doi: 10.1080/01904169409364859

Tamada, T. 2004. Effects of nitrogen sources on growth and leaf nutrient concentrations of 'Tifblue'rabbiteye blueberry under water culture. Small Fruits Rev. 3(1-2), 149-158. Doi: 10.1300/J301v03n01_15

Takamizo, T. and N. Sugiyama. 1991. Growth responses to N forms in rabbiteye and highbush blueberries. J. Jpn. Soc. Hort. Sci. 60, 41-45.

Townsend, L.R. 1967. Effect of ammonium nitrogen and nitrate nitrogen, separately and in combination, on the growth of highbush blueberry. Can. J. Plant Sci. 47, 555-562.

Townsend, L.R. 1969. Influence of form of nitrogen and pH on growth and nutrient levels in the leaves and roots of the lowbush blueberry. Can. J. Plant Sci. 49, 333-338.

Williams, L. and T.E. Martinson. 2003. Nondestructive leaf area estimation of 'Niagara' and 'DeChaunac' grapevines. Sci. Hort. 98, 493-498.

Zapata, I.C., V. Villacorta, M.E. Maldonado, D. Castro-Restrepo, and B. Rojano. 2015. Antioxidant and cytotoxic activity of black and green tea from *Vaccinium meridionale* Swartz leaves. J. Med. Plants Res. 9(13), 445-453. Doi: 10.5897/JMPR2014.5744

Zapata, I.C., S. Ochoa, M.E. Maldonado, A.D. Zapata, and B.I. Rojano. 2016. Cytotoxic effect and antioxidant activity of Andean berry (*Vaccinium meridionale* Sw) wine. J. Med. Plants Res. 10(27), 402-408. Doi: 10.5897/JMPR2016.6100

Yepes, A. and M.S. Buckeridge. 2011. Respuestas de las plantas ante los factores ambientales del cambio climático global: Revisión. Colombia Forestal 14(2), 213-232. Doi: 10.14483/issn.2256-201X

Post-harvest quality of pineapple guava [*Acca sellowiana* (O. Berg) Burret] fruits produced in two locations at different altitudes in Cundinamarca, Colombia

Calidad poscosecha de frutos de feijoa [*Acca sellowiana* (O. Berg) Burret] producidos en dos localidades de Cundinamarca, Colombia, en diferentes altitudes

Alfonso Parra-Coronado[1]*, Gerhard Fischer[2], and Jesús Camacho-Tamayo[1]

ABSTRACT

The quality of pineapple guava fruits during post-harvest storage depends directly on their quality at harvest and is influenced by climatic conditions during growth. The aim of this study was to determine the influence of climatic conditions on certain parameters of fruit quality during post-harvest storage. Twenty trees were tagged in two locations within the department of Cundinamarca (Colombia), recording the climatic conditions during fruit growth until harvest. The fruits were differentiated by place of origin and stored at 18 ± 1ºC (76 ± 5% relative humidity, RH) for 11 d or 5 ± 1ºC (87 ± 5% RH) for 31 d, evaluating several quality attributes every two d. The places of origin were San Francisco de Sales (1,800 m a.s.l., 20.6ºC, 63-97% RH, with an average annual precipitation of 1,493 mm) and Tenjo (2,580 m a.s.l., 12.5ºC, 74-86% RH, with an average annual precipitation of 765 mm). The results indicated that the fruits stored at the highest temperature were sweeter and had reduced weight and firmness, lower acidity, and faster postharvest senescence (lower post-harvest durability). The postharvest fruit characteristics were determined by considering the fruit quality during growth and the influence of climatic conditions during cultivation in each location. At the higher altitudes, the total soluble solid content in the fruits was higher and firmness decreased, and the total titratable acidity and weight loss were lower. For fruit color, significant differences were not observed that would demonstrate the effect of climatic conditions during the post-harvest period.

Key words: feijoa, weight loss, firmness, total soluble solids, total titratable acidity, maturity ratio.

RESUMEN

La calidad de los frutos de feijoa durante el almacenamiento poscosecha depende directamente de la calidad que estos tengan en el momento de la recolección, la cual está influenciada por las condiciones climáticas de cultivo. El objetivo de este estudio fue determinar la influencia de las condiciones climáticas en algunos parámetros de calidad durante el almacenamiento en poscosecha. Se marcaron veinte árboles por finca en dos localidades del departamento de Cundinamarca (Colombia), donde se registraron las condiciones climáticas durante el crecimiento de los frutos hasta la cosecha. Los frutos diferenciados por el lugar de procedencia fueron almacenados a temperaturas de 18 ± 1ºC (humedad relativa: 76 ± 5%) durante 11 días y a 5 ± 1ºC (humedad relativa: 87 ± 5%) durante 31 días, con evaluación de los atributos de calidad cada 2 días. Los lugares de procedencia fueron San Francisco de Sales (1,800 msnm, 20,6 ºC, 63-97% humedad relativa (HR), precipitación media anual 1,493 mm) y Tenjo (2,580 msnm, 12.5ºC, 74-86% HR, precipitación media anual 765 mm). Los resultados obtenidos indican que los frutos almacenados a mayor temperatura son más dulces, con mayor pérdida de peso y de firmeza, así como con menor acidez y durabilidad en poscosecha, atributos que están determinados por la calidad de estos en la cosecha, la cual está influenciada a su vez por las condiciones climáticas registradas en el cultivo. Se observó que, a mayor altitud, también es mayor el contenido de sólidos solubles totales y la pérdida de firmeza, mientras que es menor la acidez total titulable y la pérdida de peso. En las mediciones de color no se evidenciaron diferencias significativas que permitan inferir que hubo alguna influencia de las condiciones climáticas en la variación de este parámetro durante la poscosecha.

Palabras clave: feijoa, pérdida de peso, firmeza, sólidos solubles totales, acidez total titulable, relación de madurez.

Introduction

Pineapple guava or feijoa (*Acca sellowiana* (O. Berg) Burret) is a perennial fruit species of the Mirtaceae family that is native to South America, particularly southern Brazil and Uruguay (Schotsmans *et al*., 2011), with high adaptability to different climatic zones (Parra and Fischer, 2013), and is cultivated commercially between 1,800 and

Received for publication: 30 October, 2017. Accepted for publication: 28 February, 2018 Doi: 10.15446/agron.colomb.v36n1.68577

[1] Departamento de Ingeniería Civil y Agrícola, Facultad de Ingeniería, Universidad Nacional de Colombia, Bogota (Colombia).
[2] Scientific consultant; Emeritus researcher of Colciencias, Bogota (Colombia).
* Corresponding author: aparrac@unal.edu.co

CC BY NC ND

2,700 m a.s.l. in Colombia. In tropical areas, this crop can produce fruits continually throughout the year, whereas under seasonal temperature conditions, only one annual harvest occurs (Quintero, 2012). As reported by Parra and Fischer (2013), the principal commercial production of pineapple guava is found in New Zealand, Georgia, Azerbaijan, Colombia and California, with the recent addition of commercial production in Uruguay and Brazil. In Colombia, which has an estimated area of 650 ha for this crop (Quintero, 2012), different varieties of pineapple guava are cultivated, which facilitates cross pollination and yields of high-quality fruits.

After harvest, pineapple guava fruits, which are entirely green, ripen from the inside out; over-ripe fruits suffer a loss of taste and darkening of the seed and pulp (Yi *et al*., 2016). The external changes in quality that occur during post-harvest ripening are not excessive in this green fruit, which makes it difficult to determine the degree of fruit ripeness by visual, tactile, or non-destructive methods (Gaddam *et al*., 2005). According to Amarante *et al*. (2013), the physiological basis of fruit ripening is not well known, making it more difficult to determine strategies for preserving quality during the post-harvest stage.

During post-harvest, fruits undergo a series of changes that involve synthesis and degradation, which are genetically controlled and eventually lead to senescence. These changes generally include modifications of the texture and ultrastructure of cell walls, changes in turgidity, juice content, conversion of starches into sugars, increases in susceptibility to pathogens, and alterations in pigment biosynthesis and compounds that determine flavor (Kader and Yahia, 2011). The evolution of these properties determines the post-harvest quality of fruits.

Oxidative metabolism influences most of the physicochemical changes that occur in harvested fruits (Parra-Coronado and Hernández-Hernández, 2008), and respiration may have the greatest effect on fruit ripening during post-harvest through cell diffusion processes (Schouten *et al*., 2004). The respiration pattern of pineapple guava is the same as for other Mirtaceae species and is a climacteric fruit (Schotsmans *et al*., 2011). The inhibition of respiratory processes directly affects the maintenance of vegetable and fruit quality during storage and can be performed by using controlled or modified atmospheres and also by altering the temperature and relative air humidity (Yahia *et al*., 2011). General fruit quality depends directly on the stage of ripeness. Many parameters must be considered when determining quality, such as firmness, total titratable acidity, soluble solid content (Parra-Coronado *et al*., 2006; Parra-Coronado and Hernández-Hernández, 2008), and the ratio between soluble solids and titratable acidity (Parra-Coronado *et al*., 2015).

Quality parameters at the time of harvest depend upon prevailing weather conditions during growth and the levels of luminosity and temperature (Calvo, 2004). In pineapple guava fruits, these factors directly affect the soluble solid concentration and total titratable acidity but do not affect firmness and color (Parra-Coronado *et al*., 2015) or physiological and chemical changes that occur during post-harvest ripening (Mishra and Gamage, 2007). Notably, with increasing temperatures in the crop cycle and especially during maturation, the sugar content of the fruit decreases (Parra-Coronado and Miranda, 2016). Research performed on pineapple guava includes specific studies on post-harvest physiology and studies on fruit nutraceutical characteristics (Rodríguez *et al*., 2006; Velho *et al*., 2011; Amarante *et al*., 2013).

The aim of this study was to evaluate how weather conditions during the growth of pineapple guava fruits affect the quality characteristics of the produce stored under two different temperature conditions. The pineapple guava in this study was grown in two locations in the department of Cundinamarca, Colombia, with different altitudes and weather conditions.

Materials and methods

Pineapple guava fruits were collected at physiological maturity (considering the size, firmness, peduncle abscission, and color intensity described by Parra-Coronado and Fischer, 2013 and Schotsmans *et al*., 2011) from two farms located in the Andean region of the department of Cundinamarca, Colombia, where trees originating from clone 41 ('Quimba') were planted in 2006. The different crop management activities (e.g., pruning and fertilization) were performed equally on both farms following the recommendations of Quintero (2012) to eliminate the influence of cultivation variables. The soil characterization showed that the soils of the two farms had a sandy loam texture. The Ca/Mg, Mg/K, Ca/K and (Ca + Mg)/K ratios indicated that there were no K and Mg deficiencies and that Cu and Mn showed values below those considered optimal. The first farm was located in the municipality of Tenjo (4°51'23" N and 74°6'33" W) at an altitude of 2,580 m a.s.l., with a mean temperature of 12.5°C and relative humidity 74-86%. The farm had a bimodal rainfall pattern, with an average annual precipitation of 765 mm, concentrated within the periods

TABLE 1. Weather conditions in the study zones from anthesis to pineapple guava fruit harvest (Parra-Coronado *et al*., 2015).

Zone	Harvest	Days[a]	GDD[b] (ºC)	T[c] (ºC)	RH[d] (%)	P[e] (mm)	Rad[f] [W m^{-2}]
Tenjo	1	180	1,979	12.3	76.4	190	12,303
(2,580 m a.s.l.)	2	180	1,966	12.3	84.3	417	9,861
San Francisco	1	155	2,728	18.5	86.1	573	7,814
(1,800 m a.s.l.)	2	155	2,627	18.0	95.1	1,400	10,021

[a]Days: calendar days from anthesis to harvesting; [b]GDD: thermal time (growing degree-days accumulated from anthesis to harvesting); [c]T:average temperature during the study period; [d]RH: average relative humidity during the study period; [e]P: accumulated precipitation from anthesis to harvesting; [f]Rad: accumulated radiation from anthesis to harvesting.

March-May and September-November. The second farm was located in the municipality of San Francisco (4°57'57" N and 74°16'27" W) at 1,800 m a.s.l., with average temperature of 20.6°C and relative humidity between 63 and 97%. This farm had a bimodal rainfall pattern with an annual mean precipitation of 1,493 mm, concentrated within the periods February-May and September-November. For the choice of the two altitudinal sites, the authors found these sites were near the lowest and the highest elevation recommended for commercial pineapple guava cultivation in Colombia.

The climatic conditions of the locations were recorded from anthesis to harvest of the pineapple guava fruits (Tab. 1), between 2012 and 2014. The meteorological data were obtained from weather stations placed in each sampling site, which recorded hourly data on temperature, precipitation, relative air humidity, and total solar radiation.

Experimental design

Because pineapple guava is a perennial crop, 10 trees per element plot (Fernández *et al*., 2010) and two plots per farm were studied, for a total of 40 trees, planted at 4 × 4 m. The aim was to record fruit growth and development from anthesis to harvest along with the weather conditions. A total of 300 fruits free of defects and mechanical damage were collected during two harvests per plot and per farm. The fruits were transported to the laboratory for disinfection with a solution of 1 mL L^{-1} sodium hypochlorite. The fruits were differentiated per plot and site of origin for each harvest and stored at 18 ± 1°C (76 ± 5% RH, 90 fruits by 11 d) and 5 ± 1°C (87 ± 5% RH, 210 fruits by 31 d), taking into account that this fruit species can stand temperatures as low as 1.7°C (Valderrama *et al*., 2005).

Measured variables

The measured quality attributes included weight loss (WL), skin and pulp firmness, total soluble solids (TSS), total titratable acidity (TTA) and epidermal color (hue angle; °h). To determine WL, the weight variation during the storage of five samples from two fruits was determined based on a gravimetric method using an analytical precision balance, Precisa XT220A, with a capacity of 220 g and 0.0001 g precision (Precisa Instruments, Zurich, Switzerland). The skin and pulp firmness were quantified with a Brookfield CT3-4500 texturometer (Brookfield Engineering, Middleboro, MA, USA). Two readings per fruit were performed with a TA39 probe at a precision of ± 0.5%. The NTC 4624 Technical Standard (Icontec, 1999a) was used for the measurements of TSS with an Eclipse refractometer (Bellingham Stanley, Tunbridge Well, UK) using a scale from 0 to 32 and a precision of 0.2 °Brix. The TTA was determined following the NTC 4623 Technical Standard (Icontec, 1999b). The maturity ratio (MR), defined as the ratio between TSS and TTA (TSS/TTA), was determined. The epidermal color was measured using a Minolta CR-400 chroma meter (Konica Minolta, Ramsey, NJ, USA). The quality attributes of the fruits were evaluated after 1, 3, 5, 7, 9 and 11 d of fruit storage at 5 and 18°C. The quality attributes were also evaluated after 15, 19, 23 and 31 d of fruit storage at 5°C. The statistical design was entirely random, with five replicates per trial.

In order to analyze the behavior of each quality parameter and its variation over time, the statistical program IBM-SPSS v.20 (SPSS Inc., Chicago, IL, USA) was used to perform a correlation analysis between the fruit quality parameters. The data were analyzed with descriptive statistics, and the standard deviation (SD) was used as a measure of dispersion. An analysis of variance and comparison of means test (Tukey's tests) were performed for the fruit-quality characteristics during storage for each study location and harvest.

Results

Skin and pulp firmness

The pineapple guava fruit skin and pulp firmness had the same trends in behavior over time for both storage conditions and both locations, with initially high values that decreased during ripening (Tab. 2). The skin firmness was always higher than the pulp firmness for the same storage period.

At the beginning of storage, the skin firmness of the pineapple guava fruits showed mean values of 15.2 ± 1.6 N for San Francisco and 12.5 ± 3.1 N for Tenjo, with values decreasing over time. For the fruits stored at 5°C, the skin firmness at the end of the storage period reached average values of 13.8 ± 2.7 N for fruits coming from San Francisco and 7.8 ± 2.0 N for those coming from Tenjo, whereas the "San Francisco fruits" stored at 8°C showed average values of 9.3 ± 1.8 N and the "Tenjo fruits" showed mean values of 4.2 ± 0.9 N at the end of the storage period.

The pulp firmness had average values of 5.8 ± 2.0 N in fruits from San Francisco and 6.5 ± 3.1 N in fruits from Tenjo at the beginning of storage, at both temperatures. At the end of the storage period, the pulp firmness of the fruits stored at 5°C reached values of 1.7 ± 1.0 N in the fruits from San Francisco and 1.2 ± 0.5 N in the fruits from Tenjo, whereas the "San Francisco fruits" stored at 18°C had mean values of 0.8 ± 0.3 N and the "Tenjo fruits" showed average values of 0.5 ± 0.2 N.

The analysis of means (Tab. 2) indicated that, during storage, the differences were related to storage conditions and place of origin. However, differences were not observed in fruits from the same locations. The firmness behavior of the pineapple guava fruits during post-harvest depended on the storage temperature and fruit firmness at the time of harvest, which is influenced by the climatic conditions of the cultivation site. The weather conditions at the origin site influenced durability in postharvest and invariably affected the internal and external quality of the fruits and their storage capacity (Moretti *et al*., 2010).

The loss of firmness in fruit skin and pulp (Tab. 2) during storage was higher for fruits produced at the elevated altitude (Tenjo), with a lower average temperature and higher

TABLE 2. Analysis of means for skin and pulp firmness (N) of pineapple guava fruits during post-harvest under two storage conditions.

	Temperature: 5ºC; RH: 87%				Temperature: 18ºC; RH: 76%			
Day	**T-1**	**T-2**	**S.F.-1**	**S.F.-2**	**T-1**	**T-2**	**S.F.-1**	**S.F.-2**
				Skin firmness				
1	14.82 a	10.21 b	16.19 a	14.18 a	14.82 a	10.21 b	16.19 a	14.18 a
3	18.67 a	11.68 a	18.59 a	16.84 a	18.58 a	13.49 a	20.86 a	20.38 a
5	16.35 abc	11.12 bc	22.58 a	17.49 ab	17.32 ab	9.25 c	21.21 a	21.91 a
7	16.99 ab	10.79 cd	22.77 a	18.80 ab	15.59 bc	6.62 d	19.52 ab	18.39 ab
9	16.64 ab	10.99 bc	17.78 a	13.67 ab	10.88 bc	4.87 c	15.76 ab	14.75 ab
11	14.44 a	10.77 b	17.34 a	14.64 a	4.44 c	3.86 c	8.91 b	9.65 b
15	13.22 ab	10.07 c	18.59 a	16.50 ab				
19	12.45 b	8.32 c	16.86 a	14.87 ab				
23	10.42 b	7.87 b	16.09 a	16.54 a				
27	10.63 b	6.60 c	15.27 a	15.56 a				
31	9.03 bc	6.63 c	13.55 ab	14.03 a				
				Pulp firmness				
1	6.90 a	6.14 a	5.47 a	6.12 a	6.90 a	6.14 a	5.47 a	6.12 a
3	8.98 a	5.82 a	7.72 a	4.62 a	4.42 a	5.69 a	7.26 a	5.69 a
5	6.87 ab	6.49 ab	11.01 a	5.79 ab	4.15 a	2.27 a	5.88 ab	6.25 ab
7	5.56 abc	5.18 bc	10.96 a	6.75 ab	2.93 bc	1.03 c	2.81 bc	2.97 bc
9	5.29 ab	3.98 abc	5.88 a	4.05 abc	1.40 bc	0.60 c	1.87 abc	1.61 abc
11	4.43 b	3.36 bc	8.22 a	5.91 ab	0.40 c	0.60 c	0.91 dc	0.64 c
15	3.40 b	3.08 b	8.65 a	4.93 b				
19	3.18 bc	1.77 c	5.78 a	4.92 ab				
23	2.89 a	1.98 a	3.35 a	4.42 a				
27	1.75 a	1.18 a	2.95 a	2.89 a				
31	1.27 a	1.04 a	1.68 a	1.78 a				

T-1: Tenjo location, harvest 1; T-2: Tenjo location, harvest 2; S.F.-1: San Francisco location, harvest 1; S.F.-2: San Francisco location, harvest 2. Different lowercase letters in the rows show statistical differences according to the Tukey's test ($P \leq 0.05$).

Means followed by different letters for the same day indicate significant differences according to the Tukey's test ($P \leq 0.05$).

solar radiation; thus, a higher number of calendar d and lower thermal time (GDD) were required to proceed from anthesis to harvest (Tab. 1).

The average loss of skin firmness of the pineapple guava fruits from Tenjo and San Francisco was 37.1 and 8.2% for the fruits stored at 5°C, and 66.0 and 38.5% for the fruits stored at 18°C, respectively.

Contents of total soluble solids, total titratable acidity and maturity ratio

For the pineapple guava fruits stored at 18°C, the TSS (°Brix) and maturity ratio (MR) increased and TTA (% of citric acid) decreased with ripening, whereas the fruits stored at 5°C showed little variation of those parameters throughout the storage time (Tab. 3). The fruits stored at a higher temperature showed more changes because of the exponential increase in the speed of enzymatic reactions with increasing temperatures (Wills *et al*., 2007).

The TTS content showed average values of 10.8 ± 0.9 °Brix for San Francisco and 12.6 ± 1.1 °Brix for Tenjo at the beginning of storage for the two temperatures. Although the TSS in the fruits stored at 5°C showed an increasing trend, the variation was not significant, especially for the fruits from San Francisco, which was shown by comparing the values recorded at the beginning and end of the study (Tab. 3).

At the end of the storage period, the fruits reached mean values of 10.9 ± 0.7 °Brix for San Francisco and 14.2 ± 0.9 °Brix for Tenjo. The TSS of the fruits stored at 18°C increased as the fruits ripened, especially in the fruits from Tenjo. At the end of the storage period, mean TSS values of 12.0 ± 0.9 °Brix for San Francisco and 16.5 ± 0.7 °Brix for Tenjo fruits were observed.

TABLE 3. Analysis of means for total soluble solids (TSS) and total titratable acidity (TTA) in pineapple guava fruits under two storage conditions.

	Temperature: 5ºC; RH: 87%				Temperature: 18ºC; RH: 76%			
Day	**T - 1**	**T - 2**	**S.F. - 1**	**S.F. - 2**	**T - 1**	**T - 2**	**S.F. - 1**	**S.F. - 2**
				Total soluble solids (TSS, °Brix)				
1	13.35 a	11.73 ab	11.19 ab	10.25 b	13.35 a	11.73 ab	11.19 ab	10.25 b
3	12.67 abc	12.67 abc	11.32 bc	10.92 bc	14.42 a	13.21 ab	10.52 bc	10.25 c
5	13.48 ab	11.86 b	9.57 c	9.98 c	13.35 ab	14.02 a	10.11 c	10.11 c
7	14.56 a	12.13 b	9.30 c	9.30 c	14.02 a	14.15 a	11.59 b	11.05 bc
9	14.29 ab	12.54 bc	11.19 c	10.78 c	16.58 a	14.42 ab	11.46 c	11.32 c
11	15.77 b	13.48 cd	9.84 f	10.11 f	17.79 a	15.23 bc	12.67 cd	11.32 ef
15	14.83 a	12.67 b	10.92 c	11.59 bc				
19	16.04 a	13.88 b	9.71 c	10.38 c				
23	14.96 a	15.50 a	11.05 b	10.51 b				
27	14.96 a	13.88 a	11.32 b	11.19 b				
31	14.56 a	13.75 a	11.32 b	10.51 b				
				Total titratable acidity (TTA, % citric acid)				
1	1.91 a	1.68 a	1.58 a	1.80 a	1.91 a	1.68 a	1.58 a	1.91 a
3	1.77 ab	1.86 ab	1.61 b	1.81 ab	1.78 ab	2.20 a	1.65 ab	1.78 ab
5	1.86 a	2.12 a	1.66 a	1.86 a	1.82 a	1.86 a	1.88 a	1.85 a
7	1.95 a	1.81 a	1.50 a	1.90 a	1.58 a	1.49 a	1.77 a	1.80 a
9	1.87 ab	2.04 a	1.84 ab	1.97 b	1.30 b	1.49 ab	1.45 ab	1.50 ab
11	1.77 a	2.10 a	1.89 a	1.99 a	0.73 c	0.83 bc	1.20 b	1.25 b
15	1.93 a	2.10 a	1.88 a	2.16 a				
19	1.77 a	1.92 a	1.83 a	1.95 a				
23	1.45 b	2.28 a	1.92 ab	2.10 a				
27	1.50 b	1.74 ab	1.96 a	1.91 ab				
31	1.48 ab	1.57 ab	1.61 a	1.23 b				

T-1: Tenjo location, harvest 1; T-2: Tenjo location, harvest 2; S.F.-1: San Francisco location, harvest 1; S.F.-2: San Francisco location, harvest 2. Different lowercase letters in the rows show statistical differences according to the Tukey's test ($P \leq 0.05$).

Means followed by different letters for the same day indicate significant differences according to the Tukey's test ($P \leq 0.05$).

The TTA registered average values of 1.69 ± 0.18% for San Francisco and 1.80 ± 0.20% for Tenjo at the beginning of storage at both temperatures. Although the TTA in the fruits stored at 5°C showed low variation, the TTA tended to decrease during the last day of storage (Tab. 3) reaching final mean values of 1.42 ± 0.15% for San Francisco and 1.53 ± 0.19% for Tenjo. The TTA of the fruits stored at 18°C decreased as they ripened (Tab. 3), especially in fruits from Tenjo, with average values of 1.23 ± 0.25% for San Francisco and 0.78 ± 0.27% for Tenjo at the end of the storage period.

The MR increased for the two storage conditions (Fig. 1) and was elevated at the highest temperature. The MR showed average values of 6.6 ± 1.1 for San Francisco and 7.1 ± 1.2 for Tenjo at the beginning of storage for both temperatures. Although the MR at 5°C showed little variation, it generally tended to increase during the last 8 d of storage, reaching average values of 7.9 ± 0.9 for fruits from San Francisco and 9.6 ± 2.2 for fruits from Tenjo. The MR of the fruits stored at 18°C increased as the fruits ripened, presenting average values of 10.3 ± 2.6 for San Francisco and 23.9 ± 8.1 for Tenjo at the end of storage, with high dispersion for the latter location.

The analysis of means (Tab. 3) indicated that storage conditions and place of origin differed for TSS and TTA. The behavior of TSS, TTA, and MR in the pineapple guava fruits during post-harvest depended on the storage temperature and TSS and TTA values at the time of harvest, which were influenced by weather conditions during fruit growth. During storage, the fruits from the highest location (Tenjo) with an elevated accumulated solar radiation and lower mean temperature and relative air humidity (Tab. 1) had higher levels of TSS and MR, but lower TTA. These results indicate that pineapple guava fruits produced in cold climates have a better flavor than those from warm climates.

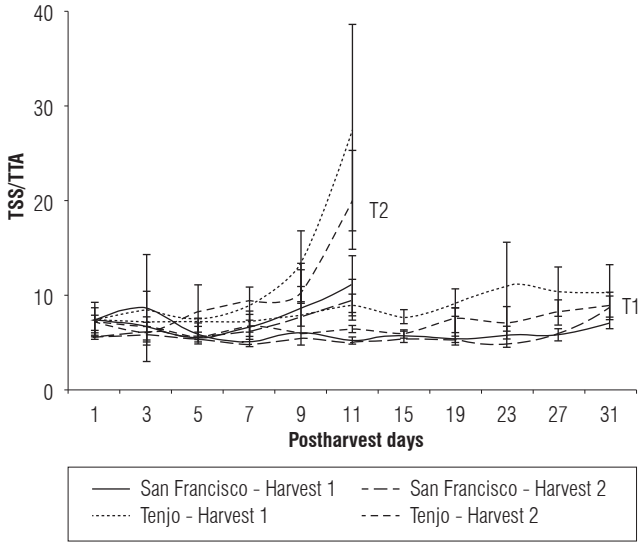


FIGURE 1. Variation of the maturity ratio (TSS/TTA) of pineapple guava fruits stored at T_1 (5ºC) and T_2 (18ºC).

The values listed in Table 3 indicate that the TTA in the pineapple guava fruits did not show differences at the time of harvest, which could help determine the influence of climatic conditions on this parameter, but differences were observed at the end of the storage period, especially for fruits stored at the highest temperature (18°C). The TTA was higher in the fruits produced at the lowest altitude (San Francisco), with less accumulated solar radiation and higher average temperatures and relative air humidity (Tab. 1).

Weight loss and color change

The weight loss (WL) in the pineapple guava fruits increased for both storage conditions (Fig. 2A). For fruits stored at 5°C, the WL at the end of the storage period showed a mean value of 8.48 ± 1.91% for San Francisco and 5.94 ± 0.75% for Tenjo, whereas those stored at 18°C presented a mean value of 20.37 ± 1.60% for fruits from San Francisco and 13.01 ± 1.98% for fruits from Tenjo. For the storage conditions (equal altitude), it was observed that, at a lower temperature, the relative humidity was lower (sensitive cooling of the air) and, therefore, the vapor pressure deficit was lower, which is the cause of the moisture loss of the product (weight loss). Transpiration and substrate consumption through respiration are the primary causes of WL of fruits during post-harvest ripening (Saladié *et al.*, 2007).

The analysis of means indicated that differences in WL occurred between storage conditions and cultivation location. The fruits stored at 5°C did not show significant differences for the site of origin or harvest when compared with fruits stored at 18°C. However, for the two storage conditions, the WL was lower in the fruits from the highest location (Tenjo), where the accumulated radiation is greater and mean temperature and relative humidity are lower (Tab. 1).

Color change occurred because of chlorophyll degradation and pigments synthesized such as anthocyanins and carotenoids (Mishra and Gamage, 2007). The color measured as the °h represented the color or tonality that varies from 0° in pure red to 180° in pure green (Hernández *et al.*, 2007). The value of the °h of the pineapple guava fruits did not show a clear trend during the first d of storage, but decreased over time for the two storage conditions (Fig. 2B). The analysis of means indicated that statistically significant differences did not occur in the °h for the pineapple guava fruits during the last d of storage for the different sites and harvests.

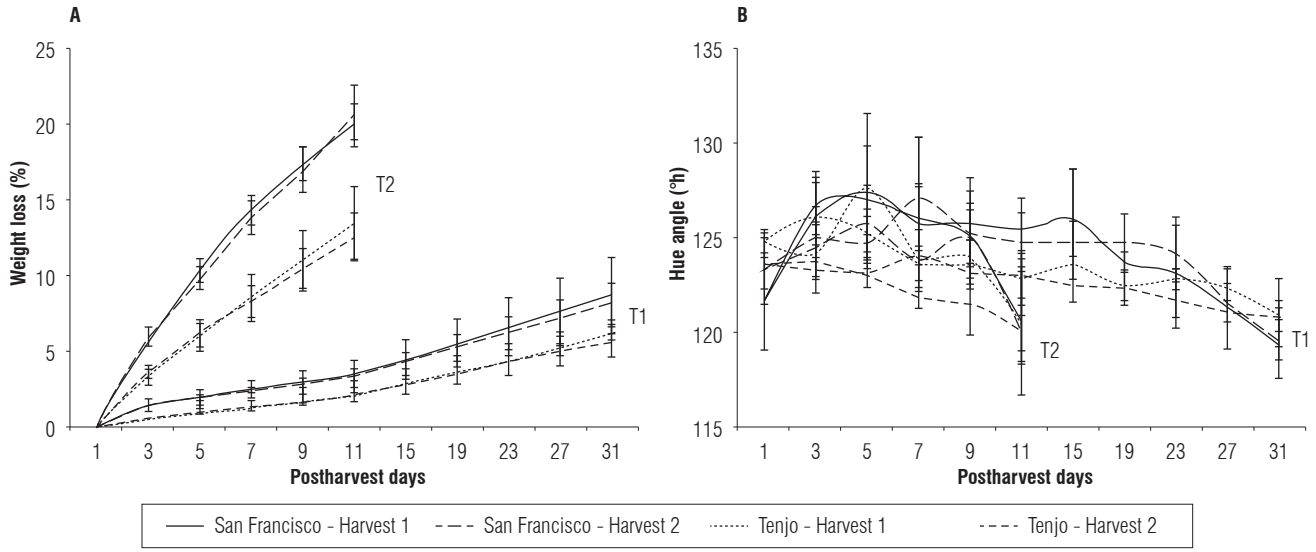


FIGURE 2. Variation in weight loss (A) and hue angle (B) of pineapple guava fruits stored at T_1 (5ºC) and T_2 (18ºC).

Discussion

Skin and pulp firmness

Different authors (Osterloh *et al*., 1996; Parra-Coronado *et al*., 2006) have indicated that decreased firmness values during fruit ripening are determined by propectin, the agglutinating substance within cells that gives turgidity to fruits and degrades along with pectin substances. This degradation process changes the texture and consistency of fruits, producing the characteristic softening during the ripening process. The decrease in firmness over the storage period has been reported by various authors for other fruits of the Mirtaceae family, such as guava (Solarte *et al*., 2010), champa (*Campomanesia lineatifolia*) (Álvarez *et al*., 2009), and araza (*Eugenia stipitata*) (Hernández *et al*., 2007). Firmness is an important fruit characteristic of consumption quality. When designing packaging and transport during the harvest and post-harvest periods (Parra and Fischer, 2013), the criteria set by Márquez *et al*. (2007) should be considered; these authors indicated that the load that a fruit can support is equivalent to 70% of its durability.

During pineapple guava fruit ripening, many enzymes are expressed, which modify the plasticity of cell walls, and one the main enzymes involved in this process is polygalacturonase (PG) (Öpik and Rolfe, 2005). The activity of PG in pineapple guava is higher inside the mesocarp, which suggests that softening progresses from the inside to the outside of the fruit (Parra and Fischer, 2013). This process is evidenced by the lower firmness value of the pulp as compared to that of the skin. For the storage conditions considered in this study, firmness decreased more rapidly for the pineapple guava fruits stored at the higher temperature (18°C). At day 11 of storage, the fruits with higher values of skin and pulp firmness were those stored at the lowest temperature (5°C) (Tab. 2). Similar results were reported by different authors for pineapple guava from clones 41 ('Quimba') and 8-4 (Rodríguez *et al*., 2006; Parra and Fischer, 2013) as well as for guava (Solarte *et al*., 2010) and pear (Parra-Coronado *et al*., 2006).

The post-harvest physiological behavior of fruits and vegetables depends on their storage conditions, with temperature being the main factor (Parra-Coronado and Hernández-Hernández, 2008). The decreasing intensity of respiration is the basis for extending the life of agricultural products through low temperatures, which reduces the speed of enzymatic activity (Wills *et al*., 2007). The speed of enzymatic reactions increases exponentially with increases of temperature, and, for every 10°C temperature increase, physiological and chemical changes increase 2 to 3 times (Q_{10}) (Mishra and Gamage, 2007). As the respiration rates increase, ethylene concentration is also increased, promoting the activation of enzymes that intervene in cell wall degradation, which causes loss of firmness and consequent softening of the fruit (Saladié *et al*., 2007).

Although studies have not been performed to determine the influence of weather conditions on the post-harvest behavior of pineapple guava fruits, the results found in this study are consistent with observations by Minas *et al*. (2018), who found that peach fruits produced at higher crop temperatures were firmer, whereas their water content was

lower. Limited studies have been performed to determine the influence of precipitation and relative humidity on fruit firmness. In this study on pineapple guava fruits, harvests with greater accumulated rain (Tenjo-2, with 417 mm; and San Francisco-2, with 1,400 mm) and average relative humidity produced fruits with lower skin firmness for the same location although the pulp firmness did not show differences (Tab. 2). This result is consistent with that of Gariglio *et al*. (2007), who reported that a high RH could severely affect fruit quality, as observed in mandarin, which quickly lost fruit consistency.

Contents of total soluble solids, total titratable acidity, and maturity ratio

The pineapple guava fruits stored at higher temperatures showed a higher and accelerated increase in TSS because the speed of enzymatic reactions increases exponentially with rises in temperature (Wills *et al*., 2007). Although there is no consensus on variations of TSS in pineapple guava fruit during post-harvest, these results are within the range reported by certain authors, who indicated that TSS values increase up to the climacteric process and subsequently decrease (Rodríguez *et al*., 2006; Parra and Fischer, 2013). Other authors have indicated that TSS decreases during post-harvest or remains constant when fruits are stored at a low temperature (Velho *et al*., 2011), with values between 5 and 15 °Brix. According to Osterloh *et al*. (1996), these dissimilar behaviors were most likely influenced by varietal characteristics, plant age, and climatic and growing conditions to which the fruits were exposed. According to Rodríguez *et al.* (2006), the pineapple guava had high levels of starch at the time of harvest, which hydrolyzed during post-harvest ripening and caused the TSS to increase.

The TTA results in this study are similar to those observed by different authors. Rodríguez *et al.* (2006) observed that, in pineapple guava fruits, the TTA increases until the climacteric process and subsequently decreases, whereas other authors (Velho *et al*., 2011) have reported that the TTA decreases during storage (4 to 23°C). Rodríguez *et al*. (2006) indicated that, in pineapple guava fruits (clones 8-4 and Quimba), acidity decreases during the ripening process because organic acids degrade during respiration. According to Wills *et al*. (2007), organic acids generally degrade during the ripening stage because they are utilized in respiration or transformed into sugars; such changes are shown in Table 3 for fruits produced in Tenjo, which presented increased TSS concentrations during storage at 18°C.

The maturity index increased for the two storage conditions (Fig. 1) and was increased at higher temperature, which is consistent with reports for the majority of fruits (Parra-Coronado *et al*., 2006; Álvarez *et al*., 2009). The MR was low and relatively constant when the product was stored at low temperatures and showed little dispersion because the starch hydrolysis was higher and more complete at low temperatures (Musacchi and Serra, 2018). The highest values for the MR occurred with the highest storage time, lowest relative air humidity and highest temperatures.

The behavior of TSS, TTA, and MR in pineapple guava fruits during post-harvest depends on the TSS and TTA values of fruits at the time of harvest, which are influenced by weather conditions at the origin site during fruit growth. The TSS content during post-harvest was higher in the fruits produced in Tenjo. These results are consistent with Kano (2015), who indicated that the TSS content in watermelon fruits would be lower at higher temperatures. Arah *et al*. (2015) also observed that, in tomatoes, the TSS content was lower at higher temperatures and relative humidity, and also at lower light intensity. Possibly, growing temperatures that are too high result in a loss of photoassimilates as a result of elevated respiration rates of carbohydrates (Wills *et al*., 2007; Taiz *et al*., 2014). However, when the higher temperature is still inside the optimum range of a fruit species, the warmer site can also promote the sugar translocation to the fruits, which was observed in Colombia in cape gooseberry (Fischer *et al*., 2007) and banana passion fruits (Mayorga, 2016) at the lower and warmer site. For accumulated solar radiation, Martínez-Vega *et al*. (2008) found similar results for pineapple guava belonging to clone 41 and indicated that the fruits with the lowest TSS values were those located within the inner-middle area of the canopy, which has a low incidence of luminous radiation. The authors supposed that the higher accumulated solar radiation at the high elevation site (Tenjo) favored photosynthetic performance and thus the TSS content (Taiz *et al*., 2014; Fischer *et al*., 2016). On the other hand, the cooler nights in Tenjo (located at a higher elevation than San Francisco) decreased the maintenance respiration of the fruits and their energy costs, increasing the positive carbon balance (Gariglio *et al*., 2007) and so, contributing to the higher TSS. Also, the lower TSS content in the fruits from San Francisco could have been influenced by the higher precipitation rates at this location (Tab. 1), especially because Osterloh *et al*. (1996) observed that high precipitation diminishes TSS production, possibly because of cloudy weather conditions and temperature decreases.

The results found in this study are consistent with those of Martínez-Vega *et al.* (2008), who observed that TTA increased slightly in pineapple guava fruits from less illuminated parts of the canopy.

Weight loss and color change

The weight loss (WL) in the pineapple guava fruits increased during both storage conditions (Fig. 2A), which is consistent with what Rodríguez *et al.* (2006) found for pineapple guava in clones 8-4 and Quimba and for champa fruits (Álvarez *et al.*, 2009). The WL was higher for the pineapple guava fruits stored at higher temperatures, a behavior also reported by Parra-Coronado *et al.* (2006) for pears. Rodríguez *et al.* (2006) found intermediate values compared with the results of this study for pineapple guava fruits of the 'Quimba' clone from the municipality of Vega (1,900 m a.s.l.) stored at ambient temperature (16.3°C, 65.1% RH); after 18 d in storage, the WL was 17.3%. Valderrama *et al.* (2005) indicated that pineapple guava fruits could be stored over long periods at low temperatures (1.7°C) with low WL and TSS.

The °h value of the green pineapple guava fruits did not show a clear trend during the first d of storage, but decreased over time for the two storage conditions (Fig. 2B). This undefined trend of °h of the pineapple guava fruit is consistent with reports by East *et al.* (2009), who indicated that significant variations might not be observed in skin color among certain cultivars as the fruit ripens. In other pineapple guava cultivars, the °h decreased, representing a loss in green color (Velho *et al.*, 2011). The results found in this study show that the pineapple guava fruit does not change color because of the genetics of the fruits and only varies in shades of green. This result should not be used to establish the influence of weather conditions during the growth stage of fruits over this parameter at post-harvest.

To date, studies reporting differences between pineapple guavas indicating the influence of weather conditions on the behavior of fruit quality parameters during the post-harvest stage have not been carried out. To our knowledge, this is the first study carried out on the subject. Further research covering a wide range of pineapple guava varieties and environments is recommended. Also, the application of research facilities such as FACE (Free-Air Carbon dioxide Enrichment), installed through weather measuring equipment in a circle around the studied trees (Jones *et al.*, 2014) could provide much more information on the direct influence of weather on the quality of fruits inside the FACE circle.

Conclusions

Storage temperature is a factor that affects the durability of pineapple guava fruits and shows a direct relationship with TSS, MR, and WL. Storage temperature has an inverse relationship with the TTA, firmness and shelf life of fruits. Therefore, stored fruits are sweeter and show a higher loss of weight and firmness and reduction in post-harvest durability.

The results obtained in this study clarify that the storage conditions and climatic conditions of origin (altitude) had a great influence on the behavior of the TSS, TTA, MR, firmness, and WL in the pineapple guava fruits during the post-harvest period. This behavior depends on the values of these parameters at the time of harvest and weather conditions during fruit growth. The conditions at the higher altitude (lower temperature but higher solar radiation) corresponded to a greater TSS content and firmness loss but lower TTA and WL. The color change (°h) of these nearly entirely green fruits did not show significant differences that might reveal the influence of weather conditions on the variation of this parameter during post-harvest.

Acknowledgments

We are grateful to the Agricultural Science Faculty of the Universidad Nacional de Colombia in Bogota for financial support, to Dr. Celsa Garcia for supplying two meteorological stations, and to biologist Omar Camilo Quintero for allowing access to his pineapple guava crops and for supplying the necessary resources for this study.

Literature cited

Álvarez, J.G., H.E. Balaguera, and J.F. Cárdenas. 2009. Caracterización fisiológica del fruto de champa (*Campomanesia lineatifolia* Ruiz and Pavón) durante la poscosecha. Rev. UDCA Act. Divul. Cient. 12(2), 125-133.

Amarante, C.V.T., C.A. Steffens, T.D.T. Benincá, C. Hackbarth, and K.L. dos Santos. 2013. Qualidade e potencial de conservação pós-colheita dos frutos em cultivares brasileiras de goiabeira-serrana. Rev. Bras. Frutic. 35(4), 990-999. Doi: 10.1590/S0100-29452013000400009

Arah, I.K., H. Amaglo, E.K. Kumah, and H. Ofori. 2015. Preharvest and postharvest factors affecting the quality and shelf life of harvested tomatoes: a mini review. Int. J. Agron. vol. 2015, Art. ID 478041, 6 p. Doi: 10.1155/2015/478041

Calvo, G. 2004. Efecto del 1-metilciclopropeno (1-MCP) en pera variedad Williams cosechadas con dos estados de madurez. Rev. Invest. Agropecu. 33(2), 3-26.

East, A.R., X.I.T. Araya, M.L.A.T.M. Hertog, S.E. Nicholson, and A.J. Mawson. 2009. The effect of controlled atmospheres on respiration and rate of quality change in 'Unique' feijoa

fruit. Postharvest Biol. Technol. 53(1), 66-71. Doi: 10.1016/j.postharvbio.2009.02.002

Fernández, R., A. Trapero, and J. Domínguez. 2010. Experimentación en agricultura. Consejería de Agricultura y Pesca, Servicio de Publicaciones y Divulgación; Sevilla, Spain.

Fischer, G., G. Ebert, and P. Lüdders. 2007. Production, seeds and carbohydrate contents of cape gooseberry (*Physalis peruviana* L.) fruits grown at two contrasting Colombian altitudes. J. Appl. Bot. Food Qual. 81(1), 29-35.

Fischer, G., F. Ramírez, and F. Casierra-Posada. 2016. Ecophysiological aspects of fruit crops in the era of climate change. A review. Agron. Colomb. 34(2), 190-199.

Gaddam, U.S., A.J. Mawson, W.C. Schotsmans, and E.W. Hewett. 2005. Segregation of feijoa fruit using acoustic impulse response. Acta Hortic. 687, 365-367.

Gariglio, N.F., R.A. Pilatti, and M. Agustí. 2007. Requerimientos ecofisiológicos de los árboles frutales. pp. 41-82. In: Sozzi, G.O. (ed.). Árboles frutales – Ecofisiología, cultivo y aprovechamiento. Editorial Facultad de Agronomía, Universidad de Buenos Aires, Buenos Aires.

Hernández, M.S., O. Martínez, and J.P. Fernández-Trujillo. 2007. Behavior of Arazá (*Eugenia stipitata* Mc Vaugh) fruit quality traits during growth, development and ripening. Sci. Hortic. 111, 220-227. Doi: 10.1016/j.scienta.2006.10.029

Icontec, 1999a. Norma técnica colombiana NTC 4624. Jugos de frutas y hortalizas. Determinación del contenido de sólidos solubles. Instituto Colombiano de Normas Técnicas y Certificación, Bogota.

Icontec, 1999b. Norma técnica colombiana NTC 4623. Productos de frutas y verduras. Determinación de la acidez titulable. Instituto Colombiano de Normas Técnicas y Certificación, Bogota.

Kader, A.A. and E.M. Yahia. 2011. Postharvest biology of tropical and subtropical fruits. pp. 79-111. In: Yahia, E.M. (ed.). Postharvest biology and technology of tropical and subtropical fruits. Vol. 1. Fundamental issues. Woodhead Publishing, Cambridge, UK.

Kano, Y. 2015. Effects of summer day-time temperature on sugar content in several portions of watermelon fruit (*Citrullus lanatus*). J. Hortic. Sci. Biotech. 79(1), 142-145. Doi: 10.1080/14620316.2004.11511728

Márquez, C.C.J., C.M. Otero, and M. Cortés. 2007. Cambios fisiológicos, texturales, fisicoquímicos y microestructurales del tomate de árbol (*Cyphomandra betacea* S.) en poscosecha. Vitae 14(2), 9-16.

Martínez-Vega, R.R., G. Fischer, A. Herrera, B. Chaves, and O.C. Quintero. 2008. Características físico-químicas de frutos de feijoa influenciadas por la posición en el canopi. Rev. Colomb. Cienc. Hortic. 2(1), 21-32. Doi: 10.17584/rcch.2008v2i1.1170

Mayorga, M.J. 2016. Caracterización ecofisiológica de curuba (*Passiflora tripartita* var. *mollissima*) en dos condiciones ambientales. MSc thesis. Faculty of Agronomy, Universidad Nacional de Colombia, Bogota.

Minas, I.S., G. Tanou, and A. Molassiotis. 2018. Environmental and orchard bases of peach fruit quality. Sci. Hortic. 235, 307-322. Doi: 10.1016/j.scienta.2018.01.028

Mishra, V. K. and T.V. Gamage. 2007. Postharvest physiology of fruit and vegetables. pp. 19-48. In: Shafiur Rahman, M. (ed.). Handbook of food preservation. 2nd ed. Taylor & Francis Group, New York, USA.

Moretti, C.L., L.M. Mattos, A.G. Calbo, and S.A. Sargent. 2010. Climate changes and potential impacts on postharvest quality of fruit and vegetable crops: A review. Food Res. Int. 43, 1824-1832. Doi: 10.1016/j.foodres.2009.10.013

Musacchi, S. and S. Serra. 2018. Apple fruit quality: Overview on pre-harvest factors. Sci. Hortic. 234, 409-430. Doi: 10.1016/j.scienta.2017.12.057

Öpik, H. and S. Rolfe. 2005. The physiology of flowering plants. 4th ed. Cambridge University Press, Cambridge, UK.

Osterloh, A., G. Ebert, W.H. Held, H. Schulz, and E. Urban. 1996. Lagerung von Obst und Südfrüchten. Verlag Ulmer, Stuttgart, Germany.

Parra, A. and G. Fischer. 2013. Maduración y comportamiento poscosecha de la feijoa (*Acca sellowiana* (O. Berg) Burret). Una revisión. Rev. Colomb. Cienc. Hortic. 7(1), 98-11. Doi: 10.17584/rcch.2013v7i1.2039

Parra-Coronado, A., G. Fischer, and J.H. Camacho-Tamayo. 2015. Development and quality of pineapple guava fruit in two locations with different altitudes in Cundinamarca, Colombia. Bragantia 74(3), 359-366. Doi: 10.1590/1678-4499.0459

Parra-Coronado, A. and D. Miranda. 2016. La calidad poscosecha de los frutos en respuesta a los factores climáticos en el cultivo. Agron. Colomb. 34(1Supl.), S1415-S1418.

Parra-Coronado, A. and J.H. Hernández-Hernández. 2008. Fisiología postcosecha de frutas y hortalizas. 4th ed. Facultad de Ingeniería, Universidad Nacional de Colombia, Bogota.

Parra-Coronado, A., J.E. Hernández-Hernández, and J.H. Camacho-Tamayo. 2006. Comportamiento fisiológico de la pera variedad Triunfo de Viena (*Pyrus communis* L.) durante el período poscosecha. Rev. Bras. Frutic. 28(1), 46-50. Doi: 10.1590/S0100-29452006000100017

Quintero, O.C. 2012. Feijoa (*Acca sellowiana* Berg). pp. 443-473. In: Fischer, G. (ed.). Manual para el cultivo de frutales en el trópico. Produmedios, Bogota.

Rodríguez, M., H.E. Arjona, and J.A. Galvis. 2006. Maduración del fruto de feijoa (*Acca sellowiana* Berg) en los clones 41 (Quimba) y 8-4 a temperatura ambiente en condiciones de la Sabana de Bogotá. Agron. Colomb. 24(1), 68-76.

Saladié, M., A.J. Matas, T. Isaacson, M.A. Jenks, S.M. Goodwin, K.J. Niklas, R. Xiaolin, J.M. Labavitch, K.A. Shackel, A.R. Fernie, A. Lytovchenko, M.A. O'Neill, C.B. Watkins, and J.K.C. Rose. 2007. A reevaluation of the key factors that influence tomato fruit softening and integrity. Plant Physiol. 144, 1012-1028. Doi: 10.1104/pp.107.097477

Schotsmans, W.C., A. East, G. Thorp, and A. Woolf. 2011. Feijoa (*Acca sellowiana* [Berg] Burret). pp. 115-135. In: Yahia, E.M. (ed.). Postharvest biology and technology of tropical and subtropical fruits. Vol. 3. Cocona to mango. Postharvest biology and technology of tropical and subtropical fruits. Woodhead Publishing, Cambridge, UK. Doi: 10.1533/9780857092885.115

Schouten, R.E., R.H. Veltman, H.P.J. De Wild, T.J. Koopen, M.G. Staal, and L.M.M. Tijskens. 2004. Determination of O_2 y CO_2 permeance, internal respiration and fermentation for a batch

of pears (cv. Conference). Postharvest Biol. Technol. 32, 289-298. Doi: 10.1016/j.postharvbio.2003.12.006

Solarte, M.E., M.D. Hernández, A.L. Morales, J.P. Fernández, and L.M. Melgarejo. 2010. Caracterización fisiológica y bioquímica del fruto de guayaba durante la maduración. pp. 85-119. In: Morales, A.L. and L.M. Melgarejo (eds.). Desarrollo de productos funcionales promisorios a partir de la guayaba (*Psidium guajava* L.) para el fortalecimiento de la cadena productiva. Facultad de Ciencias, Universidad Nacional de Colombia, Bogota.

Taiz, L., E. Zeiger, I.M. Møller, and A. Murphy. 2014. Plant physiology and development, 6th ed. Sinauer Associates, Sunderland, MA, USA.

Valderrama, J.K., G. Fischer, and M.S. Serrano. 2005. Fisiología poscosecha en frutos de dos cultivares de feijoa (*Acca sellowiana* [O. Berg] Burret) sometidos a un tratamiento cuarentenario de frío. Agron. Colomb. 23(2), 276-282.

Velho, A.C., C.V.T. do Amarante, L.C. Argenta, and C.A. Steffens. 2011. Influência da temperatura de armazenamento na qualidade pós-colheita de goiabas serranas. Rev. Bras. Frutic. 33(1), 14-20. Doi: 10.1590/S0100-29452011005000016

Wills, R., B. McGlasson, D. Graham, and D. Joyce. 2007. Postharvest: An introduction to the physiology and handling of fruit, vegetables and ornamentals. 5th ed. CAB International, Wallingford, UK.

Yahia, E.M., J. Ornelas-Paz, and A. de J. Elansari. 2011. Postharvest technologies to maintain the quality of tropical and subtropical fruits. pp. 142-193. In: Yahia, E.M. (ed.). Postharvest biology and technology of tropical and subtropical fruits. Vol. 1. Fundamental issues. Woodhead Publishing, Cambridge, UK.

Yi, J., B.T. Kebede, T. Grauwet, A. Van Loey, X. Hu, and M. Hendrickx. 2016. A multivariate approach into physicochemical, biochemical and aromatic quality changes of purée based on Hayward kiwifruit during the final phase of ripening. Postharvest Biol. Technol. 117, 206-216. Doi: 10.1016/j.postharvbio.2016.03.007

How to improve smallholder market access: Evaluation of Mercados Campesinos in Colombia

Cómo mejorar el acceso al mercado de los pequeños productores: Evaluación de los Mercados Campesinos en Colombia

Federica Romagnoli[1], Juan Molina[2], and Álvaro Parrado[2]*

ABSTRACT

This paper presents an analysis of the results obtained in the project called "Mercados Campesinos" carried out in the central region of Colombia between 2004 and 2015. This analysis was performed to evaluate the impacts of this short food value chain experience and its influence on economic, social and political dimensions of smallholders' market access. The analysis included two complementary research methods: quantitative and qualitative approaches. These methods were stated to identify the benefits accrued to participants using three different methodologies: i) t-test analysis; ii) an impact evaluation known as difference-in-difference and iii) multi-level regressions. On the other hand, qualitative analysis was based on semi-structured interviews and informal dialogues to investigate the perceptions of a selected group of beneficiaries regarding how project goals have been achieved. The results showed that one of the most influential elements in smallholders' market access has been the role of peasant organizations, associations and local farmer committees. These administrative structures greatly affected the economic efficiency, political participation and, to a lesser extent, commercial improvements. Project outcomes have been extremely influenced by participants' expectations of improvements in wellbeing, life quality, production rates, and income. The project was not able to reach a good level of financial sustainability; however, it provided peasants with well-designed tools to self-coordinate their actions. Proof of that is that farmers started to organize themselves autonomously to exert pressure at municipal and local levels.

Key words: short food supply chain, rural territorial development, impact analysis, smallholders' organizations.

RESUMEN

El artículo presenta un análisis de los resultados del proyecto "Mercados Campesinos" desarrollado en la región central de Colombia entre 2004 y 2015, evaluando el impacto que esta experiencia de circuitos cortos de comercialización tuvo en las dimensiones política, económica y social. El análisis incluye dos métodos complementarios de investigación: enfoque cualitativo y cuantitativo. El primero pretende identificar los beneficios alcanzados a través de tres metodologías: i) análisis t-test; ii) valoración de impacto utilizando la técnica difference-in-difference y iii) regresión multivariada. El análisis cualitativo se basó en entrevistas semi-estructuradas y diálogos informales para investigar la percepción de un grupo de participantes sobre cómo los objetivos han sido alcanzados. Los resultados muestran que uno de los elementos más influyentes ha sido el rol de las fundaciones y asociaciones campesinas y de los comités campesinos locales. Ellos tuvieron fuerte influencia tanto en alcanzar eficiencia económica como en aumentar la participación política de los campesinos y, en menor manera, en alcanzar mejoramientos en la comercialización. La estructura del proceso ha sido enormemente afectada por las expectativas de los participantes en términos de bienestar, calidad de vida, producción e ingreso. El proyecto no alcanzó un buen nivel de sostenibilidad financiera; igualmente pudo proveer herramientas para coordinar autónomamente tanto la acción económica como la política, tanto que los campesinos empezaron a organizarse autónomamente para ejercer presión a nivel municipal.

Palabras clave: cadenas cortas de comercialización de alimentos, desarrollo rural territorial, análisis de impacto, organizaciones de campesinos.

Introduction

Considering its agro-ecological potential, Colombia is one of the strongest agricultural countries of Latin America (Velez *et al.*, 2010). The agricultural sector could potentially be one of the main driving forces of social and economic development of the country. However, the sector suffers great structural problems that affect agricultural productivity causing gradual marginalization and stagnation (Cano *et al.*, 2016). The main reasons for the sector's backwardness have been the high poverty levels, several adversities, functional

Received for publication: 27 September, 2017. Accepted for publication: 23 February, 2018 Doi: 10.15446/agron.colomb.v36n1.67970

[1] Master in Economics and Development, Faculty of Economy and Management, University of Florence, Italy.
[2] Research Group on Rural Management and Development, Faculty of Agricultural Sciences, Universidad Nacional de Colombia. Bogotá.
* Corresponding author: aparradob@unal.edu.co

CC BY NC ND

instability and social violence events that Colombian smallholders have experienced for several decades (Osorio and Riva, 2017). However, despite their shortage of means, smallholders play a key role in the Colombian agricultural sector constituting 70.9% of total farms (DANE, 2016). This is why peasants can still be considered the main agricultural product suppliers, even if their market access and products commercialization are extremely compromised and strongly dependent on intermediaries (Parrado and Molina, 2014). In recent years, a new solution is arising to improve farmers market access, promote smallholders agriculture and create conditions for a sustainable rural development (Prado, 2014). In that framework, there is a growing interest for short food value chains (ECLAC-FAO-IICA, 2014) as a valuable possibility to create a profitable situation for both smallholders and consumers. Farmers' markets are a great opportunity to foster small scale agriculture and family farming and, at the same time, satisfy consumer expectations of high quality, fresh and safe food, and chemical free products (Diaz, 2014). Short food value chains create conditions for an equitable and sustainable development, reducing the gap between rural and urban areas and increasing market access opportunities for family farming. Through them, smallholders are able to diversify commercial channels, increase their product value and obtain higher and more stable income by reducing intermediaries' role (CEPAL, 2014).

Along with short food supply chain, smallholders' organizations are one of the elements that contribute to boost the productivity and competitiveness of the agricultural sector. Indeed, several researches have shown how systematization of farmers' organization has stimulated the small farmers' integration in the local and regional food supply system (Romero *et al*., 2017). Smallholders' organizations have improved cooperation among farmers to face economic challenges but also stand social and political injustices (Machado, 2003). Short food value chains combined with the support and systematization of farmers' associations have led to important achievements in increasing smallholders' wellbeing, improving not only living conditions of rural families but also fostering rural communities (Escobar *et al.,* 2010).

In a context of social and political transformation of Colombian smallholders' reality, a short food supply chain experience developed in Bogota is outstanding. "Mercados Campesinos" project implemented efficient solutions to overcome bottlenecks and constraints that were affecting the smallholders of the Central region of Colombia and improved the food supply of the capital city. The project started in 2004 and ended in 2015; during this period it passed through various phases and achieved different objectives. In particular, it launched a new market channel in Bogota that allowed direct contact between producers and consumers. Thanks to the project, every two weeks farmer markets were organized in 16 squares of Bogota and smallholders coming from the rural areas surrounding the Capital could directly sell their products to customers (Gutiérrez *et al*., 2012). Additionally, smallholders could participate in several workshops and training workshops to better comprehend market dynamics and commercialization, improve product quality and introduce new production methods.

The project was able to combine productive transformation and institutional change using a theoretical framework based on the rural territorial development approach (Schejtman and Berdegué, 2003). With this focus, the "Mercados Campesinos" project introduced changes in the smallholders' access to Bogota food supply chain and consequently encouraged cooperation processes between local actors, among themselves and external actors with the purpose of modifying the food policy patterns of the capital (Parrado and Molina, 2014).

With its participatory approach, "Mercados Campesinos" aimed at positively influence the social, economical and political dimensions of beneficiaries providing means to achieve not only economic improvements but also social and political ameliorations (Gutierrez *et al*., 2012). To reach these goals, two main sub-objectives were set: i) to ensure fairer smallholders' participation in production, commercialization and product transformation and ii) to highlight the key role played by family famers in food supply and increase their political recognition, representativeness and influence in public policy definition (Ordoñez and Montoya, 2011). To achieve the latter dimension and ensure participants' engagement in all project phases, a complex organizational structure was set. All project participants were engaged in a local smallholder farmer group in which there was at least one local representative of project leading organizations (Parrado and Molina, 2014). Participation in farmers' organizations stressed the importance of collective work to obtain political recognition, facilitated organizations of farmers markets and strengthened local rural communities (Romero *et al.,* 2017).

"Mercados Campesinos" has been an extremely complex short food supply chain experience that introduced important changes to the wellbeing of smallholders of the Central region of Colombia and redefined food supply of the capital. The research objective was to identify the overall project impact on smallholders' living conditions.

To accomplish this objective, a series of quantitative and qualitative investigation have been undertaken. An in depth and comprehensive statistical analysis has been implemented to measure the achievements of the project objectives in the three different dimensions that "Mercados Campesinos" initiative wants to improve.

Following main pillars of rural territorial development, this study was designed to identify project effects on three important dimensions: i) improvement in commercialization, ii) increase of economic efficiency, and iii) increase the farmers' political and social involvement. Additionally, the quantitative analysis was carried out to identify exogenous and endogenous factors that have affected most project implementation. Considering the participatory nature of the project, further than the achievement of project goals, the analysis aimed at measuring the participant satisfaction of the actions implemented and their engagement in the different project steps. These data were collected using qualitative research methods such as semi-structured interviews and focus groups.

The combination of the two methodologies allowed a detailed examination of the elements in line with the evaluation goals (EU-AID, 2004). The implementation of both methods was designed to avoid and clarify eventual errors derived by the impact evaluation and to understand accurately the environment in which the analysis was undertaken.

Materials and methods

The research methodology chosen was designed to identify the overall objectives achievement and the beneficiaries' satisfaction and engagement in the measures implemented. To accomplish these goals, a combination of quantitative and qualitative research methods was implemented.

To undertake the first analysis, a dataset was created based on an evaluation questionnaire implemented on February 2014 during a monitoring phase of the project. The survey was conducted by the Research Group in Management and Rural Development (GIGDR) of the of the Agricultural Sciences Faculty, National University of Colombia, Bogota, along with OXFAM GB and the European Union support. It included 488 observations: 158 formed the treated group and 330 the control group. People interviewed were settled in Colombia Central region, specifically in the departments of Cundinamarca, Tolima and Boyaca and in the rural areas surrounding Bogota. The assessed regions are situated in the central geographic area of Colombia, but differ in climate, types of crops and infrastructures. The beneficiaries selected for the questionnaire were involved in the project for at least one year, while the control group was chosen using a propensity score matching method based on common socio-economic characteristics between the treated and the control group. The survey was constructed including two periods of time: 2007 as baseline year and 2014 as follow up year. It's important to highlight that some questions were not retrospective, in that case they refer to the follow-up year. Table 1 represents participants and control group main features.

TABLE 1. Descriptive statistics from treated and control groups.

	Treated group	Control group
Activity led by women	35%	0%
Average HH size	5	4
Average wealth level	3.5	2.8
Average education level	Primary	Primary
Dependency ratio	1.09	1.12
Average age	52	51
Average property areas (ha)	2	1.7
Percentage of small farmers <1 hc	64.77%	59.03%
Percentage of small farmers 1<hc<5	31.21%	35.83%
Percentage of small farmers 5<hc<10	3.36%	4.21%
Percentage of small farmers 10<hc<50	0.67%	0.62%
Percentage of small farmers hc>50	0%	0.31%
Tractor	20%	23%
Irrigation	0.63%	1.53%
Average savings 2013	1,347,003.3	944,166.67

A full impact evaluation was implemented on the previous dataset to study the effects of project interventions on final welfare outcomes (ADB, 2006). The different statistical analysis that constituted the impact evaluation provided robust evidence on performances and revealed to what extent the project has achieved its desired outcomes (Gertler *et al.*, 2011). The statistical analysis was based on three different methods, according to the rationale of the analysis and the types of data available. For each method and estimation, an ad hoc dataset was prepared to define the dependent and independent variables considered, especially to avoid data loss. The investigations implemented were: i) t-test; ii) difference-in-difference and iii) multinomial analysis.

The t-test analysis was performed to identify how the project influenced the price definition. The difference-in-difference investigation was implemented to isolate the treatment effect and evaluate eventual indicator improvements derived by project participation. This method allowed the identification of the outcome rates between

the treated and control group over time and describe the project's real impact (Gertler *et al.*, 2011). Considering the time line of "Mercados Campesinos", an analytical representation of the model was obtained:

$$Y = (2014\text{-}2007) - (2014\text{-}2007)$$

The analysis was implemented using a probit model considering the type of data used and the characteristics of the indicators. Probit model implementation was justified by the fixed effect identified in the Hausmann test.

$$Y = (\overline{Y}_t 2014\text{-}\overline{Y}_t 2007) - (\overline{Y}_{nt} 2014\text{-}\overline{Y}_{nt} 2007)$$

The last step of the statistical analysis was the implementation of a multinomial probit / logit regression. Multinomial logistic regression is used to predict categorical placement in or the probability of category membership on a dependent variable based on multiple independent variables. For this research, this model was implemented to identify the probability of being involved in one or more situations described by the project analysis.

All the data elaborations related to the quantitative analysis were implemented using STATA software (StataCorp LLC, USA).

In the second level of the analysis, a qualitative research was carried out. In particular, in depth interviews were performed, complemented by participatory observation. The Interviewees were five women living in Tuta and Duitama, two villages of Boyaca rural area that have been involved in "Mercados Campesinos" project since its beginning. Interviews were focused on understanding participant perceptions about the project and the objectives achieved, to have a better comprehension of their living conditions and the main problems affecting their businesses. Interviews were focused on four different topics: i) project participation determinants and main goals reached, ii) challenges characterizing agricultural business, iii) commercialization and production improvements, iv) project weaknesses. During the field trip, a meticulous observation of the environment permitted a profound comprehension of the external context and further in-depth observation of family dynamics and peer relations were useful to integrate the information already collected. A qualitative investigation was implemented during a one-week field camp in Tuta on November 2015 and during 4 visits to Mercados Campesinos in Bogota in September and October. The last step of the qualitative analysis was a focus group in which smallholders from Tuta analyzed their engagement in local and Bogota markets. Participatory observation was also implemented during the visits to "Mercados Campesinos" in Bogota, focusing mainly on understanding the market dynamics, observing participants' commercial abilities and relations between smallholders and costumers.

Results and discussion

The research was performed around three main topics that coincide with the project's main objectives: commercial abilities improvement, economic efficiency, and smallholders' political participation. Analyzing the development of the "Mercados Campesinos" process, it was possible to identify the dimensions that have been mostly affected by the short value chain experience and the aspects of smallholders' livelihood that have been improved.

Commercial Improvements

This topic identified those activities designed to improve smallholders' abilities to sell products autonomously, deal with costumers and define prices (Romagnoli, 2016). Previous studies (Ordoñez and Montoya, 2011; CEPAL, 2014; Molina and Parrado, 2015) observed that smallholders' business is extremely subjected to the control of intermediaries, who have strong market and price power. By reducing the intermediaries influence, peasants will increase their income, enhance self-confidence and their abilities to deal with costumers, and improve overall business management. To monitor improvements in these dimensions, three core variables were identified: selling products directly to consumers, the ability to establish selling prices and quality definition. Figure 1 shows a brief comparison of these aspects between the project beneficiaries and the control group.

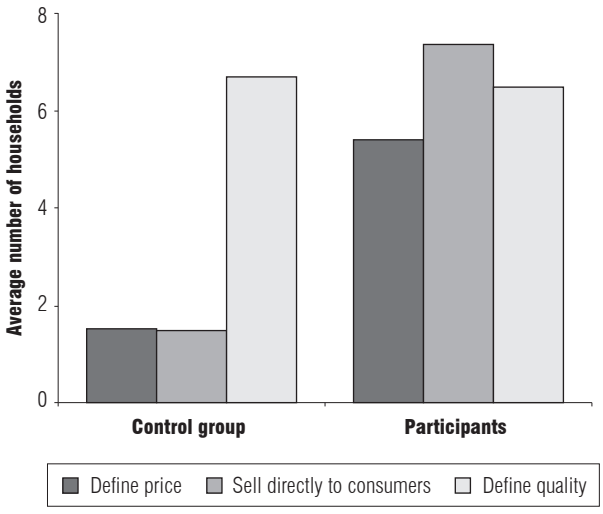


FIGURE 1. Descriptive statistics of the commercial improvement indicators.

In the X axis, the variables analyzed are represented highlighting the differences between the treated and non-treated groups. On average, project participants are more inclined to sell directly to consumers and to define the price of their goods than the control group, while a remarkable difference in product quality definition is not registered. Figure 2 shows the distribution of the two groups among market channels considered in the analysis.

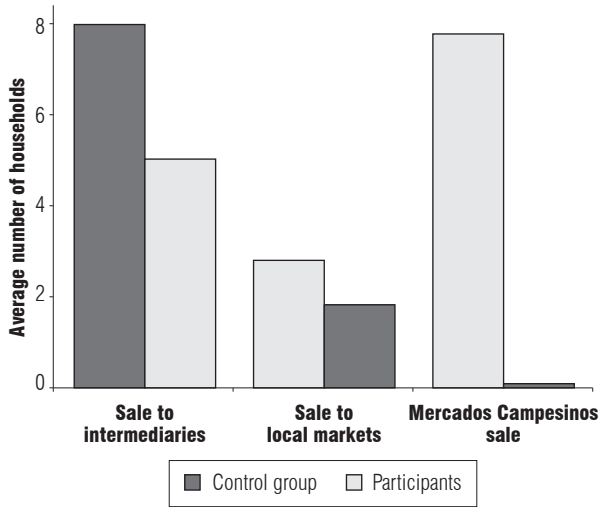


FIGURE 2. Descriptive statistics of involvement in different market channels.

The graph confirms the positive trends pointed out in the previous figure, but registers milder improvements. In fact, Figure 1 shows that participants were generally able to avoid intermediaries and establish selling prices autonomously, whereas Figure 2 shows that intermediaries still represent an important buyer for both group categories. Interviews helped to clarify the complexity linked with price determination and the intermediaries' role. Regarding the first issue, participants reported strong difficulties in extending price increase out of "Mercados Campesinos" channels and only a small percentage acquired enough bargaining abilities to negotiate prices with intermediaries and at a village market level. These results were confirmed by a t-test analysis which showed that in a basket composed by 15 products, on average, participants succeeded in setting higher prices for just a few goods in all market channels in comparison to the control group. Thus, the only commercial channel where all beneficiaries succeeded in defining "fair" prices were "Mercados Campesinos" markets. In particular Bogota markets are identified as the most profitable channel and participants' main source of income.

Positive project impact on commercial activities has been better explained by difference-in-difference analysis that assessed the correlation between project involvement and goals achieved. Table 2 summarizes the project influence comparing treated and control groups.

TABLE 2. Impact analysis results of commercial improvement indicators.

x \ y	Consumer direct sale	Price definition	Quality definition
Treatment effect	1.278***	0.459*	0.434*
Project participation	0.751***	0,762***	-0.525***
Male	-0.189*	0.055	0.337**
Wealth	0.066*	0.029	0.0135
Education	-0.036	0.0301	-0.075*
Savings	0.286*	0.150	0.060
Products sold	0.565**	0.034*	0.094***

*** $P<0.001$; ** $P<0.005$; * $P<0.1$.

The variable that fully represents project impact is *treatment effect*; as expected, it has a positive and valuable significance in all aspects analyzed, showing the highest positive influence on direct sale to consumers. That outcome confirmed our expectations and also the results of the interviews, proving the project key role in improving direct sale and its smoother influence on price and quality determination. Socio-economic variables registered ambiguous influence and significance. A univocal conclusion can be drawn only for *quantity of good sold* that has a remarkable and positive significance in all aspects, confirming that the involvement in this activities is positively correlated with the quantity and types of goods produced. Impact analysis results were complemented by multinomial analysis. This investigation confirmed that the treated group is more likely to be involved in activities to ameliorate trade. Indeed, the percentage of beneficiaries introducing business improvement is higher than non-participants.

Furthermore, the notable role played by variables *wealth* and *farmers' group participation* was stressed. Activity engagement is positively linked with wealth level, which means that richer farmers have more possibilities or more interest to increase market abilities. Equally significant is local producers' group participation that boosts project activities involvement.

Interviews showed that commercial improvements not only included economic and business enhancements but also several intangible and non-economic aspects that increased overall farmers' wellbeing. The majority of non-economic dimensions were not analyzed with quantitative approach. However, participants considered these aspects equally important as income increase and market diversification. Thanks to project participation and attendance

to workshops, beneficiaries not only developed an entrepreneurial mentality and soft skills which are extremely useful for the management of their businesses, but also private life organization. Additionally, direct relation with costumers was fundamental to increase the participants' self-esteem and confidence, valorize family farming activities and peasants' role, and raise consumers' awareness of smallholders' claims.

Economic performance

Economic efficiency embraces those activities aiming at introducing novelty and innovation to smallholders' businesses, increasing productivity and supply, and positively influencing their profits (Parrado and Molina, 2014). This dimension is composed by the following variables: introduction of organic cultivation practices, introduction of product exchange among project participants, and supply diversification given by sale of uncommon products in the local area.

Interviews clarified that improvements in these dimensions were achieved mainly through participation to the workshops organized within the project framework that mainly dealt with more efficient production techniques and market practices. Through these workshops, participants obtained a set of tools and knowledge to boost productivity and increase yields, as well as a full comprehension of overall market processes that clarified price definition mechanisms, market negotiation, costumer care and sale arrangement practices. An important remark is that workshops effectiveness was strongly determined by project participants' initial features. Beneficiaries were extremely differentiated in relation to management abilities and mechanization. Qualitative analysis showed that the most benefitted participants were those smallholders who lacked strong productive mechanization and had poor organizational abilities. However, all people interviewed agreed that trainings positively affected their businesses.

Participants' diversity highly influenced economic efficiency enhancements. Consequently, improvements in this dimension have not been as straightforward as in the previous topic. As shown in Figure 3, there is not a strong difference between treated and control groups regarding organic practices implementation and commercialization of non-regional products, while there is an outstanding discrepancy in relation to product exchange practices.

Impact analysis confirms descriptive statistics results (Tab. 3). Indeed, the investigation showed strong project significance on the product exchange / barter dimension. However, no statistical correlation was found between project participation and implementation of organic cultivation practices even if outcomes showed positive project influence. Regarding cultivation of non-local products, project participation seems to have a negative impact. Nevertheless, this result was not statistically confirmed. Socio-economic variables have an ambiguous impact and a weak influence, the only exception is represented by *quantity of product sold* that had a strong and proved significance in all aspects considered.

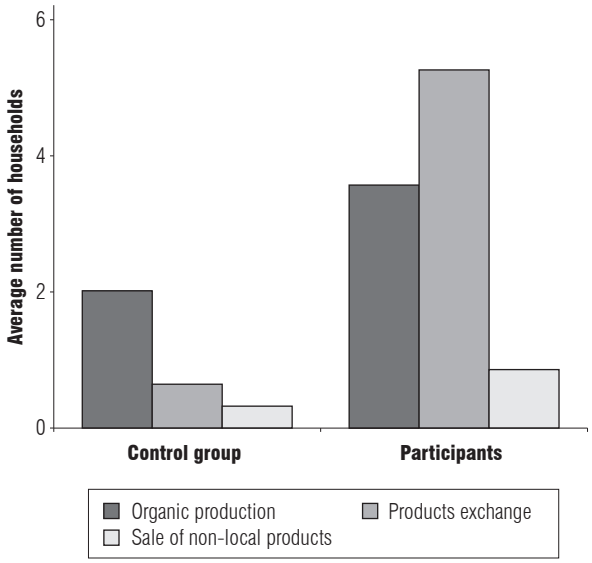


FIGURE 3. Descriptive representation of the economic efficiency indicator.

TABLE 3. Impact analysis results of economic efficiency indicator

x \ y	Product exchange	Nontraditional product	Organic
Treatment effect	0.819***	-0.173	0.245
Project participation	0.818	0.273	-0.061
Male	0.070	-0.274*	0.082
Wealth	0.081*	0.083	-0.033
Education	-0.135*	0.011	-0.119*
Savings	0.297*	-0.009	0.178
Products sold	0.040 *	0.071***	0.116***

*** $P<0.001$; ** $P<0.005$; *$P<0.1$.

Impact analysis outcomes were complemented by multinomial analysis. In particular, it clarified the role of socio-economic variables. The analysis confirmed higher beneficiaries' engagement in activities that led to business returns. It is important to highlight the positive influence of *farmers' group participation*, volume *of products sold* and *wealth level* on activity involvement.

The different analyses showed that together with project participation, participant's features had a crucial role in achieving economic improvements. Additionally, several

unobservable variables affected outcomes of this dimension, in particular the project leading foundation and associations' role. Given that they were in charge of training organization and management, workshops contents were often influenced by organization sensitiveness and commitment to different topics. A clear example of this is given by the leading role that "Fundación San Isidro" had in sensitizing and educating project participants on agro-ecological practices. In comparison with other beneficiaries, smallholders that attended to trainings organized by this association were more informed and committed to agro-ecological cultivation, preservation of traditional products and food sovereignty.

Political participation

The project aimed at to ameliorate all aspects of smallholders' livelihoods in order to reach sustainable and comprehensive improvements in beneficiaries' well-being. To meet this goal, activities that influenced social and political dimensions were implemented, besides economic and technical ones. In particular "Mercados Campesinos" focused its efforts on decreasing peasants' underrepresentation and low political recognition of the smallholders' needs and claims, increasing participants' advocacy power and influence on political decisions. These goals were achieved mainly thanks to the organizational structure of the project, namely the creation of local producer groups in each village that ensured active beneficiaries' involvement in project activities definition and management. Through direct involvement in the decision making process, participants acquired the abilities needed to express their requests and understood collective action strength and efficiency. Farmers' group constituted the milestone to build confidence and collective organization structure necessary to pressure at local and regional levels.

Political participation improvements were measured with the following variables: local committees' participation, advocacy activities participation, meetings with local politicians, and participation in the creation of a law proposal draft. The analysis undertaken expressed ambiguous results. Descriptive statistics showed a marked difference between beneficiaries and the control group regarding commitment to advocacy activities that becomes particularly significant in relation to the participation level of the farmers group (Fig. 4). This result is particularly relevant because it indicates that smallholders are rarely used to gather into groups and act collectively.

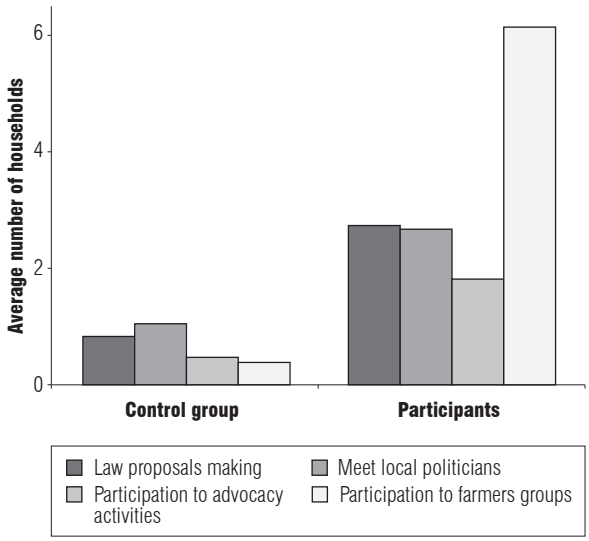


FIGURE 4. Descriptive statistics of political participation.

The impact analysis recorded a positive influence of the project without stating a significant correlation between project participation and the improvements reached. No relevant influence of the variables on participants' political engagement was recorded, except for the local producers' group membership. However, it is worthy to highlight that if the *participation of the producers*' group is considered an independent variable and not an indicator, it has a fundamental role in boosting participation to project advocacy

TABLE 4. Impact analysis result of political improvements.

x \ y	Meeting with local politicians	Advocacy activities	Participation in law proposal draft	Farmers group participation
Treatment effect	0.299	0.149	-0.336	2.705***
Project participation	-0.222	0.121*	0.036*	1.176***
Wealth	0.053	0.029	0.127*	0.081*
Education level	0.025	0.042	-0.020	-0.041*
Farmers group participation	0.0637**	0.825***	0.881***	Not analyzed
Dependency rate	-0.114*	-0.589	0.111*	0.113
Products sold	0.048**	0.039*	0.046*	0.135
Governmental program awareness	0.448**	0.458**	0.555**	0.402

Source: own elaboration.

*** $P<0.001$; ** $P<0.005$; * $P<0.1$.

activities. The combined effect of these results with the key role of the treatment variable on the farmers group participation indicator, would suggest that project participation indirectly influences all the other activities. Indeed, if the independent variable *participation to producer group* is eliminated, treatment effect acquires a positive and significant role. It is also worth mentioning the strong correlation between *governmental program awareness* and engagement in political activities.

Impact analysis results have been cleared up by the multinomial investigation that confirmed a high positive influence of governmental programs awareness on peasant active citizenship. Additionally, it highlights the significant role of project participation on beneficiaries' political engagement. Outcomes of descriptive statistics and multinomial analysis are totally in line with the interviews results, which confirmed that "Mercados Campesinos" increased knowledge of agricultural political issues, peasant confidence in exerting pressure at the local level and in promoting sensitizing activities with market costumers.

Outcomes of the interviews clarified that the participation of local producers' groups had a positive influence on several dimensions besides improving active citizenship and political awareness. Initially, smallholders developed a sense of belonging and attachment to traditions and community and, and simultaneously, they gained managerial and organizational competencies and full comprehension of the market structure.

Conclusions

The impact analysis of the results in "Mercados Campesinos" project shows that this initiative offered tools and activities to improve peasants' livelihood through a process that changed smallholders' perceptions about themselves and their occupation.

The quantitative and qualitative methodologies of this study indicated that the process had some contradictory results in terms of economic and commercial improvements. The results ensure that farmer markets in Bogota have been extremely successful and have led to an increase in beneficiaries' income and productivity. However, not all beneficiaries obtained an equal profit from the participation in the project. Socio-economic variables such as family incomes, agricultural tools endowment and organizational abilities played an important role in defining the degree of satisfaction and engagement in project activities. Additionally, the results achieved in Bogota farmer markets, such as the ability to overcome market intermediaries and to define good prices, were not easily replicable out of the "Mercados Campesinos" structure.

Smallholders' organizations played an important role not only in project organization, but also in creating a cultural and political base for cooperation among smallholders that strengthened producers and the entire rural community. This condition has been fundamental to create smallholders' awareness of their crucial role in the food supply chain and, consequently, start consumers' sensitization.

Lastly, it is important to stress that the project pointed out how productive and institutional dimensions are strongly interlinked. Indeed, peasant foundations and associations and local farmers committees not only influenced notoriously the political participation but also increased the economic efficiency. Regarding the social and political role of agriculture as a source of local development, the project strengthened rural economies and valorized indigenous and local products. Mercados Campesinos provided few actions that could be implemented to employ the political role of agriculture to reach territorial development. How to delineate and design this last element should be studied and investigated in further researches. In particular, strategies that could foster smallholders' associations advocacy and collective activities independently from projects implementation should be identified. Rural extension programs should focus their efforts on promoting short food supply chain based on a bottom up approach that starts with the direct involvement of farmers' associations and smallholders in order to ensure public policy efficiency.

Literature cited

ADB. 2006. Impact evaluation: methodological and operational issues. Asian Development Bank. Economic Analysis and Operations Support Division Economics and Research Department. Mandaluyong City, Philippines.

Beland, E. 2013. Dinámicas regionales, economía y pobreza: Departamento de Boyacá. Documento de Trabajo Nº21 Serie Estudios Territoriales. RIMISP. Santiago de Chile.

Cámara de Comercio de Bogotá. 2006. Plan Maestro de Abastecimiento y seguridad alimentaria. Series mesas de Trabajo Bogotá Como Vamos. Nº 19. Bogota.

Cano, C.G., A.M. Iregui, M.T. Ramírez, and A.M. Tribín. 2016. El desarrollo competitivo, equitativo y sostenible del sector agropecuario en Colombia. Banco de la República – CAF. Bogota.

CEPAL. 2014. Agricultura familiar y circuitos cortos. Nuevos esquemas de producción, comercialización y nutrición. Santiago de Chile: Series Seminarios y conferencias 77. United Nations Publication, République Française.

Díaz, T. 2014. La visión de la FAO. pp. 11-12. In: Agricultura Familiar y circuito cortos. Nuevos esquemas de producción, comercialización y nutrición. Naciones Unidas, Santiago de Chile.

DANE. 2016. Tercer Censo nacional Agropecuario. Tomo 2. Resultados. Departamento Administrativo Nacional de Estadística, Bogota.

Escobar, G., O. Paz, and E. Jara. 2010. La participación en organizaciones rurales y los ingresos de pequeños productores en América Latina. Centro Latinoamericano para el Desarrollo Rural Rimisp. Santiago de Chile.

EU-AID European Aid Co-operation Office. 2004. Evaluation methods for the European Union's external assistance. Evaluation tool, vol. 4. Joint Evaluation Unit. Luxemburg.

FLACSO, OXFAM, UNAL. 2014. Modelos de acceso al mercado para pequeños productores en tres contextos de América Latina. Informe ejecutivo. Guatemala.

Gertler, J.P., S. Martinez, P. Premand, L.B. Rawlings, and M.J. Veermesch. 2011. Impact evaluation in practice. World Bank.

Guereña, A. 2014. Mercados Campesinos locales en la región central: un ejemplo de Apalancamiento. Series de aprendizajes Nº 3. OXFAM.

Gutiérrez, O., A. Parrado, and J.P. Molina. 2012. Mapa de actores y circuitos de mercados campesinos. Documento de trabajo. Universidad Nacional de Colombia, Grupo de Investigación en Gestión y Desarrollo Rural, Facultad de Agronomía. Bogota.

Machado, A. 2003. Ensayos sobre seguridad alimentaria. Universidad Nacional de Colombia. Bogota.

Ordoñez, F. and G. Montoya. 2011. Economía campesina, soberanía y seguridad alimentarias. La experiencia de Mercados Campesinos en Bogotá y la región central de Colombia. ILSA. Bogota.

Osorio, A.A. and A.G. Rivas. 2017. Contribution of local peasant innovations to the re-configuration of endogenous rural development. Agron. Col. 35(3), 365-373. Doi: 10.15446/agron.colomb.v35n3.63551

Parrado, A. and J.P. Molina. 2014. Mercados Campesinos: modelos de acceso a mercados y seguridad alimentaria en la región central de Colombia. Universidad Nacional de Colombia, Grupo de Investigación en Gestión y Desarrollo Rural, Facultad de Ciencias Agrarias. Primera edición. Bogota.

Pereira, F.A. 2014. La experiencia del proyecto "Mercados Campesinos" en el apoyo a la economía campesina y el consumo urbano. Universidad Nacional de Colombia, Facultad de Agronomía. Bogota.

Prado, A. 2014. La visión de CEPAL. pp. 13-14. In: Agricultura Familiar y circuito cortos. Nuevos esquemas de producción, comercialización y nutrición. Naciones Unidas. Santiago de Chile.

Romagnoli, F. 2016. Small-holders market participation in Colombia: An Assessment of *Mercados Campesinos* Project in Boyaca Region. Master thesis. Università degli studi di Firenze, Florence, Italy.

Romero, N.M., N.R. Escobar, and J.P. Molina. 2017. Organizaciones de productores campesinos: un eje transversal en el desarrollo rural. pp. 437-459. In: El desarrollo competitivo, equitativo y sostenible del sector agropecuario en Colombia. Banco de la República – CAF. Bogota.

Schejtman, A. and J.A. Berdegué. 2003. Desarrollo Rural Territorial. Centro Latinoamericano para el Desarrollo Rural (RIMISP). Santiago de Chile.

Optimization of fermentation process conditions for chili pepper (*Capsicum frutescens*) fruit using Response Surface Methodology

Optimización del proceso de fermentación de frutos de ají (*Capsicum frutescens*) utilizando el Método de Superficie Respuesta

Carlos Vegas[1, 2]*, Amparo Iris Zavaleta[1], and Braulio Zarzoso[3]

ABSTRACT

The consumption of chili pepper fruits (CPF) is widespread throughout the world. However, countries without tropical climates can only consume few CPF varieties. The lactic fermentation (LF) of CPF is a good alternative for their preservation and consumption in those regions where they are not cultivated. The main objective of this research was to optimize the fermentation process conditions for a CPF variety (*Capsicum frutescens*) modifying the Sodium Chloride (NaCl) and glucose concentrations to increase the acidification rate. The Response Surface Methodology was used applying a Central Composite Design to integrate a desirability approach. The growth of the microorganisms responsible for the fermentation process was also evaluated. The addition of NaCl and glucose significantly affected the acidification rate for LF of CPF. The optimum fermentation parameters determined to maximize the acidification rate were 6.25% NaCl and 1.77% glucose concentrations with an acidification rate of 0.113% acidity/day. However, this value was slightly lower than predicted. Lactic acid bacteria and yeasts were the main microorganisms throughout the fermentations.

Key words: yeasts, lactic fermentation, lactic acid bacteria, glucose, NaCl.

RESUMEN

El consumo de ajíes está ampliamente extendido en el mundo. Sin embargo, países con climas no tropicales pueden consumir una restringida cantidad de variedades de ajíes. La fermentación láctica (FL) de ajíes es una buena alternativa para su conservación y consumo en regiones donde no son cultivados. El objetivo de este trabajo fue optimizar el proceso de fermentación para una variedad de ají (*Capsicum frutescens*), modificando las concentraciones del Cloruro de Sodio (NaCl) y glucosa para incrementar la velocidad de acidificación. Se utilizó el método de Superficie Respuesta usando el Diseño Compuesto Central e integrando la función de deseabilidad. El crecimiento de los microorganismos responsables de la fermentación también fue evaluado. La adición de NaCl y glucosa influyó significativamente en la velocidad de acidificación para la FL de los ajíes. Los parámetros de fermentación óptimos determinados para maximizar la velocidad de acidificación fueron las concentraciones de 6.25% de NaCl y 1.77% de glucosa con una velocidad de acidificación de 0.113% de acidez/día. Sin embargo, este valor fue ligeramente menor al predicho. Las bacterias ácido lácticas y levaduras fueron los principales microorganismos identificados durante el proceso de fermentación.

Palabras clave: levaduras, fermentación láctica, bacterias ácido lácticas, glucosa, NaCl.

Introduction

Chili peppers (*Capsicum* sp.) belong to the Solanaceae family which includes at least 37 species (Bosland *et al*., 2012), being five of them domesticated species: *Capsicum annum* L. var. *annum*, *C. baccatum* L. var. *pendulum* (Willd.) Eshbaugh, *C. pubescens* Ruiz and Pavon, *C. chinense* Jacquin and *C. frutescens* L. (Pickersgill, 2007). Chili pepper fruits (CPF) are consumed raw, cooked and as a spice (Sherman and Billing, 1999). Furthermore, CPF are used as additives in the food industry and are even used in traditional medicine around the world (Di Scala and Crapiste, 2008). The CPF consumption may provide some health benefits due to their high content of carotenoids (provitamin A), ascorbic acid (vitamin C), tocopherols (vitamin E), flavonoids and capsaicinoids (Howard and Wildman, 2007; Topuz and Ozdemir, 2007).

Nowadays, CPF are cultivated around the world, particularly in the tropical and subtropical countries. Peru is one of the few countries in the world with the highest diversity of CPF due to a local high crop diversity (five-cultivated species are grown) and to the traditional consumption by the native population (Meckelmann *et al*., 2013).

Received for publication: 1 December, 2017. Accepted for publication: 28 February, 2018 Doi: 10.15446/agron.colomb.v36n1.69164

[1] Laboratorio de Biología Molecular, Facultad de Farmacia y Bioquímica, Universidad Nacional Mayor de San Marcos, Lima (Peru).
[2] Universidad Privada San Juan Bautista, Lima (Peru).
[3] Departament de Bioquímica i Biotecnologia, Facultat d' Enologia, Universitat Rovira i Virgili,Tarragona (Spain).
* Corresponding author: carlosvegas@hotmail.com

CC BY NC ND

"Ají Charapita" (*C. frutescens*) is the common name of the main cultivated chili pepper in the Peruvian Amazon region. This fruit has high economic value due to its high consumption in the local gastronomy as fresh fruit, powder, pickles, and sauce at national and international levels. Considering the consumer trends towards the demand for natural food, in which high sensorial quality, safety, high nutritional value, healthy options, low prices, and minimum processing with environmentally-friendly production processes are highly appreciated, the lactic fermentation (LF) methodology of CPF is considered a good alternative for food preservation. In addition, LF represents a good alternative for improving the smallholder farmers and rural communities' incomes through the artisanal production of low-value commodities.

LF is an ancient biotechnology technique of food preservation used to maintain and improve food safety, keeping its nutritional, sensory and shelf life properties (Steinkraus, 1996; Buckenhüskes, 1997; Karovicová and Kohajdová, 2003; Demir *et al*., 2006). Additionally, lactic acid bacteria (LAB) are considered the main microorganisms involved during LF. They contribute with positive effects on human health through the development of probiotics and natural food products which lead inhibitory metabolites and bacteriocin toxins to act as antimicrobial agents, thus replacing chemical preservatives (Ross, 2002; Gaggia *et al*., 2011). Cabbages, cucumbers and olives are the main fermented and commercialized vegetables and fruits worldwide (Montet *et al*., 2006; Rodríguez *et al*., 2009). Several factors such as pH, water activity, oxygen availability, temperature, nutrients, selected starter culture, inoculum concentration, and salt concentration, could influence LF optimization and affect the growth and activity of LAB during the fermentation process (Lee and Salminen, 1995; Ballesteros *et al*., 1999). Sodium Chloride (NaCl) is one of the most historically important factors. Its concentration stimulates salt tolerant LAB growth, and, moreover, it inhibits growth of unwanted microorganisms (Rao *et al*., 2004). Availability of fermentable carbohydrates has been described as an important factor in the preparation of vegetables and fruits for LF (glucose and fructose) (Ray and Panda, 2007; Wouters *et al*., 2013).

Response surface methodology (RSM) is a statistical method used for developing, improving and optimizing complex processes. Box and Wilson (1951) described this method and it can be defined as the assessed interaction between independent variables and response to find out the best conditions for a multivariable system (Gomes *et al*., 2013). The main advantage is to reduce the number of experiments required even when a large number of variables is involved, being less laborious and time consuming than other methods (Wu, 2002). This method has been extensively applied in the optimization of several food fermentations, such as York cabbage, cassava, apple, palm, mulberry, and radish (Ghosh *et al*., 2012; Jaiswal *et al*., 2012; Nwabueze and Odunsi, 2013; Wang *et al*., 2013; Joshi *et al*., 2015; Peng *et al*., 2015). Central Composite Design (CCD) provides favorable predictions from a second order fitting with a stable variance. This design combines the factorial design with axial runs and center points to produce CCD experiments. Axial runs are included in the design to introduce quadratic terms in the model as long as the center points are used to check the curvature of the response surface. The model equation is obtained after performing the regression analysis of experimental results, and the desirability function is further applied to obtain the optimal parameters (Jou *et al*., 2014).

The aim of this study was to optimize the LF of CPF using RSM. A CCD was conducted to evaluate the effect of NaCl and glucose concentrations added on the acidification rate during LF, and to obtain the optimum parameters to improve the CPF fermentation process.

Materials and methods

Native Peruvian CPF

CPF belonging to "Ají Charapita" variety were aseptically hand-recollected from rural areas in Iquitos city and transported to a lab under controlled conditions. Samples were fermented in the laboratory of Molecular Biology in the Faculty of Farmacia y Bioquímica at the Universidad Mayor de San Marcos (Lima, Peru).

Preparation of LF

CPF were selected under sterile conditions, according to their size and ripeness stage. The stalks from CPF were separated. CPF were rinsed two times with distilled sterile water and then drained off. LF was prepared in sterile plastic containers. Then, 35% (w/v) of CPF was placed into each container. A brine solution, according to each tested condition, was added up to 100 mL.

LF conditions and sampling

The brine solution for LF was added using three values for two variables: 0, 5 and 10% NaCl (Calbiochem, Darmstadt, Germany) with 0, 2.5 and 5% glucose (Amresco, Ohio, USA), as indicated in the experimental design (see below). LF was considered finalized when the reducing sugar content was below 1 gL^{-1}. LF was carried out at room

temperature. Brine samples from the starting mixture (d 0) and throughout the LF were taken at: 0, 3, 6, 12, 18, 27 and 37 d.

Chemical analysis

Acidity, sugar concentration and pH were monitored throughout the process (at the frequency mentioned above). Total titratable acidity was determined according to the Official Methods of analysis by the Association of Official Analytical Chemists (AOAC). Reducing sugars were measured by a 3,5-dinitrosalicylic acid (DNS) assay (Miller, 1959).

Microorganism count

The total microorganism cells were counted under a microscope using a Neubauer counting chamber. The different brine samples were grown on four specific culture media: MRS agar (Merck, Darmstadt, Germany) supplemented with nystatin (Merck, Darmstadt, Germany) and sodium azide (Merck, Darmstadt, Germany) (both 100 mg L^{-1}) for the selective enumeration of LAB. YPD agar (containing 2% glucose, 2% peptone, 1% yeast extract, 2% agar; w/v) was supplemented with chloramphenicol (Applichem GmbH, Darmstadt, Germany) (100 mg L^{-1}) specially for the yeast count. McConkey agar (Oxoid, Basingstoke, United Kingdom) was used to enumerate enterobacteriaceae and Cetrimide agar (Merck, Darmstadt, Germany) for *Pseudomonas*. All plates were incubated at 30°C for 1-3 d in aerobic conditions; with the exception of MRS media in anaerobic conditions using Anaerocult A (Merck, Darmstadt, Germany), anaerobic bags and anaerobic indicators (Oxoid, Basingstoke, United Kingdom).

Experimental design (ED)

A factorial design 3^2 was used. NaCl (X_1) and glucose (X_2) concentrations were considered as independent variables to study the concentration effect on the LF of CPF. Each variable was studied at three different levels (-1, 0, +1) represented in Table 1. The selection of level for both independent variables was chosen after assessing the recovery of viable LAB and the further acidity production.

In this study, the CCD was assumed as a limit value (equal to 1). Points outside the "safe" area were not tested. The dependent variable was the acidification rate (% acidity/day), which was estimated as the maximal slope obtained from the representation of acidity production during LF time.

The analysis of the ED data and the calculation of the predicted response were carried out using the RSM, Minitab software. The analysis of variance (ANOVA) was used to determine the statistical significance of the model

TABLE 1. Process variables and levels used in this study.

Independent variables	Coded symbols	Coded variable level		
		-1	0	1
NaCl (%; w/v)	X_1	0	5	10
Glucose (%; w/v)	X_2	0	2.5	5

(P-value<0.05). In order to understand the effect of NaCl (variable X_1) and glucose (variable X_2), concentrations on the acidification rate, experimental data were fitted using the model described by the Eq. (1) that includes linear, quadratic and interaction terms:

$$Y = B_0 + B_1x_1 + B_2x_2 + B_{12}x_1x_2 + B_{11}x^2_1 + B_{22}x^2_2 \qquad (1)$$

Where Y is the dependent variable (acidification rate), B corresponds to the regression coefficients: B_0 is a constant, B_1 and B_2 are the linear coefficients, B_{11} and B_{22} are the quadratic coefficients, and B_{12} is the interaction coefficient between variables 1 and 2. X_1 and X_2 correspond to the independent variables. Twelve LF conditions (Tab. 2) were tested and the sequence was randomly established to limit the influence of systematic error in the interpretation of results. Experiments 1-9 (Tab. 2) allowed the calculation of regression coefficients, while experiments 10-12 (Tab. 2) were replicates at the central point of the ED in order to estimate the influence of the experimental error. Each LF of ED point was carried out with one replicate and the mean values were reported as observed responses.

Results and discussion

LF of vegetables and fruits can be influenced by the addition of salt, glucose and some acids to the brine, which finally influences the pH (Pérez-Díaz *et al*., 2013). Microbiologically, LAB are the main microorganisms responsible for this type of fermentation process. However, yeasts are also involved, but depending on the salt concentration and other environmental factors (Arroyo-López *et al*., 2008). In this research, the hypothesis that the selection of adequate fermentation parameters favors the growth of LAB was stated, and, therefore, an increase of the acidification rate. Prior to this study, the variation of NaCl, glucose and acidity concentrations were evaluated independently. Due that there was no variation of the acidity concentration in terms of acidification rate, this variable was discarded. (results not shown).

Chemical analysis

Twelve LF of CPF were performed using different NaCl and glucose concentrations (Tab. 2). One of the replicates

of LF at initial stages was chemically analyzed. The LF were considered as finished when the reducing sugar content was below 1 g L^{-1}, and the total titratable acidity remained stable. Thus, LF conditions containing 5% NaCl with 0 and 2.5% glucose, and 10% NaCl with 0% glucose finished faster (12 d). Furthermore, LF process without NaCl but containing 2.5 and 5% glucose were slower (37 d), despite the reducing sugars of the last LF condition were tested above 2 g L^{-1}. It is known that a strong brine solution draws sugar and water out of the vegetables (Panda *et al*., 2009). This effect was noted mainly in those LF conditions without glucose, where an increase of reducing sugars was determined on the third day (Fig. 1). A critical factor in sensorial quality and reduction of the microbial action of many fermented vegetables and fruits is the pH (Spyropoulou *et al*., 2001; Muyanja *et al*., 2003; Rao *et al*., 2004; Ogunjobi *et al*., 2005). Therefore, pH values at the final of all LF conditions ranged between 3.10 and 3.87 decreasing the initial pH by two units respect to their initial value (Tab. 2). A decrease, not correlated to the LF conditions, was also observed on the microbial biodiversity in relation to the starting population, being present at the beginning of the LF the four microbial groups analyzed, while at the end only two or three groups were observed (Fig. 1). The total titratable acidity determined during LF is normally associated with an increase in organic acids, mainly lactic acid which minimizes the presence of contaminant microorganisms (Steinkraus, 1997; Spyropoulou *et al*., 2001). The total titratable acidity at the initial stage of all LF conditions was 0.225%, while the highest concentrations were obtained on LF conditions containing 10% NaCl with 2.5 and 5% glucose (1.91 ± 0.159%) followed by 5% NaCl with 2.5% glucose (between 1.69-1.80%). By contrast, the lowest concentrations were obtained in those LF without NaCl and glucose (Tab. 2, LF 2, 3, 4, 6 and 8).

A fast acidification rate is desirable in fermentations because rapid fermentation of raw materials could prevent the growth of undesirable microorganisms which may affect the aroma, texture, and taste of the final product (Akabanda *et al*., 2014). The fastest acidification rates (determined as the ratio between the difference of the final and initial acidy values, and the LF time) were recorded in the LF conditions containing 5% NaCl with 2.5% glucose (0.122-0.131% acidity/day) followed by 5% NaCl with 0% glucose, 10% NaCl with 0% and 2.5% glucose (0.094% acidity/day).

Microbiological analysis

The microbiological results of nine LF conditions are shown, due to the similar values among the central point replicates (5% NaCl with 2.5% glucose).

The microbial counting was performed by microscopy and plating. Total cell counting by microscope showed populations ranging from 10^6 to 10^9 cells mL^{-1}. However, when plated in four specific culture media, the population recovered in these media ranged from 10^3 to 10^8 CFU mL^{-1}. The reasons for the differences on the microbial count between microscopy and plating could be due to: (1) the viable but non-culturable state adopted as a strategy by

TABLE 2. Chemical analysis at initial and final stages of twelve LF conditions and CCD with the observed response for acidification rate.

Run	Coded variable levels		LF time (days)	Initial LF		Final LF			Acidification rate (Y) (% acidity/day)*
	NaCl (%; X_1)	Glucose (%; X_2)		pH	Reducing sugars (g L^{-1})	pH	Reducing sugars (g L^{-1})	Total titratable acidity (%; w/v)	
1	5 (0)	5 (1)	18	5.68	49.1	3.50 ± 0.057	0.24 ± 0.049	1.58 ± <0.001	0.075 ± <0.001
2	10 (1)	0 (-1)	12	5.62	0.35	3.87 ± 0.078	0.39 ± 0.009	1.35 ± <0.001	0.094 ± <0.001
3	5 (0)	0 (-1)	12	5.65	0.62	3.75 ± 0.085	0.33 ± <0.001	1.35 ± <0.001	0.094 ± <0.001
4	0 (-1)	0 (-1)	18	5.85	0.24	3.65 ± 0.012	0.75 ± 0.046	1.20 ± 0.212	0.054 ± 0.012
5	10 (1)	5 (1)	27	5.54	44.66	3.45 ± 0.057	0.28 ± 0.014	1.91 ± 0.159	0.063 ± 0.006
6	0 (-1)	5 (1)	37	5.94	45.44	3.10 ± 0.014	2.47 ± 0.882	1.24 ± 0.159	0.027 ± 0.004
7	5 (0)	2.5 (0)	12	5.60	27.13	3.60 ± 0.061	0.27 ± 0.024	1.76 ± 0.053	0.128 ± 0.004
8	0 (-1)	2.5 (0)	37	5.71	23.49	3.12 ± 0.014	0.96 ± 0.086	1.35 ± <0.001	0.030 ± <0.001
9	10 (1)	2.5 (0)	18	5.52	23.89	3.57 ± 0.049	0.33 ± 0.023	1.91 ± 0.159	0.094 ± 0.009
10	5 (0)	2.5 (0)	12	5.60	27.13	3.56 ± 0.057	0.27 ± 0.032	1.80 ± <0.001	0.131 ± <0.001
11	5 (0)	2.5 (0)	12	5.60	27.13	3.51 ± 0.042	0.30 ± 0.009	1.69 ± 0.159	0.122 ± 0.013
12	5 (0)	2.5 (0)	12	5.60	27.13	3.59 ± 0.071	0.28 ± 0.041	1.69 ± 0.159	0.122 ± 0.013

* Acidification rate: determined as difference between the final and initial acidity, then dividing by LF time.

microorganisms in response to adverse environmental conditions (Ramamurthy *et al*., 2014), and (2) the presence of different microorganisms able to grow on the culture media used in this research.

The first day of sampling (d 0) was characterized by a higher presence of enterobacteria and *Pseudomonas* bacteria compared to the remaining microorganisms studied (Fig. 1). However, according to viable counts, LAB were considered the main microbial group responsible for LF containing NaCl because in all assays with these conditions it was the principal isolated microbial group. Yeasts were mostly recovered after the 6th d of LF process with 10% NaCl. However, yeasts along with *Enterobacteriaceae* were the second most recovered microbial population during all the LF process. These results agree partially with those

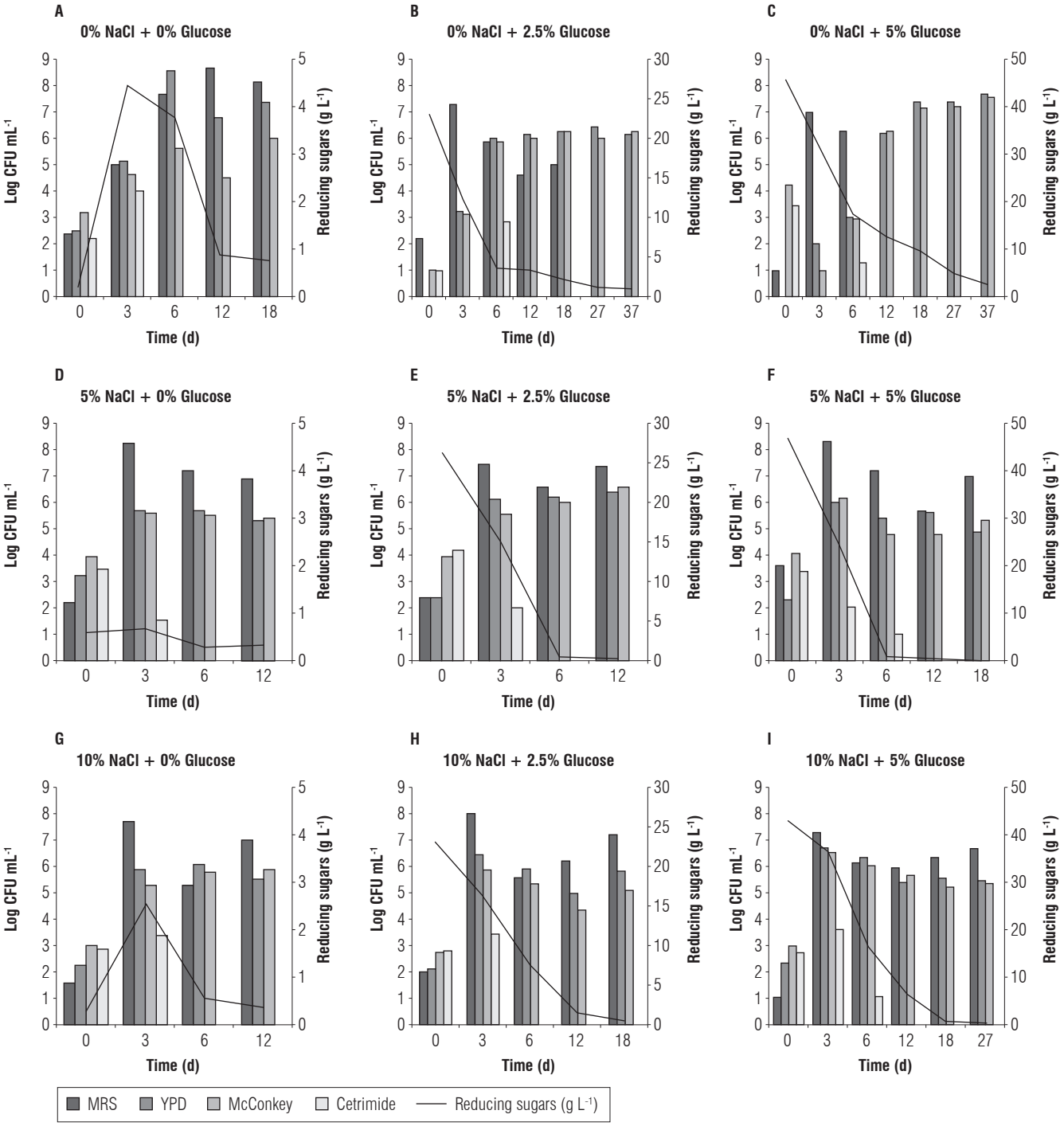


FIGURE 1. Evolution of the microbial populations for LF of CPF. Effect of NaCl or glucose concentrations on microbial recovery and sugar consumption.

reported by Fleming (1982), where the Gram-positive and Gram-negative bacteria were the main organisms responsible for the fermentation process at the initial stages, and the lactic acid bacteria with or without fermentative yeasts were leading the primary fermentation process until the fermentable carbohydrates were consumed or until the LAB were inhibited at low pH values. Finally, acid-tolerant fermentative yeasts were responsible for the secondary fermentation until the fermentable carbohydrates were totally consumed.

For LF condition without NaCl and glucose, yeasts and LB were the microorganisms responsible for starting and ending the LF, respectively; but, while the LF condition without NaCl with 2.5 and 5% glucose remained, the LAB were predominant until d 6 leading yeasts and *Enterobacteriaceae* to finish the LF process.

Enterobacteriaceae and *Pseudomonas* are gram negative fermentative and non-sugar fermentative bacteria, respectively. These microorganisms come from the farm and their presence at the initial stages of the fermentation process is harmful because they could spoil vegetables and fruits as well as they could produce undesirable metabolites that would affect their sensorial quality. It is possible that the recovery of *Enterobacteriaceae* populations would have declined if the CPF had previously been disinfected with a sodium hypochlorite solution following the food industry standard procedure. (Fukuzaki, 2006). LF with 5 and 10% of NaCl seemed to be more adequate because these conditions favored the recovery of LAB and yeasts.

RSM analysis

RSM analysis was applied to the acidification rate resulting in the twelve LF performed (Tab. 2). The regression coefficients of the model and their statistical significance are described in Table 3. The "fitness" of the model was studied through coefficients of determination (R^2), probability values (*P*) and lack-of-fit values (Tab. 3 and 4).

TABLE 3. Regression coefficient, significance level (*P*), R^2 and *F* values of the quadratic model determined for acidification rate (Y).

Parameter	Acidification rate (% acidity/day) (Y)	*P-Value* (Y)
B_0	0.0415	0.02
B_1	0.02262	0.002
B_{11}	-0.001775	0.003
B_2	0.01277	0.171
B_{22}	-0.0035	0.053
B_{12}	-0.00008	0.897
R^2	0.8371	
F	12.31	0.004

From the variables presented in Table 3, the linear term (B_1) of NaCl had the most significant effect ($P = 0.002$) on the acidification rate, followed by the quadratic term (B_{11}) of NaCl ($P = 0.003$). On the contrary, the linear and quadratic term of glucose and their interaction with NaCl did not have any significant contribution towards acidification rate. The R^2 of the predicted model was considered appropriate ($R^2 = 0.8371$; $P = 0.004$); however, the *P*-value for the lack-of-fit was less than 0.05 (*P*-value = 0.017) (Tab. 4), indicating that the predicted model of acidification rate can be reasonably represented by the mathematical model in the Eq. (2).

$$\text{Acidification rate (\% acidity/day)} = 0.0415 + 0.02262\ X_1 - 0.001775\ X^2_1 \quad (2)$$

Contour plots for acidification rate are represented in Figure 2. In this plot, it is shown that the best LF conditions ranged from 4.3 to 8.4% NaCl and from 1 to 3.45% glucose, theoretically obtaining an acidification rate higher than 0.12% acidity/day.

TABLE 4. ANOVA for the response surface quadratic model determined between response variable (Y) and independent variables (X_1, X_2).

Source	DF*	Sum of squares (SS)	Mean square	*F*-value	*P*-value
Regression	5	0.013543	0.002709	12.31	0.004
Linear	2	0.007828	0.003914	17.78	0.003
quadratic	2	0.009284	0.004642	21.09	0.002
Interaction	1	0.000004	0.000004	0.02	0.897
Residual error	6	0.00132	0.00022		
Lack-of-fit	3	0.00126	0.00042	20.74	0.017
Pure error	3	0.000061	0.00002		
Total	11	0.014864			

* DF: Degrees of Freedom.

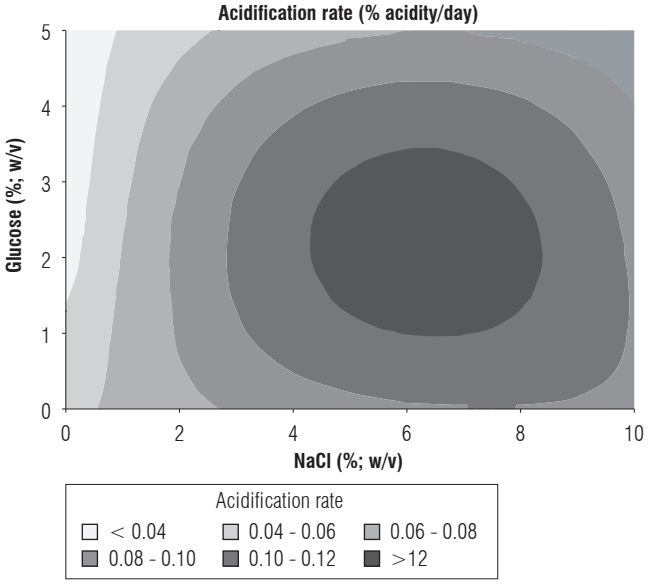


FIGURE 2. Contour plots for acidification rate (% acidity/day) as a function of NaCl and glucose concentrations.

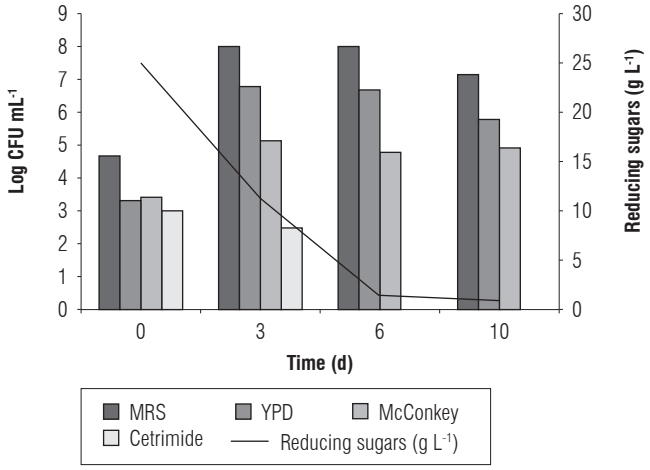


FIGURE 3. Evolution of the microbial populations during LF of CPF at theoretically optimal conditions. Plate counting on different culture media and sugar content of the brine during LF were included.

Optimal conditions

The "Response Optimizer" option of the program MINITAB was used to identify the best LF conditions for increasing the acidification rate. It was evaluated by the "Composite Desirability" where a value equals to 1 corresponds to the ideal case, while 0 indicates that the response is outside the acceptable limits. The specified targets (maximize) in order to increase the acidification rate were 0.027 and 0.131% acidity/day as minimum and maximum value, respectively. Thus, the optimized conditions were as follows: 6.25% NaCl and 1.77% glucose. The "composite desirability" determined by the software was equal to 0.9357 with an acidification rate of 0.1243% acidity/day.

Finally, a new experiment was done by triplicate with the optimized LF conditions as described above. The pH value, reducing sugars and total titratable acidity at the initial stages of the LF process were 5.30±0.193, 24.74±3.978 g L^{-1} and 1.04±0.054% (w/v), respectively. LF time was slightly shorter (10 d) (Fig. 3). The final pH values, reducing sugar and total titratable acidity were 3.09±0.015, 1.04±0.054 g L^{-1} and 1.35%±0.001 (w/v) respectively, while the acidification rate was 0.113%±0.001 acidity/day. This LF, the values of the final total titratable acidity and acidification rate were lower than those obtained with 5% NaCl and 2.5% glucose together. The low total titratable acidity values and acidification rates can be explained due to the different crops the assessed fruits came from compared to those that were analyzed on the prior LF process.

Microbiologically, LAB remained as the most recovered microorganism during the LF. Yeasts were the second most recovered microbial population followed by *Enterobacteriaceae*. In contrast, *Enterobacteriaceae* and *Pseudomonas* were not the most predominant microorganisms at d 0 (Fig. 3).

Conclusions

NaCl and glucose, as the main parameters involved in LF, were studied carefully. The suitable concentrations for both compounds were determined using RSM. The optimum conditions based on the maximum acidification rate as response were 6.25% NaCl and 1.77% glucose, but experimentally the acidification rate was slightly lower that other LF conditions. Microbiologically, LAB were the microorganisms responsible during LF, followed by yeasts. Finally, studies on the microbiota of LAB and yeasts at strain and species levels involved during the LF of CPF are recommended in order to continue with fermentation process optimization.

Acknowledgments

This work was supported by INNOVATE-PERU, agreement N° 230-FINCyT-IA-2013 and CIENCIACTIVA, agreement N° 007- FONDECYT-2014.

Literature cited

Akabanda, F., J. Owusu-Kwarteng, K. Tano-Debrah, C. Parkouda, and L. Jespersen. 2014. The use of lactic acid bacteria starter culture in the production of Nunu, a spontaneously fermented milk product in Ghana. Int. J. Food Sci. Doi: 10.1155/2014/721067

Arroyo-López, F.N., A. Querol, J. Bautista-Gallego, and A. Garrido-Fernández. 2008. Role of yeasts in table olive production. Int. J. Food Microbiol. 128, 189-196. Doi: 10.1016/j.ijfoodmicro.2008.08.018

Ballesteros, C., L. Palop, and I. Sánchez. 1999. Influence of sodium chloride concentration on the controlled lactic acid fermentation of "Almagro" eggplants. Int. J. Food Microbiol. 53, 13-20. Doi: 10.1016/S0168-1605(99)00130-0

Bosland, P.W. and E.J. Votava. (eds.). 2012. Peppers: Vegetable and spice capsicums. Cambridge, UK. Doi: 10.1079/9781845938253.0000

Box, G.E.P. and K.B. Wilson. 1951. Experimental attainment of optimum conditions. J. R. Stat. Soc. 13, 1-45. Doi: 10.1007/978-1-4612-4380-9_23

Buckenhüskes, H.J. 1997. Fermented vegetables. pp. 595-609. In: P.D. Doyle, L.R. Beuchat, T.J. Montville (eds.). Food microbiology: fundamentals and frontiers. ASM Press, Washington, DC.

Demir, N., K.S. Bachçeci, and J. Acar. 2006. The effects of different initial *Lactobacillus plantarum* concentrations on some properties of fermented carrot juice. J. Food Process. Pres. 30, 352-363. Doi: 10.1111/j.1745-4549.2006.00070.x

Di Scala, K. and G. Crapiste. 2008. Drying kinetics and quality changes during drying of red pepper. LWT – Food Sci. Technol. 41, 789-795. Doi: 10.1016/j.lwt.2007.06.007

Fleming, H.P. 1982. Fermented vegetables. pp. 52-70. In: Rose, A.H., (ed.). Economic microbiology of fermented foods. Academic Press, New York, USA.

Fukuzaki, S. 2006. Mechanisms of actions of sodium hypochlorite in cleaning and disinfection processes. Biocontrol Sci. 11, 147-157. Doi: 10.4265/bio.11.147

Gaggia, F., D. Di Gioia, L. Baffoni, and B. Biavati. 2011. The role of protective and probiotic cultures in food and feed and their impact on food safety. Trends Food Sci. Technol. 22, 58-66. Doi: 10.1016/j.tifs.2011.03.003

Ghosh, S., R. Chakraborty, and U. Raychaudhuri. 2012. Optimizing process conditions for palm (*Borassus flabelliffer*) wine fermentation using response surface methodology. Int. Food Res. J. 19, 1633-1639. Doi: 10.1002/jib.153

Gomes, T., C. Barradas, T. Dias, J. Verdial, J. Sá Morais, E. Ramalhosa, and L.M. Estevinho. 2013. Optimization of mead production using Response Surface Methodology. Food Chem. Toxicol. 59, 680-686. Doi: 10.1016/j.fct.2013.06.034

Howard, L.R., and R.E.C. Wildman. 2007. Antioxidant vitamin and phytochemical content of fresh and processed pepper fruit (*Capsicum annuum*). pp. 165-191. In: R.E.C. Wildman (ed.). Handbook of nutraceuticals and functional foods (2nd ed.). CRC Press, Boca Raton, USA.

Jaiswal, A.K., S. Gupta, and N. Abu-Ghannam. 2012. Optimization of lactic acid fermentation of York cabbage for the development of potential probiotic products. Int. J. Food Sci. Technol. 47, 1605-1612. Doi: 10.1111/j.1365-2621.2012.03010.x

Joshi, V.K., A. Chauhan, S. Devi, and V. Kumar. 2015. Application of response surface methodology in optimization of lactic acid fermentation of radish: effect of addition of salt, additives and growth stimulators. J. Food Sci. Technol. 52, 4935-4944. Doi: 10.1007/s13197-014-1570-9

Jou, Y.T., W.T. Lin, W.C. Lee, and T.M. Yeh. 2014. Integrating the Taguchi method and response surface methodology for process parameter optimization of the injection molding. Appl. Math. Inf. Sci. 8, 1277-1285. Doi: 10.12785/amis/080342

Karovicová, J. and Z. Kohajdová. 2003. Lactic acid fermented vegetable juices. Hortic. Sci. 30, 152-158. Doi: 10.1556/AAlim.34.2005.3.5

Lee, Y.K. and S. Salminen. 1995. The coming age of probiotics. Trends Food Sci. Technol. 6, 241–245. Doi: 10.1016/S0924-2244(00)89085-8

Meckelmann, S.W., D.W. Riegel, M.J. Van Zonneveld, L. Ríos, K. Peña, R. Ugas, L. Quiñonez, E. Mueller-Seitz, and M. Petz. 2013. Compositional characterization of native Peruvian chili peppers (*Capsicum* spp.). J. Agric. Food Chem. 61, 2530-2537. Doi: 10.1021/jf304986q

Miller, G.L. 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Analyt. Chem. 31, 426-428. Doi: 10.1021/ac60147a030

Montet, D., G. Loiseau, and N. Kakhia-Rozis. 2006. Microbial technology of fermented vegetables. In R.C. Ray, O.P. Ward (eds.). Microbial biotechnology in horticulture (Vol. 1, 309-344). Science Publishers, Inc. New Hampshire, USA.

Muyanja, C.M., J.A., Narvhus, J., Treimo, and T., Langsrud. 2003. Isolation, characterization and identification of lactic acid bacteria from bushera: An Ugandan traditionally fermented beverage. Int. J. Food Microbiol. 80, 201-210. Doi: 10.1016/S0168-1605(02)00148-4

Nwabueze, T.U., and F.O. Odunsi. 2013. Optimization of process conditions for cassava (*Manihot esculenta*) Lafun production. Afr. J. Food Sci. Res. 1, 1-9.

Ogunjobi, A.A., B.C. Adebayo-Tayo, and A.A. Ogunshe. 2005. Microbiological, proximate analysis and sensory evaluation of processed Irish potato fermented in brine solution. Afr. J.Biotechnol. 4, 1409-1412.

Panda, S.H., S. Panda, P.S. Shiva Kumar, and R.C. Ray. 2009. Anthocyanin-rich sweet potato lacto-pickle: production, nutritional and proximate composition. Int. J. Food Sci. Technol. 44, 445-455. Doi: 10.1111/j.1365-2621.2008.01730.x

Peng, B., Y. Lei, H. Zhao, and L. Cui. 2015. Response surface methodology for optimization of fermentation process parameters for improving apple wine quality. J. Food Sci. Technol. 52, 7513-7518. Doi: 10.1007/s13197-015-1872-6

Pérez-Díaz, I.M., F. Breidt, R.W. Buescher, F.N. Arroyo-López, R. Jiménez-Díaz, A. Garrido-Fernández, J. Bautista-Gallego, S.S. Yoon, and S.D. Johanningsmeier. 2013. Fermented and acidified vegetables. pp. 521-532. In: Downes, F.P. and K. Ito (eds.). Compendium of methods for the microbiological examination of foods. American Public Health Association, Washington DC.

Pickersgill, B. 2007. Domestication of plants in the Americas: insights from mendelian and molecular genetics. Ann. Bot. 100, 925-940. Doi: 10.1093/aob/mcm193

Ramamurthy, T., A. Ghosh, G.P. Pazhani, and S. Shinoda. 2014. Current perspectives on viable but non-culturable (VBNC) pathogenic bacteria. Front. Public Health. 2, 1-9. Doi: 10.3389/fpubh.2014.00103

Rao, M.S., J. Pintado, W.F. Stevens, and J.P. Guyot. 2004. Kinetic growth parameters of different amylolytic and non-amylolytic *Lactobacillus* strains under various salt and pH conditions. Bioresour Technol. 94, 331-337. Doi: 10.1016/j.biortech.2003.11.028

Ray, R.C. and S.H. Panda. 2007. Lactic acid fermented fruits and vegetables: an overview. In M.V. Palino (ed.). Food microbiology research trends. Nova Science Publishers Inc., Hauppauge, New York, USA.

Rodríguez, H., J.A. Curiel, J.M. Landete, B. De las Rivas, F.L. De Felipe, C. Gómez-Cordovés, J.M. Mancheño, and R. Muñoz. 2009. Food phenolics and lactic acid bacteria. Int. J. Food Microbiol. 132, 78-90. Doi: 10.1016/j.ijfoodmicro.2009.03.025

Ross, R.P., S. Morgan, and C. Hill. 2002. Preservation and fermentation: past, present and future. Int. J. Food Microbiol.79, 3-16. Doi: 10.1016/S0168-1605(02)00174-5

Sherman, P.W., and J. Billing. 1999. Darwinian Gastronomy: Why we use spices: Spices taste good because they are good for us. BioScience 49, 453-463. Doi: 10.2307/1313553

Spyropoulou, K.E., N.G. Chorianopoulos, P.N. Skandamis, and G.J.E. Nychas. 2001. Control of *Escherichia coli* O157: H7 during the fermentation of Spanish-style green table olives (conservolea variety) supplemented with different carbon sources. Int. J. Food Microbiol. 66, 3-11. Doi: 10.1016/S0168-1605(00)00510-9

Steinkraus, K.H. (ed.). (1996). Handbook of indigenous fermented foods. New York, USA.

Steinkraus, K.H. 1997. Classification of fermented foods: worldwide review of household fermentation techniques. Food Control. 8, 311-317. Doi: 10.1016/S0956-7135(97)00050-9

Topuz, A. and F. Ozdemir. 2007. Assessment of carotenoids, capsaicinoids and ascorbic acid composition of some selected pepper cultivars (*Capsicum annuum* L.) grown in Turkey. J. Food Compost Anal. 20, 596-602. Doi: 10.1016/j.jfca.2007.03.007

Wang, C.Y., Y.W. Liu, J.Q. Jia, T.R. Sivakumar, T. Fan, and Z.Z. Gui. 2013. Optimization of fermentation process for preparation of mulberry fruit wine by response surface methodology. Afr. J. Microbiol. Res., 7, 227-236.

Wouters, D., N. Bernaertb, W. Conjaertsa, B. Van Droogenbroeckb, M. De Looseb, and L. De Vuyst. 2013. Species diversity, community dynamics, and metabolite kinetics of spontaneous leek fermentations. Food Microbiol. 33, 185-196. Doi: 10.1016/j.fm.2012.09.016

Wu, Y.W. 2002. Testing design and data processing. Suzhou University Press, Suzhou, China.

Editorial policy

Agronomia Colombiana is a scientific and technical publication of the agricultural sector, edited by the Faculty of Agricultural Sciences of Universidad Nacional de Colombia - Bogota campus. It is directed to agricultural science researchers, extension workers and to all professionals involved in science development and technological applications for the benefit of agricultural producers and their activity.

Issued as a four monthly journal, it is intended to transfer research results in different areas of tropical agronomy. Original unpublished papers are therefore accepted in the following areas: physiology, crop nutrition and fertilization, genetics and plant breeding, entomology, phytopathology, integrated crop protection, agro ecology, weed science, environmental management, geomatics, soils, water and irrigation, agroclimatology and climate change, post-harvest and agricultural industrialization, rural and agricultural entrepreneurial development, agrarian economy, and agricultural marketing.

The authors of the articles submitted to the journal Agronomía Colombiana must be aware of and avoid scientific misconduct (code of conduct by Committee on Publication Ethics, COPE) related to: scientific fraud in all or part of the data of the study and data falsification and manipulation; dishonesty due to fictitious authorship or gifting or exchange of co-authorship, duplicate publications, partial or complete, in different journals and self-plagiarism by reusing portions of previous writings; citation omission, citation copying without consultation and excessive self-citation, among others. The authors have the following rights: fair and impartial evaluation of articles done in a reasonable amount of time, correspondence shall be taken seriously and requests for changes and corrections shall be respected, subject to review by the evaluators, and articles shall remain unaltered.

Agronomia Colombiana uses double blind peer review process with a previous quick preliminary review. The articles must be submitted according to the rules established in the instructions to authors. If the articles fulfill minimum criteria established for the preliminary review in terms of language and scope, they will be sent to three or more expert reviewers in the specific area of knowledge, up to obtain two or three evaluations; two of them must be external to Universidad Nacional de Colombia, and the third one belonging to the research and teaching staff of the University. When the manuscript reaches two approval evaluations, the article is accepted for publication. If two of the reviewers consider that the scientific level of the manuscript is not acceptable for publication, the article will be rejected. When accepted, the author(s) must introduce the suggested corrections and answer all the questions from the reviewers or the Editor Finally, the Editor in Chief or the Editorial Committee reserves the right to accept or reject the submitted manuscripts.

For additional information, correspondence, subscriptions and journal swap, you may contact Universidad Nacional de Colombia, Facultad de Ciencias Agrarias, Journal *Agronomia Colombiana*, Bogota campus; P.O. Box 14490, Bogota-Colombia; phone numbers: (571) 316 5355 / 316 5000 extension 10265; e-mail: agrocol_fabog@unal.edu.co; the digital version of the journal is available at http://www.scielo.org.co; http://www.revistas.unal.edu.co/index.php/agrocol

Instructions to authors

The articles sent for publication to the editorial committee must be unpublished. In consequence, those that have been previously published in other journals, technical or scientific publications, will be rejected. To submit manuscripts, authors must be registered in our platform (https://revistas.unal.edu.co/index.php/agrocol/about/submissions#onlineSubmissions) and follow the submission instructions. Corresponding authors will be required to use an ORCID iD when submitting a manuscript. After manuscript submission, papers can be screened for plagiarism using specialized software. In case of finding significant level of duplication, the manuscript will be rejected.

In a preliminary review stage, two aspects, English grammar and scope, will be assessed in order to ensure mini-

mum requirements of any manuscript to be peer reviewed. If the manuscript is rejected in the preliminary review because English grammar aspect, authors are encouraged to edit for language their manuscript using professional services and submit the manuscript again. When reason for preliminary reject is a mismatch of the scope between the manuscript and journal interests, resubmission should be avoided.

Agronomia Colombiana accepts the following two types of original articles:

- ***Scientific and technological research papers:*** those documents presenting detailed original research results. The most generally applied structure has four main parts: introduction, materials and methods, results and discussion, and conclusions.
- ***Scientific note:*** brief document introducing original preliminary or partial results of a scientific or technical research, which usually need immediate publication.

Format and organization of the text

Research article length should not exceed 5,200 words, whereas scientific notes should not have more than 4,000 words. Tables and figures, that is to say, diagrams, drawings, schematic and flow diagrams, pictures and maps, should be consecutively numbered (Table 1 ...Table n; Figure 1... Figure n, etc).

Texts and tables should be prepared using an MS-Word® processor. Double-space all text including table head, figure captions and cited literature. All pages must be numbered consecutively and line numbering on each page is mandatory, Tables and diagrams of frequency (bar and circular diagrams) should be included in the mentioned Word file, as well as in their original MS-Excel® or any different format. Other figures, including photographs and drawings should be submitted in digital JPG (or JPEG) digital compression format, with a minimum resolution of 300 dpi.

As a general rule, tables and figures should only be submitted in black and white, except for those intended for the cover page of the Journal, for those cases in which it is absolutely necessary to present them in color (at the judgment and discretion of the editor), or when the costs of color publication are covered by the authors.

Languages, units, and style

The journal's official language is English. Regarding measurement units, the metric system (SI) should be consistently used through the manuscript, unless the need is seen to apply any specific units that are of frequent use by the scientific community. Multiplication followed by negative superscript (e.g., kg ha-1) can only be used with SI units. The slash (/) is a mathematical operation symbol that indicates "divided by". Anyway, in sciences it is used as a substitute of the word "per", and it is used to indicate rates. Use the slash to connect SI to non-SI units (e.g., 10°C/h or 10 L/pot).

All abbreviations should be explained in full length when first mentioned in the manuscript.

With regards to the tenses, the most commonly used ones are the past, for the introduction, procedures and results; and the present, for the discussion.

Title and authors

The title in English, as well as its corresponding Spanish translation, shall not exceed 15 words. The scientific names of plants and animals shall be italicized and lower cased, except for the first letter of the genus (and of the species author), which must be upper cased.

The authors (including first and second names) shall be listed in order of their contribution to the research and preparation of the manuscript, in completely justified text format (filling the whole line, or, if necessary, the next one below) under the translated version of the title. At the bottom of the article's first page include only the name and city location of the employer or supporting institution(s), and the e-mail address of the corresponding author.

Abstract, resumen, and key words

The abstract should be written in English with Spanish translation for the Summary. Both texts should contain brief (no longer than 200 words in a single paragraph) and accurate descriptions of the paper's premise, justification, methods, results and significance. Both language versions shall be mandatorily provided with a list of (maximum six) key words that have not appeared in the title or abstract, and included in the Agrovoc thesaurus by Agris (FAO).

Introduction

In the introduction, include the delimitation and current status of the problem, the theoretical or conceptual basis of the research, the literature review on the topic, and the objectives and justification of the research. Common names must be accompanied with the corresponding scientific ones, plus the abbreviation of the species author surname when mentioned for the first time.

Materials and methods

Besides a clear, precise and sequential description of the materials used for the research (plant or animal materials, plus agricultural or laboratory tools), this section illustrates

the procedures and protocols followed, and the experimental design chosen for the statistical analysis of the data.

Results and discussion

Results and discussion can be displayed in two different sections or in a single section at the authors convenience. The results shall be presented in a logical, objective, and sequential order, using text, tables (abbreviated as Tab.) and figures (abbreviated as Fig.). The latter two should be easily understandable and self-explaining, in spite of having been thoroughly explained in the text. The charts should be two-dimensional and prepared in black and white, resorting to a tone intensity degradation to illustrate variations between columns. Diagram curves must be prepared in black, dashed or continuous lines (- - - - or ———), using the following conventions: ▪, ▲, ◆, ●, □, △, ◇, ○. The tables should contain few columns and lines.

Averages should be accompanied by their corresponding Standard Error (SE) values. The discussion shall be complete and exhaustive, emphasizing the highlights and comparing them to the literature.

This section should briefly and concisely summarize the most important findings of the research.

Conclusion (optional)

A short conclusion section is useful for long or complex discussion. It should provide readers with a brief summary of the main achievements from the results of the study. It also can contain final remarks and a brief description of future complementary studies which should be addressed.

Acknowledgements

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

Citations and literature cited

The system (author(s), year) will be consistently applied to all citations intended to support affirmations made in the article's text. When the cited reference has three or more authors, the citation shall only mention the name of the first author, accompanied by the Latin expression et al. (which means 'and others'), italicized and followed by a period, and separated from the year by a comma: (García et al., 2003). Alternatively, you can leave just the year in parenthesis: García et al. (2003). In case of references with only two authors, citations should include both names separated by 'and': (García and López, 2012) or García and López (2012).

The complete list of cited references in alphabetical order, according to the authors' surnames, is to be included at the end of the article. When the list includes various publications of the same author(s), they shall be listed in chronological order. When they correspond to the same year, they must be differentiated with lower case letters: 2008a, 2008b, etc. Each citation must contain a DOI (digital object identifier) at the end. Furthermore, the text of the manuscript must contain a minimum of 30% of the citations found in the Literature Cited section with their respective DOIs. Only original citations will be accepted. Avoid web citations unless their future availability is guaranteed

Illustrative cases:

- ***Books:*** author(s). Year. Book title. Edition. Publisher, city (and country, if the city is not a capital) of publication.

  E.g., Dubey, N.K. (ed.). 2015. Plants as a source of natural antioxidants. CABI, Wallingford, UK. Doi: 10.1079/9781780642666.0000.

- ***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

- ***Journals:*** Author(s). Year. Title of the article. Abbreviated name of the journal. Volume (number), page-page. E.g.: Zhu, J.K. 2001. Plant salt tolerance. Trends Plant Sci. 6, 66-71. Doi: 10.1016/S1360-1385(00)01838-0.

- ***Reference to a website:*** Author(s). Year. Title of the document. Retrieved from: URL; consulted date. E.g.: FAO. 1994. Neglected crops 1492 from a different perspective. Retrieved from: http://www.fao.org/docrep/t0646e/T0646E00.htm#Contents; consulted: August, 2017.

AGRONOMIA COLOMBIANA

VOLUME XXXVI, No. 1 JANUARY-APRIL 2018 ISSN (print): 0120-9965 / ISSN (online): 2357-3732

AGRONOMIA COLOMBIANA

P.O. Box 14490, Bogota-Colombia
Phones: (571) 316 5498 / (571) 316 5000 ext. 19095
Fax: (571) 316 5176
E-mail: agrocol_fabog@unal.edu.co
http://www.scielo.org.co
http://www.revistas.unal.edu.co/index.php/agrocol
http://agronomia.unal.edu.co