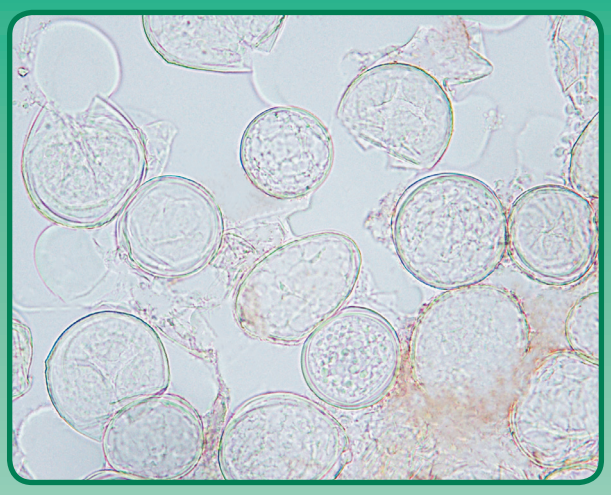
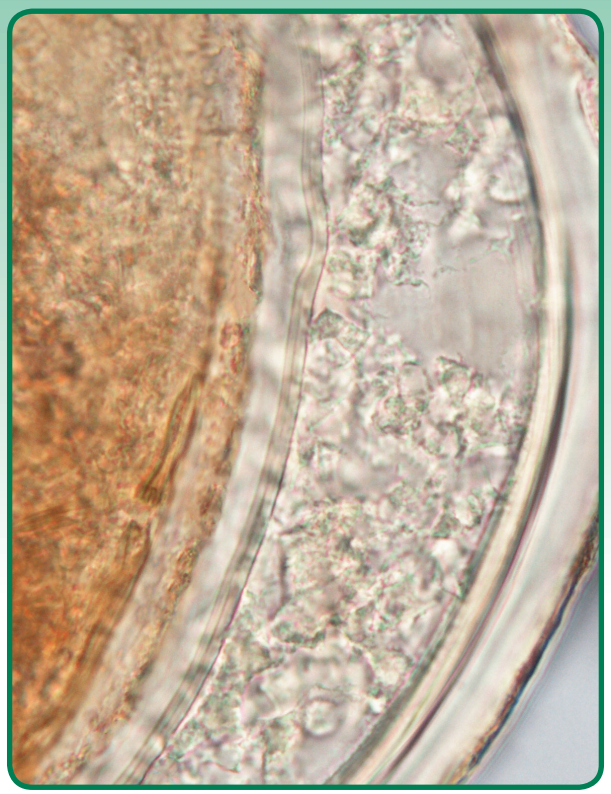
AGRONOMIA COLOMBIANA

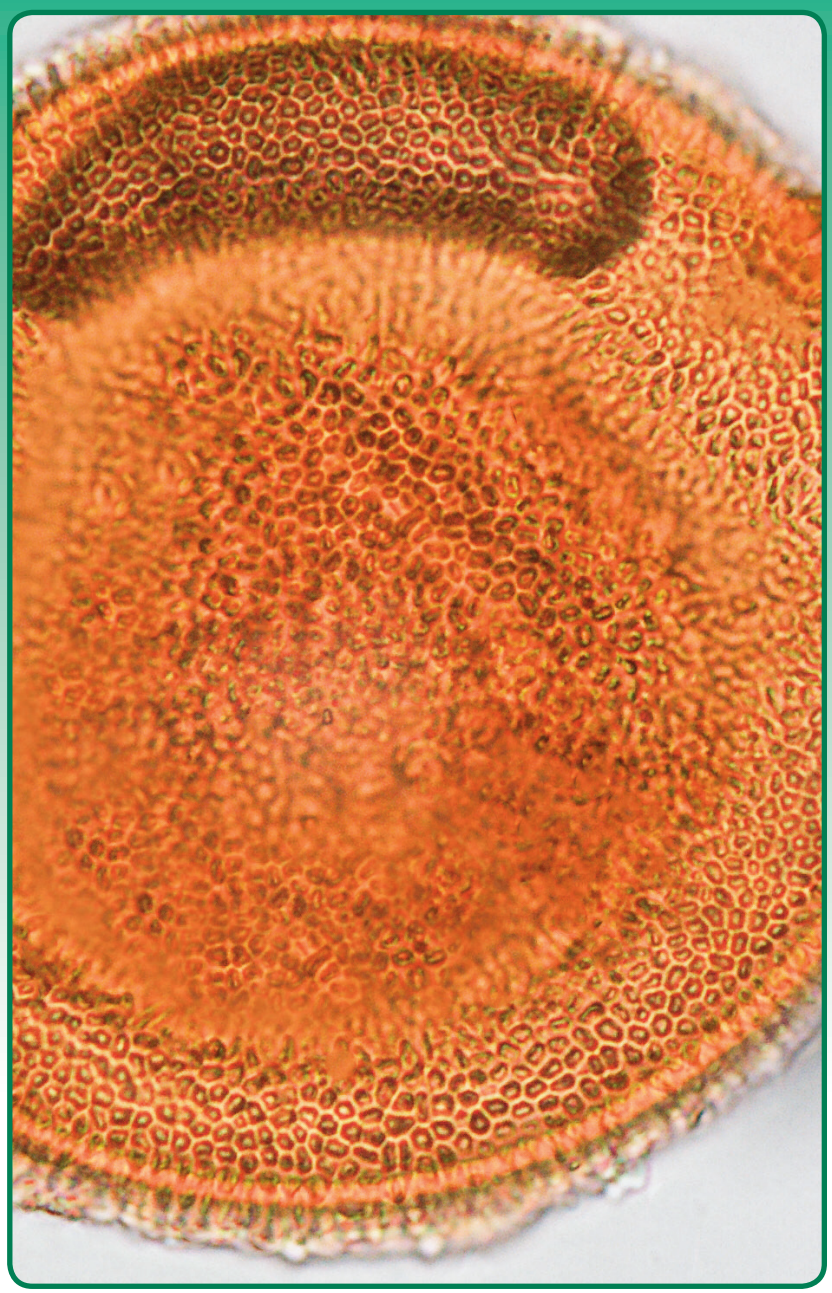
Doi: 10.15446/agron.colomb

VOLUME XXXVII, No. 3 SEPTEMBER-DECEMBER 2019 ISSN 0120-9965

Tarifa Postal Reducida No. 2015-404 4-72 La Red Postal de Colombia, vence 31 de Dic. 2019







Centro Editorial
Facultad de Ciencias Agrarias
Sede Bogotá

UNIVERSIDAD NACIONAL DE COLOMBIA

AGRONOMIA COLOMBIANA

VOLUME XXXVII | No. 3 | SEPTEMBER-DECEMBER 2019 | 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

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)

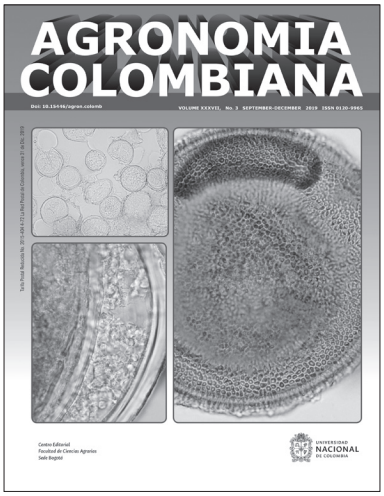
Published: Triannual
Number of copies: 20

Editorial assistance
César Albarracín
Juan Carlos Anduckia Ávila
Ana María Díaz
Luz Grass Bernal
Stanislav Magnitskiy

Translator and English proofreader
Thomas Defler
César Cabra

Electronic Design and Development
Isabel Sandoval

Printer
Digiprint



Our cover:

Diversity of arbuscular mycorrhizal fungi communities associated with cape gooseberry (*Physalis peruviana* L.) crops
Article on pages: 239-254

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.

AGRONOMIA COLOMBIANA

VOLUME XXXVII | No. 3 | SEPTEMBER-DECEMBER 2019 | ISSN (print): 0120-9965 / ISSN (online): 2357-3732

TABLE OF CONTENTS

Effect of nitrogen and potassium on plant height and stem diameter of *Jatropha curcas* L. in Colombian tropical dry forest

Efecto del nitrógeno y el potasio en la altura de la planta y el diámetro del tallo de *Jatropha curcas* L. en el bosque seco tropical de Colombia

Omar Montenegro[1], Stanislav Magnitskiy[2]*, and Aquiles Darghan[2]

ABSTRACT

The use of raw materials of *Jatropha curcas* L. to produce biofuel is of increasing interest in Colombia; little information is available on this species as a crop. This research evaluated plant height (Hp) and basal stem diameter (BSD) of *J. curcas* as affected by different rates of nitrogen (N) and potassium (K) during the first 435 days of growth (DAP). The experiment involved a repeated measures design with inter-subject factors of fertilization (N dose, K_2O dose nested in N dose) and an intra-subject factor of evaluation time. The Hp varied both as a function of N dose and the K_2O dose nested in the N dose. The tallest plants were obtained with 150 kg ha^{-1} N and 180 kg ha^{-1} K_2O treatment. With simultaneous confidence intervals of Bonferroni, we compared the evaluation times indicating for Hp non-overlapping intervals at 435 DAP (150 kg ha^{-1} N and remaining levels of this factor) and at 255 DAP (180 kg ha^{-1} K_2O and remaining levels of this factor). The application of N fertilizers resulted in thicker stems as compared to control plants. Mathematical predictive models were obtained for Hp and BSD in *J. curcas* using a multiple regression analysis. These models permitted future rapid and non-invasive predictions for *J. curcas* growth in the field.

Key words: growth, modeling, macronutrients, mineral nutrition, Euphorbiaceae.

RESUMEN

El uso de materias primas vegetales de *Jatropha curcas* L. para producir biocombustibles es de creciente interés en Colombia, mientras que hay poca información disponible sobre esta especie como cultivo. Este estudio evaluó la altura de la planta (Hp) y el diámetro del tallo basal (BSD) de *J. curcas* según lo afectado por las diferentes dosis de nitrógeno (N) y potasio (K) durante los primeros 435 días de crecimiento (DAP). El experimento incluyó un diseño de medidas repetidas con factores de fertilización entre sujetos (dosis de N, dosis de K_2O anidadas en la dosis de N) y un factor de tiempo de evaluación. La Hp varió tanto en función de la dosis de N como de la dosis de K_2O anidada en la dosis de N, obteniéndose las plantas más altas con aplicación de 150 kg ha^{-1} N y 180 kg ha^{-1} K_2O. Con los intervalos de confianza simultáneos de Bonferroni, se compararon los tiempos de evaluación indicando para Hp intervalos no superpuestos a 435 DAP (150 kg ha^{-1} N y niveles restantes de este factor) y a 255 DAP (180 kg ha^{-1} K_2O y el resto niveles de este factor). La aplicación de fertilizantes N dio como resultado tallos más gruesos en comparación con las plantas de control. Los modelos de predicción matemática se obtuvieron para Hp y BSD en *J. curcas* utilizando un análisis de regresión múltiple. Estos modelos permiten en futuro predicciones rápidas y no invasivas del crecimiento de *J. curcas* en campo.

Palabras clave: crecimiento, modelación, macronutrientes, nutrición mineral, Euphorbiaceae.

Introduction

In the search for sustainable sources of energy, plant materials for the production of biofuel have recently attracted attention. *Jatropha curcas* (Euphorbiaceae) produces seeds containing 27-43% inedible oil that can be converted into biodiesel (Chhetri *et al*., 2008). This shrub adapts easily to various tropical climatic conditions and can grow on marginal lands of low fertility and does not compete with traditional crops (Matos *et al*., 2014). These characteristics extend perspectives for the plant's cultivation in northwestern South America. However, these features (low requirements in mineral nutrients and water, low labor demand, and tolerance for salinity, pests and diseases) were recorded for genotypes from the wild. Little information on these characteristics is available in the field under Colombian conditions (Campuzano, 2008; Arévalo *et al*., 2011).

Received for publication: 28 February, 2019. Accepted for publication: 14 October, 2019 Doi: 10.15446/agron.colomb.v37n3.78172

[1] Corporación Colombiana de Investigación Agropecuaria AGROSAVIA, Nataima, El Espinal, Tolima (Colombia).
[2] Facultad de Ciencias Agrarias, Universidad Nacional de Colombia, Bogota (Colombia).
* Corresponding author: svmagnitskiy@unal.edu.co

One of the factors that limits growth and dry mass production in *J. curcas* is a deficiency of macronutrients in the soil (Silva *et al*., 2009; Ahmed *et al*., 2016). Fertilization that is nitrogen-poor could severely diminish biomass production in *J. curcas,* reducing plant height and affecting leaf formation (Wang *et al*., 2011). Mohapatra and Panda (2011) found that growth and yield of *J. curcas* in poor soils were highly influenced by the application of NPK fertilizers. According to Kalannavar *et al*. (2009), seed yield in *J. curcas* treated with 100-100-150 kg ha^{-1} per year of N-P_2O_5-K_2O increases 4.5-fold, reaching 3,937 kg seeds ha^{-1} per year as compared to absolute control without fertilization (875 kg ha^{-1} per year). Also, Patil and Parameshwarappa (2007) report significant increases in plant height, basal stem diameter, number of branches, and a respective seed yield of 1,475 kg ha^{-1} per year following the application of 80-80-80 kg ha^{-1} N-P_2O_5-K_2O. In Thailand, a significant increase in seed yield up to 1,559 kg ha^{-1} per year was achieved in plants of 75 cm height and fertilized with 15-15-15 N-P_2O_5-K_2O at a rate of 312.5 kg ha^{-1} (Suriharn *et al*., 2011). Several studies carried out in Brazil emphasize the need for N fertilization in *J. curcas*, mainly due to the high losses of N caused by its volatilization and leaching in tropical soils (Collier *et al*., 2006; Lara and Souza, 2008).

Plant height (Hp) and basal stem diameter (BSD) are used in *J. curcas* to estimate plant biomass as reported by Makungwa *et al*. (2013), who obtained an allometric model to estimate woody biomass using BSD under field conditions. These authors do not recommend a generalized application of models since these are determined for each specific site and, as far as possible, according to the authors should be applied on fully developed trees. In the wild this species can reach a height of more than 5 m with stems of a sympodial growth, since several stems produce branches close to the soil surface (Tigere *et al*., 2006). Bártoli (2008) states that a *J. curcas* crop should be pruned to a maximum of 2.5 m height to induce lateral branching. This increases the number of fruits per plant, facilitates control of pests and weeds, and provides easy harvesting by gaining access to all sectors of the crop canopy. According to de Lima *et al*. (2016), *J. curcas* tends to increase height at high sowing densities due to interspecific competition, a condition that accelerates plant development and fruit production in this species. The same authors obtain the minimum seed yield per plant at the highest sowing density (2,500 plants ha^{-1}). In this context, plant height is determined by agronomic practices that influence growth and dry mass allocation and in *J. curcas* has a direct influence on crop yield (Tjeuw *et al*., 2015).

In Colombia, there are few studies on *J. curcas* growth. Pedraza and Cayón (2010) evaluate environmental effects on growth and plant morphometry of *J. curcas* (Brazil ecotypes) in the provinces of Vichada and Santander. The plants at these locations did not significantly differ in height or number of secondary stems per plant; however, leaf dry weight and leaf area vary according to leaf strata (Pedraza and Cayón, 2010). These results suggest that *J. curcas* could increase leaf area to intercept more solar radiation, an aspect reported in other woody species of C3 photosynthesis (Larcher, 2003).

More than 600,000 ha of land in Colombia are considered to be appropriate for the cultivation of *J. curcas*, with the upper Magdalena region of the country exhibiting high potential for crop production (Campuzano, 2008). The growth response of *J. curcas* to fertilizer treatments should be studied with emphasis on arid soils of low fertility, such as the ones present in this region. The objective of the present study was to evaluate the effect of N and K rates on Hp and BSD of *J. curcas* under agroclimatic conditions of Colombian tropical dry forests in order to contribute to agronomic practices that would allow predictable levels of productivity.

Materials and methods

Experimental site and plant material

The study was conducted between March 2012 and August 2013 beginning from planting at day one to day 435 of field growth. The research took place at the Nataima Experimental Center, Colombian Corporation of Agricultural Research (AGROSAVIA) located in Espinal (Tolima province, Colombia). The crop was established in a tropical dry forest (bs-T) zone according to Holdridge (1967). The area was located at coordinates 4°11'14" N and 74°57'22" W at 371 m a.s.l. and characterized by mean 28°C air temperature, 70% relative air humidity, and 1,270 mm annual precipitation (IDEAM, 2017).

Jatropha curcas seeds of elite ecotype M-3 were obtained from the "Genetic Improvement Program of *Jatropha curcas*" of Corpoica 2006-2011. The seeds were planted in black plastic bags with a 4 kg capacity and were placed under a plastic mesh providing 60% shading. The substrate was a 2:1:1 mixture (v/v/v) of sandy loam soil: rice husk 60% burnt: compost. The substrate had pH 5.5, 4.1% organic matter, and available contents of 67.7 mg kg^{-1} phosphorus and 0.55 $cmol^{+}$ kg^{-1} potassium.

Sprinkler irrigation was applied twice a week and seedlings emerged 4 d after planting (DAP). Eight days later, the plastic mesh was removed and seedlings were left free to solar exposure. At 45 DAP the plants had between 5 and 6 true leaves and a height of 35 cm and were transplanted into a field during the rainy season that was characterized by a mean air temperature of 28°C and a mean precipitation rate of 80 mm m^{-2}. From May 2012 to July 2013, the mean air temperature was 27.8°C and relative air humidity oscillated between 70 and 85%. In June-August 2012, the experimental area had supplementary irrigation to avoid a water deficit balance. During 2013, the dry season had low precipitation during January and February (28.9°C mean air temperature and 65.3 mm m^{-2} monthly precipitation) and a rainy period during March-May had a mean air temperature of 27.0°C and a monthly precipitation of 160.4 mm m^{-2}.

Plant establishment in the field and fertilizer treatments

The planting distance in the field was 3x2 m which is considered adequate for monoculture of *J. curcas* for providing high yields, reducing intraspecific competition, and facilitating fruit harvest and weed control (Ghosh *et al*., 2007). A randomized complete block design was established with 4 replicates and 24 plants per experimental unit, separated by a border row, for a total of 1,184 plants ha^{-1}. The experiment was carried out in an inceptisol sandy loam soil identified as Typic Haplusterts with 1.2% organic matter (Tab. 1) and good drainage capacity.

Considering the soil characteristics and responses to fertilizer applications reported for *J. curcas* in earlier studies (Patil and Parameshwarappa, 2007; Kalannavar *et al*., 2009; Suriharn *et al*., 2011), the doses of fertilizers were established between 0 and 150 kg ha^{-1} per year for N and between 0 and 180 kg ha^{-1} per year for K_2O. These doses were arranged in a scheme comprising 12 treatments including control treatments with either N or K applied and an absolute control without NK application (Tab. 2). The sources of fertilizers were urea and potassium chloride. Other mineral elements were applied pre-transplant in equal doses for all treatments: 46 kg ha^{-1} P_2O_5, 44 kg ha^{-1} Ca, 18.8 kg ha^{-1} Mg, 27.2 kg ha^{-1} S, 3.18 kg ha^{-1} Mn, 2.4 kg ha^{-1} Zn, and 1 kg ha^{-1} B.

The annual doses of fertilizers in each treatment were fractioned into four applications starting one month after transplanting; fertilizers were applied evenly around the stem and covered with a soil layer of approximately 1 cm. Weed control was accomplished manually and complemented with a tractor-operated drag scraper between the rows. No pruning was performed on plants during the development of the experiment.

Plant sampling

Five destructive plant samplings (45 d after each fertilization) were carried out at 75, 165, 255, 345, and 435 DAP. Four plants were evaluated in each treatment for a total of 48 plants at each sampling moment. The plants were cut at soil level and plant height (Hp) was measured (cm) from the stem base at soil level to the apex of the principal orthotropic stem. Basal stem diameter (BSD) was measured at the base of the principal stem with an electronic digital caliper (Mitutoyo Absolute Digital Caliper 500-197-20, Japan).

Data analysis

Statistical analyses involved descriptive and inferential components using bivariate and univariate approaches. Initially, descriptive statistics (mean, standard error of mean, and standard deviation) for Hp and BSD were applied for each treatment and each moment of evaluation associated with factors of N and K_2O doses. A three-dimensional dispersion diagram visualized Hp and BSD at each evaluation time for two contrasting treatments, absolute control and a treatment combining high rates of N (150 kg ha^{-1}) and K_2O (120 kg ha^{-1}). The experiment involved a repeated measures design with inter-subject factors of fertilization rates (K_2O dose was nested in N dose due to the origin

TABLE 1. Chemical characteristics of Ap horizon of soil at the experimental site.

Horizon	pH	Organic matter (%)	P	S	Exchangeable cations				CEC	Micronutrients				
					Ca	Mg	K	Na		Fe	Cu	Mn	Zn	B
			(mg kg^{-1})		($cmol^+$ kg^{-1})					(mg kg^{-1})				
Ap (0-18 cm)	6.3	1.21	39.8	2.6	4.7	1.12	0.24	0.13	6.19	36.0	1.27	2.6	0.14	0.2

TABLE 2. Combination of N and K_2O doses in fertilizer treatments.

	N (kg ha^{-1})											
	0		50			100				150		
K_2O (kg ha^{-1})	0	120	60	120	180	0	60	120	180	60	120	180

of the K_2O dose, so that different levels of the K_2O factor shared a single level of the N factor) and an intra-subject factor of evaluation time (75, 165, 255, 345, and 435 DAP) (Davis, 2002; Montgomery, 2013). The Hp and BSD were used as response variables. A bivariate analysis of variance was applied to adjusted linear model. *P* values less than 5% were considered significant but the effects with significance less than 10% were taken into account; this was done in order not to rule out any effect that might be significant in the model. With simultaneous confidence intervals of Bonferroni, the evaluation moments were compared using error bars, since it is usual in growth models to search for significant interactions between inter-subject factors and evaluation time. Comparisons using error bars were made on the response profiles for each factor (Cumming *et al*., 2007). To complement the descriptive analysis, bar diagrams for Hp and BSD were constructed at the end of the evaluation period (435 DAP), combining factors to generate a single label of "treatments". This was done to obtain information on the behavior of 12 treatments (Tab. 2) at the end of the experiment for each response variable separately; in this case, the Tukey's test ($P<0.05$) was applied to compare the mean values. Finally, a multiple regression model was fitted for each response variable (Hp, BSD) using as predictors the N and of K_2O doses and the moments of evaluation; this was done only by predictive interest and not to compare the effects. With this, while setting the evaluation time to 435 d, a cross-table was generated to present the estimates of combinations of N and K_2O doses that were not originally used in the experiment.

Results and discussion

Effects of N and K applications on plant height and stem diameter

The vegetative growth phase of *J. curcas* in the field comprised 165 DAP indicating the moment at which the first flowering appeared. Mean Hp of *J. curcas* varied significantly between absolute control and the treatments with joint application of high N doses (100 or 150 kg ha^{-1}) and K_2O (120 or 180 kg ha^{-1}) at 75, 165, 255, and 345 DAP, while at 435 DAP these differences were not observed (Tab. 3). The BSD had minimum values in plants deprived of N fertilization throughout the experiment (Tab. 3). For both Hp and BSD variables, their behavior against NK rates presented significant differences at 435 DAP, in which the absolute control had the lowest Hp and BSD (Fig. 1). In the final evaluation, the plants treated with the highest doses of N and K_2O had maximum Hp and BSD compared to absolute control (Fig. 1).

A three-dimensional diagram indicated the simultaneous relationship between variables Hp and BSD as a function of fertilizer rates and moments of evaluation (Fig. 2). Two treatments contrasting in Hp and BSD behavior, such as absolute control and N=150 kg ha^{-1} plus K_2O=120 kg ha^{-1} (Tab. 3 and Fig. 1), were used in the diagram. It showed progressive increases in each variable in time and confirmed that plants were taller and thicker when treated with NK, while the lowest Hp and BSD were observed in absolute control without NK fertilization (Fig. 2). The

TABLE 3. Average plant heights (Hp, cm) and basal stem diameters (BSD, cm) of *J. curcas* at five evaluation moments.

Dose N+K_2O kg ha^{-1}	Days after planting (DAP)									
	75		165		255		345		435	
	Hp±SD	BSD±SD	Hp±SD	BSD±SD	Hp±SD	BSD±SD	Hp±SD	BSD±SD	Hp±SD	BSD±SD
50+60	52.0 ±6.5abc	2.4±0.1 ab	121.5±12.3 ab	5.1±0.2 a	184.5±10.8 cde	8.7±0.7 a	230.0±7.1 abc	10.1±0.5 ab	244.0±6.4 ab	12.2±1.1 abc
50+120	53.5± 10.8abc	2.4±0.2 ab	129.8±16.0 ab	4.5±0.3 abc	189.0±2.7bcd	8.1± 0.5ab	245.3±26.1 ab	10.5± 0.4ab	266.3±15.5 ab	11.8±0.8 bc
50+180	56.3±1.0 abc	2.0±0.3 bc	126.8± 2.5ab	4.8±0.4 ab	207.3±5.7ab	7.8 ±0.3 a b	254.0±4.2 a	10.7± 0.5ab	255.0±12.9 ab	12.2±0.4 abc
100+60	61.8± 9.8ab	2.6±0.3 a	130.8±6.4 ab	4.9±0.1 ab	195.8±7.5 abcd	8.5±0.3 a	224.8± 17.5abc	10.0±0.5 ab	247.0±17.4ab	13.0±0.3 ab
100+120	59.5±5.1 ab	2.0±0.2 abc	132.0±7.3a	5.1±0.5 a	209.5±6.7 ab	8.2±0.7 a	250.0±1.6 ab	10.1±0.7 ab	248.8±13.1 ab	12.3±0.7abc
100+180	61.0±2.2 ab	2.0±0.2 bc	133.0±4.8 a	4.6±0.3 abc	204.0 ±5.7 abc	7.9± 0.4ab	241.5±16.4 ab	10.5±0.7 ab	263.8±37.7 ab	11.7±0.5 bc
150+60	62.0±4.6 ab	1.9±0.3bc	129.8± 5.1ab	4.8±0.5 ab	205.3± 18.0abc	8.3±0.4 a	256.3± 13.1a	10.9± 0.6ab	274.5±8.8 a	11.3± 0.4cd
150+120	65.0±5.8 a	1.9±0.1 bc	134.3± 3.3a	4.8± 0.2ab	191.0±8.4 bcd	8.1±0.6 ab	244.3±14.4 ab	11.1±0.4 a	263.5±8.5 ab	12.4±0.5abc
150+180	65.5±3.9 a	1.9±0.1bc	136.0 ±2.2a	4.8±0.3 ab	213.8±7.1 a	8.4±0.3 a	247.0±18.6 ab	10.6±0.4 ab	267.5±41.7 ab	13.4±0.3 a
0+120	49.5±3.1 bc	2.02±0.1 bc	119.5±6.9 a	4.2±0.1 bc	180.3±7.5 de	7.0±0.2 b	220.0± 3.6bc	9.8±0.7 bc	220.5±20.2 b	10.2±0.5 d
100+0	58.3±2.2 ab	2.2± 0.1ab	122.0±7.6 ab	4.8±0.4 ab	195.0±10.5 abcd	8.6±0.4 a	233.3±8.5 abc	10.5±0.5 ab	252.8±13.5 ab	13.2±0.4 ab
0+0	43.8±2.2 c	1.6±0.3 c	110.0±13.2 b	3.8±0.3 c	165.3±4.9 e	7.1± 0.3b	208.0±7.6c	8.7±0.1 c	221.8±14.3 b	10.2±0.4 d

Hp - Plant height (cm), BSD - Basal stem diameter (cm), SD - standard deviation. Letters following standard deviations of each treatment identify the homogeneous groups generated from multiple comparisons (Tukey's test, $P<0.05$).

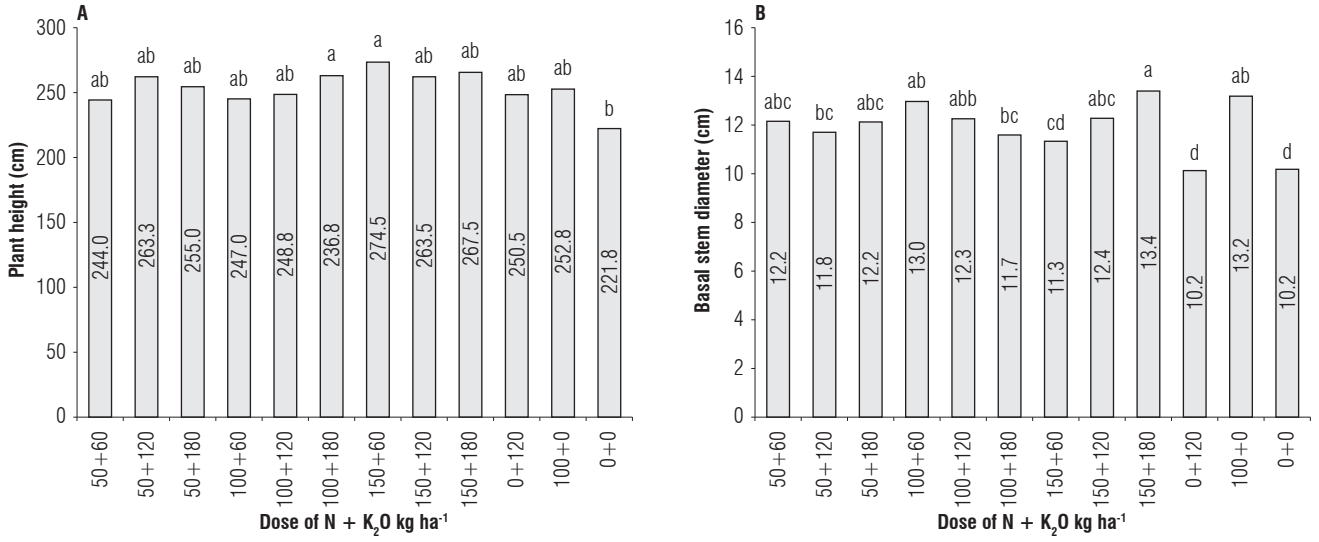


FIGURE 1. Plant height (A) and basal stem diameter (B) of *J. curcas* at 435 DAP treated with different doses of N and K_2O (Tukey's test, $P<0.05$).

highest dispersion in variables Hp and BSD occurred in the reproductive phase of growth at 345 and 435 DAP. Both variables possessed a similar growth pattern over time, as shown by Reis *et al*. (2018), but stood out in the treatment with the highest rate of N (150 kg ha^{-1}) at 345 and 435 DAP. The diagram of dispersion did not reflect a trend, since the variable "treatments" was of nominal scale; however, an increasing trend in time for Hp and BSD could be observed for both treatments (Fig. 2).

To evaluate the effect of N and of K_2O doses nested in N (K_2O(N)) on Hp and BSD at different moments of growth, Wilks' Lambda statistics was used. It revealed the interactions of second and third order, both significant ($P<0.05$) and highly significant ($P<0.01$) for the main effects associated with doses of N, doses of K_2O nested in N, and evaluation moments (Tab. 4). In order to use the results of the bivariate analysis of variance, it was necessary to recognize the presence of significant interactions; these interactions made it impossible to interpret the main effects (Tab. 4), so that one could appeal for the graphs of interaction (Sokal and Rohlf, 2012). Table 4 presents the summary of the two-way ANOVA of repeated measures design of some of the effects. It highlights the interactions and justifies the use of interaction profiles to select the best N doses at each evaluation time. Table 4 shows the effects of the reduced model eliminating effects with a significance level greater than 5%. The values close to 5% were left in the model, although they were higher than this limit. In double interactions of inter-subject factors with "time", the effect of K_2O(N) could be interpreted,

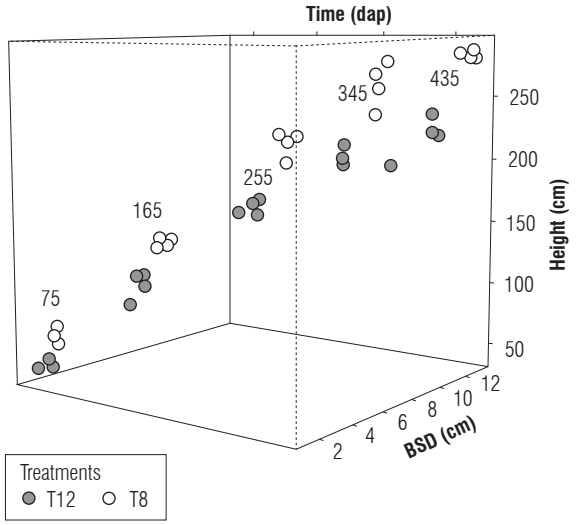


FIGURE 2. Three-dimensional dispersion diagram of plant height (cm) and basal stem diameter (cm) according to the moment of evaluation (DAP) and N+K_2O treatments (kg ha^{-1}). T8 denotes the treatment N=150 kg ha^{-1} plus K_2O=120 kg ha^{-1} and T12 corresponds to absolute control.

since the interaction was not significant ($P=0.0589$) (Tab. 4). In the absence of a significant interaction between "time" and "dose of K_2O nested in N", the values of Hp and BSD at 435 DAP corresponded to 266.7 and 12.2 cm, respectively, for 180 kg ha^{-1} K_2O nested in different N doses, while control plants had Hp of 239.1 cm and BSD of 11.2 cm. As for the effect of N, the interaction of "N dose" with "time" was revealed. This makes it impossible to select the best dose of N for all moments, so that the use of profiles facilitated choosing the best dose at each evaluation time (Figs. 3 and 4).

TABLE 4. Significance levels for individual effects and interactions in bivariate analysis of variance.

Effect	Pr<F
Responses (Hp; BSD)	<.0001
Responses (Hp; BSD) x (N)	<.0001
Responses (Hp; BSD) x K_2O(N)	0.0017
Time	<.0001
Time x N	<.0001
Time x K_2O(N)	0.0573
Responses (Hp; BSD) x Time	<.0001
Responses (Hp; BSD) x Time x N	<.0001
Responses (Hp; BSD) x Time x K_2O(N)	0.0589

Hp - plant height (cm); BSD - basal stem diameter (cm); N - dose of N; K_2O(N) - dose of K_2O nested in dose of N. The effects, whose significance was higher than 5%, were not considered. The effects close to 5% significance were left in the model, although being higher than this limit.

The behavior of Hp discriminated by moments of evaluation (DAP), dose of N, and dose of K_2O nested in N confirmed the major increases in plant height of *J. curcas* during the vegetative phase between 75 and 255 DAP (Fig. 3), a period when plants allocated most of their carbon resources to new branches and leaves. According to the slopes of the curves, the gain in height of 0.78 cm/day was observed for plants treated with N=150 kg ha^{-1} plus K_2O=180 kg ha^{-1}, while the absolute control gained 0.71 cm/day in height (Fig. 3A). A slower growth was detected during the reproductive phase between 345 and 435 DAP, probably due to translocation of nutrients from leaves, branches, and stem towards flowers and fruits (Larcher, 2003). The plants gained only 0.35 cm/day in height in

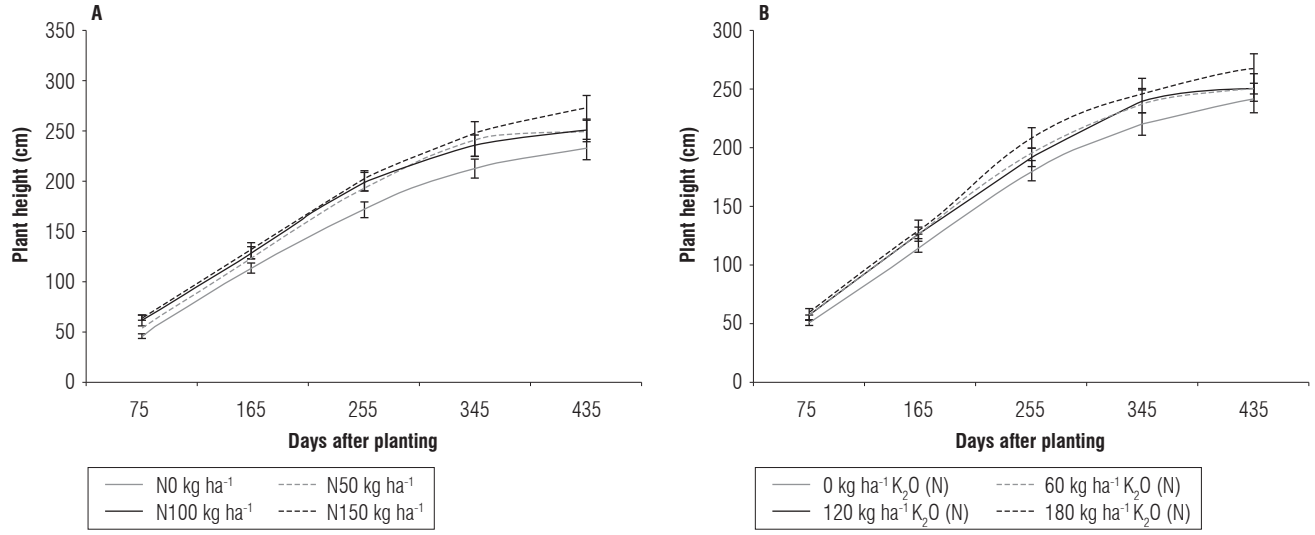


FIGURE 3. Plant height (cm) of *J. curcas* affected by different doses of N (**A**) and doses of K_2O nested in N (**B**). The bars correspond to standard errors of the means.

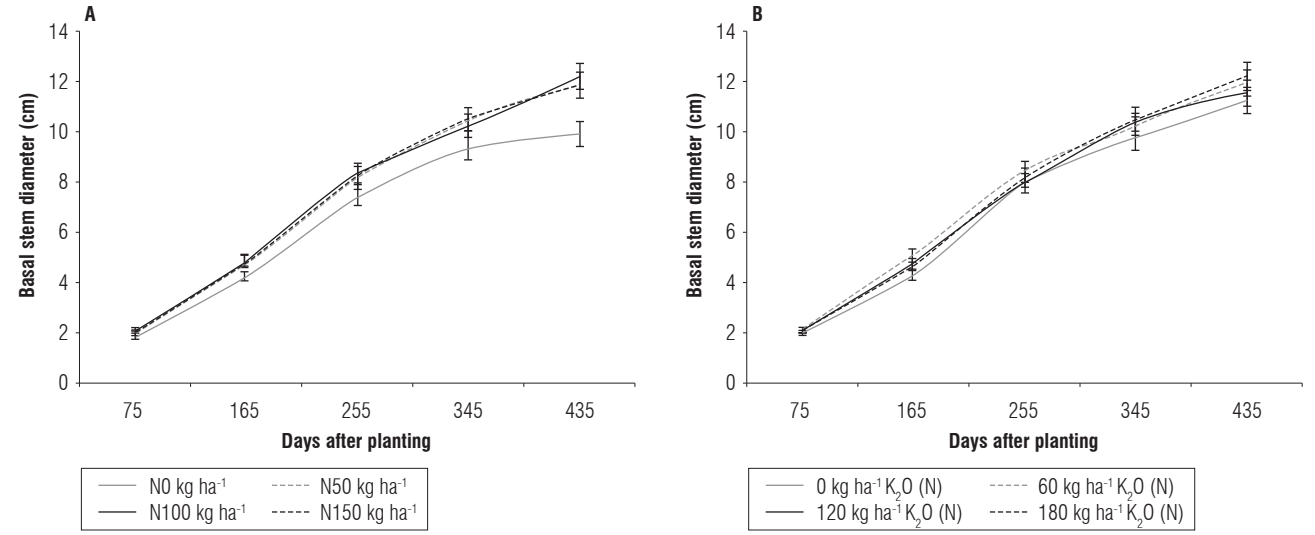


FIGURE 4. Basal stem diameter (cm) of *J. curcas* affected by different doses of N (**A**) and doses of K_2O nested in N (**B**). The bars correspond to standard errors of the means.

this period when treated with 150 kg ha^{-1} N and 180 kg ha^{-1} K_2O and only 0.17 cm/day in control plants without NK fertilization (Fig. 3A). The plants were taller in all treatments with N application presenting the highest Hp at all moments of the evaluation and reaching 285 cm at 435 DAP when treated with 150 kg ha^{-1} N. In contrast, the lowest heights of 228 cm corresponded to the treatments without N (Fig. 3A), which agrees with Su *et al*. (2013), who reports increases in height, stem diameter, and total number of branches in *J. curcas* as the doses of 15-15-15 NPK fertilizer increase. However, very high doses of N could prolong the vegetative phase of growth, thus, delaying the start of fruit production (Larcher, 2003). Likewise, increases in height above 2.5 m might affect agronomic practices in *J. curcas* by requiring pruning and obstructing crop harvest, since fertilization could affect branching in *J. curcas* (Nirala *et al*., 2017). When analyzing the effect of the K_2O dose nested in N (K_2O(N)) on Hp, a similar trend was observed, with the largest heights (267 cm) obtained with application of 180 kg ha^{-1} K_2O for the average N doses, whereas the lowest heights (239 cm) corresponded to control plants without K application (Fig. 3B).

The plants were not significantly different in BSD during the first two evaluation moments according to values of standard errors (Fig. 4). It should be noted that BSD increased progressively in time. Thus, starting from 165 DAP all plants treated with NK differed in BSD from the absolute control; however, no differences in BSD were detected among the plants treated with non-zero doses of N or K (Fig. 4A). A similar result in BSD was observed affected by K_2O dose nested in N but with fewer differences, with smaller BSD corresponding to control plants without K fertilization (Fig. 4B).

Multiple regression models for prediction of plant height and stem diameter

Multiple regression models for Hp and BSD were developed. These permitted predicting responses according to the levels of each factor and the development of two formulae as presented in Table 5. The models served to assess untested levels of factors (dose of N or dose of K_2O or moment of evaluation) fitting these within their range of operation (an example highlighted in Table 6). According to the multiple linear regression analysis, the behavior of Hp fit this model well and explained 92.99% of the variability and, analogously, the model for BSD adjusted to 94.68% of the variability. These mathematical models allow estimating Hp and BSD for any dose of N and K_2O at five evaluation moments within the evaluated range (Tab. 5). Therefore, N and K fertilization rates can be adjusted to a desired plant height and, thereby, they can be used to schedule some agronomic labors, such as pruning. In *J. curcas*, Hp and BSD could be further used to estimate the plant biomass (Makungwa *et al*., 2013).

Hp had a highly significant (P<5%) correlation with BSD. As the doses of N and K increased, both Hp and BSD increased progressively, an aspect observed for all moments of evaluation. These allometric relationships vary considerably by species (Clough and Scott, 1989), but are especially important for trees (Lott *et al*., 2000; Wang *et al*., 2000; Salis *et al*., 2006; Levia, 2008), where stem diameter is used to calculate some parameters, such as plant height and biomass, an aspect that could be used in *J. curcas*. In this sense, tree growth is supported by the trunk and must be biomechanically balanced (Montagu *et al*., 2005); in other words, stem diameter is related to the length and distribution of branches and should prevent uprooting or branch split. In *J. curcas*, stem diameter and number of branches (Laviola *et al*., 2012) as well as plant height (Laviola *et al*., 2012; Shabanimofrad *et al*., 2013; De Lima *et al*., 2016) were shown to correlate positively with seed yield per plant.

Employing the models obtained, the values of Hp and BSD for *J. curcas* at 435 DAP were estimated for different levels of N and K fertilizers. Also, randomly chosen and experimentally untested doses (N=75 kg ha^{-1} and K_2O=90 kg ha^{-1}) were introduced within the operational ranges of each dose to verify the predictive behavior of the models (Tab. 6).

TABLE 5. Multiple regression models for the prediction of plant height and basal stem diameter of *J. curcas* as a function of N dose (kg ha^{-1}), K_2O dose (kg ha^{-1}), and moment of evaluation (DAP).

Response	Model	R^2
$\hat{Y}_1$: Hp	$\hat{Y}_1 = 8.71659 + 0.07722 \times K_2O + 0.17786 \times N + 0.56276 \times t$	0.93
$\hat{Y}_2$: BSD	$\hat{Y}_2 = -0.60192 + 0.00232 \times K_2O + 0.00803 \times N + 0.02738 \times t$	0.95

Range of settings of prediction models: $0 \leq K_2O \leq 180$, $0 \leq N \leq 150$, $75 \leq t \leq 435$, where K_2O - dose of K_2O (kg ha^{-1}), N - dose of N (kg ha^{-1}), t - time (DAP). $\hat{Y}$ corresponds to estimated model and R^2 denotes the adjusted coefficient of determination.

TABLE 6. Plant height (cm) and basal stem diameter (cm) of *J. curcas* estimated at 435 DAP with different combinations of N and K_2O doses including a dose not tested experimentally (highlighted).

K_2O (kg ha^{-1}) N (kg ha^{-1})	Variables	0	60	(90)	120	180
0	Hp	253.52	258.15	(260.47)	262.78	267.42
	BSD	11.31	11.44	(11.52)	11.58	11.73
50	Hp	262.41	267.04	(269.36)	271.68	276.31
	BSD	11.70	11.84	(11.91)	11.98	12.12
(75)	Hp	(266.86)	(271.49)	(273.81)	276.12	280.76
	BSD	(11.91)	(12.04)	(12.11)	12.18	12.32
100	Hp	271.30	275.94	278.25	280.57	285.20
	BSD	12.11	12.25	12.32	12.38	12.52
150	Hp	280.20	284.83	287.15	289.46	294.10
	BSD	12.51	12.65	12.72	12.79	12.93

The values estimated by the models for both evaluated doses and untested doses experimentally showed a clear increase in Hp and BSD for *J. curcas* with increasing doses of N and/or K fertilizer (Tab. 6). Therefore, these models could be applied for easy, rapid and non-invasive predictions of *J. curcas* growth in the field for the region.

Conclusions

The growth of *J. curcas* was influenced by doses of N and doses of K_2O nested in N during the vegetative and reproductive phases. The tallest plants with the largest stem diameters were those subjected to the highest rates of N and K fertilizers. For the management of fertilization of *J. curcas* in the given edaphoclimatic conditions of Colombia, doses of 100 kg ha^{-1} N and 120 kg ha^{-1} K_2O can be recommended. Both basal stem diameter and plant height in *J. curcas* increased proportionally during growth. The predictive models for plant height and basal stem diameter integrated rates of N, rates of K_2O, and time had an adjusted coefficient of determination R^2 over 92%. The fertilizer rates N=75 kg ha^{-1} and K_2O=90 kg ha^{-1} were not evaluated in the field, but they fitted well within the operational ranges of the models and data generated were consistent with the behavioral tendencies of the studied variables. The models obtained could be used for rapid and non-invasive predictions of *J. curcas* growth in the field.

Acknowledgments

The authors thank the Colombian Corporation for Agricultural Research (AGROSAVIA), the Directorate of the Nataima Research Center, and the Faculty of Agricultural Sciences of the Universidad Nacional de Colombia for facilitating the development of this research. The authors greatly acknowledge the help of Dr. Martha Cecilia Henao (Faculty of Agricultural Sciences, Universidad Nacional de Colombia) for guiding the research. Likewise, we acknowledge the help of Dr. Eduardo Barragán Quijano and Dr. Fernando Campuzano Duque for the financial support through the Transitional Crop Programs and the National Agroenergy Program of AGROSAVIA. Our gratitude is extended to Mr. Gentil García Prada for his support in the agronomic management and data collection during this study.

Literature cited

Ahmed, D.M.M., A.R.M. Yousef, E.A.M. Ali, and M.A. El-Hady. 2016. Potassium forms as a macronutrient application to maximize fruit and oil productivity of *Jatropha curcas* (Part 1: The use of mono potassium phosphate (MKP)). Int. J. Agric. Res. 11, 116-125. Doi: 10.3923/ijar.2016.116.125

Arévalo, L.P., P.A. Méndez, and P.R. Linares. 2011. Propuesta para desarrollar una plantación forestal de la especie *Jatropha curcas* para extracción de biodiesel en el municipio de Guaduas, Cundinamarca. Revista Gestión. Integral Ingen. Neogranadina 3, 35-50.

Bártoli, J.A.A. 2008. Manual para el cultivo de Piñon (*Jatropha curcas*) en Honduras. Centro de Comunicación Agrícola de la Fundación Hondureña de Investigación Agrícola (FHIA), Cortes, Honduras.

Campuzano, L.F. 2008. Programa Jatropha Colombia: genética, agronomía, poscosecha y desarrollo agroindustrial del piñón en Colombia (2008-2012). Rev. Innov. Cambio Tecnol. 7, 64-68.

Chhetri, A.B., M.S. Tango, S.M. Budge, K.C. Watts, and M.R. Islam. 2008. Non-edible plant oils as new sources for biodiesel production. Int. J. Molec. Sci. 9, 169-180. Doi: 10.3390/ijms9020169

Clough, B.F. and K. Scott. 1989. Allometric relationships for estimating above-ground biomass in six mangrove species. Forest Ecol. Manage. 27, 117-127. Doi: 10.1016/0378-1127(89)90034-0

Collier, L.S., D.V. Castro, J.J. Dias Neto, D.R. Brito, and P.A.D.A. Ribeiro. 2006. Nitrogen fertilization management for maize

on legume straw crop with no tillage cultivation in Gurupi, Tocantins State. Cienc. Rural 36, 1100-1105. Doi: 10.1590/S0103-84782006000400009

Cumming, G., F. Fidler, L. David, and D.L. Vaux. 2007. Error bars in experimental biology. J. Cell Biol. 177, 7-11. Doi: 10.1083/jcb.200611141

Davis, C.S. 2002. Statistical methods for the analysis of repeated measurements. Springer-Verlag, New York, USA. Doi: 10.1093/ije/dyg249

De Lima, R.D., A.R. Campos, C.A. de Azevedo, J.A. Calado, S. de Sousa Silva, and R. do Nascimento. 2016. Effects of planting density on vegetative growth and production components of jatropha (*Physic nut* L). Austral. J. Crop Sci. 10, 632-636. Doi: 10.21475/ajcs.2016.10.05.p7148

Ghosh, A., J.S. Patolia, D.R. Chaudhary, J. Chikara, S.N. Rao, D. Kumar, G. Boricha, and A. Zala. 2007. Response of *Jatropha curcas* under different spacing to Jatropha de-oiled cake, pp. 26-28. In: Expert seminar on *Jatropha curcas* L. agronomy and genetics. Wageningen, The Netherlands.

Holdridge, L.R. 1967. Life zone ecology. 1st ed. Tropical Science Center, San Jose.

IDEAM. 2017. Instituto de hidrología, meteorología y estudios ambientales, Bogota.

Kalannavar, V.N., S.S. Angadi, V.C. Patil, S.G. Angadi, A.S. Byadagi, and S.J. Patil. 2009. Effect of major nutrients on growth and yield of *Jatropha curcas*. Int. J. Agric. Sci. 5, 390-393.

Lara, W.A.R. and M.A. Souza. 2008. Ammonia volatilization, leaching of nitrogen and corn yield in response to the application of mix of urea and ammonium sulphate or gypsum. Rev. Bras. Cienc. Solo 32, 2331-2342. Doi: 10.1590/S0100-06832008000600012

Larcher, W. 2003. Carbon utilization and dry matter production. In: Physiological plant ecology: ecophysiology and plant physiology of functional groups. Springer-Verlag, New York.

Laviola, B.G., A.A. Alves, F.D.L. Gurgel, T.B. Rosado, R.D. Costa, and R.B. Rocha. 2012. Estimate of genetic parameters and predicted gains with early selection of physic nut families. Cienc. Agrotec. 36, 163-170. Doi: 10.1590/S1413-70542012000200004

Levia, D.F. 2008. A generalized allometric equation to predict foliar dry weight on the basis of trunk diameter for eastern white pine (*Pinus strobus* L.). For. Ecol. Manage. 255, 1789-1792. Doi: 10.1016/j.foreco.2007.12.001

Lott, J.E., S.B. Howard, C.R. Black, and C.K. Ong. 2000. Allometric estimation of above-ground biomass and leaf area in managed *Grevillea robusta* agroforestry systems. Agrofor. Syst. 49, 1-15. Doi: 10.1023/A:1006330830109

Makungwa, S.D., A. Chittock, D.L. Skole, G.Y. Kanyama-Phiri, and I.H. Woodhouse. 2013. Allometry for biomass estimation in *Jatropha* trees planted as boundary hedge in farmers' fields. Forests 4, 218-233. Doi: 10.3390/f4020218

Matos, F.S., V.R. Rosa, L.F.O. Borges, R.P. Ribeiro, C.K.L. Crucinel, and L.A.S. Dias. 2014. Response of *Jatropha curcas* plants to changes in the availability of nitrogen and phosphorus in Oxissol. Afric. J. Agric. Res. 9, 3581-3586. Doi: 10.5897/AJAR2014.9188

Mohapatra, S. and P.K. Panda. 2011. Effects of fertilizer application on growth and yield of *Jatropha curcas* L. in an aeric tropaquept of Eastern India. Not. Sci. Biol. 3, 95-100. Doi: 10.15835/nsb.3.1.5497

Montagu, K.D., K. Düttmer, C.V.M. Barton, and A.L. Cowie. 2005. Developing general allometric relationships for regional estimates of carbon sequestration - an example using *Eucalyptus pilularis* from seven contrasting sites. For. Ecol. Manage. 204, 113-127. Doi: 10.1016/j.foreco.2004.09.003

Montgomery, D.C. 2013. Design and analysis of experiments. 8th ed. Arizona State University, John Wiley and Sons, USA.

Nirala, D.P., P. Santosh, A. Sinha, and K. Rajneesh. 2017. Effect of pruning and fertilizer application to optimize branching and fruiting in *Jatropha curcas* L. Life Sci. Leafl. 92, 42-49.

Patil, S.J. and K. G. Parameshwarappa. 2007. Silvicultural management of jatropha (*Jatropha curcas* L.) under rainfed conditions. pp. 371-372. In: National seminar on changing global vegetable oil scenario: Issues and challenges before India. ISOR, Hyderabad, India.

Pedraza, E.A. and D.G. Cayón. 2010. Caracterización morfofisiológica de *Jatropha curcas* L. variedad Brasil cultivada en dos zonas de Colombia. Acta Agron. 59, 30-36. Doi: 10.15446/acag

Reis, A.D.S., T.R.B. da Silva, J. Gonçalves, D. Secco, C.Z. Alves, and R.F. Santos. 2018. Organic and mineral fertilizer on the initial development and nutrition of jatropha. J. Agric. Sci. 10, 274-281. Doi: 10.5539/jas.v10n10p274

Salis, S.M., M.A. Assis, P.P. Mattos, and A.C.S. Pião. 2006. Estimating the aboveground biomass and wood volume of savanna woodlands in Brazil's Pantanal wetlands based on allometric correlations. For. Ecol. Manag. 228, 61-68. Doi: 10.1016/j.foreco.2006.02.025

Shabanimofrad, M., M.Y. Rafii, P.M. Wahab, A.R. Biabani, and M.A. Latif. 2013. Phenotypic, genotypic and genetic divergence found in 48 newly collected Malaysian accessions of *Jatropha curcas* L. Ind. Crop. Prod. 42, 543-551. Doi: 10.1016/j.indcrop.2012.06.023

Silva, E.B., L.P. Patente, S.R. Santos, and P.S. Resende. 2009. Sintomas visuais de deficiências nutricionais em pinhão -manso. Pesq. Agropec. Bras. 44, 392-397. Doi: 10.1590/S0100-204X2009000400009

Sokal, R.R. and F.J. Rohlf. 2012. Biometry: the principles and practice of statistics in biological research. 4th ed., Freeman, USA.

Su, L., F. Qin, T. Su, T. He, Y. Zhang, Y. Zeng, L. Hie, Y. Huang, and Y. Liu. 2013. Effects of fertilization on growth and dynamic of leaf nutrients of *Jatropha curcas*. J. Southern Agric. 44, 1517-1523.

Suriharn, B., J. Sanitchon, P. Songsri, and T. Kemala. 2011. Effect of pruning levels and fertilizer rates on yield of physic nut (*Jatropha curcas* L.). Asian J. Plant Sci. 10, 52-29. Doi: 10.3923/ajps.2011.52.59

Tigere, T.A., T.C. Gatsi, I.I. Mudita, T.J. Chikuvire, S. Thamangani, and Z. Mavunganidze. 2006. Potential of *Jatropha curcas* in improving smallholder farmers' livelihoods in Zimbabwe: an exploratory study of Makosa ward, Mutoko district. J. Sust. Dev. Africa 8, 1-9.

Tjeuw, J., M. Slingerland, and K. Giller. 2015. Relationships among *Jatropha curcas* seed yield and vegetative plant components under different management and cropping systems in Indonesia. Biomass Bioenergy 80, 128-139. Doi: 10.1016/j.biombioe.2015.05.003

Wang, J.R., T. Letchford, P. Comeau, and J.P. Kimmins. 2000. Above- and below-ground biomass and nutrient distribution of a paper birch and subalpine fir mixed-species stand in the Sub-Boreal Spruce zone of British Columbia. For. Ecol. Manage. 130, 17-26. Doi: 10.1016/S0378-1127(99)00193-0

Wang, W.G., R. Li, B. Liu, L. Li, S. H. Wang, and F. Chen. 2011. Effects of low nitrogen and drought stresses on proline synthesis of *Jatropha curcas* seedling. Acta Physiol. Plant. 33, 1591-159. Doi: 10.1007/s11738-010-0692-6

Polyphasic identification of preharvest pathologies and disorders in avocado cv. Hass

Identificación polifásica de patologías y trastornos precosecha en aguacate cv. Hass

Joaquín Guillermo Ramírez-Gil[1]* and Juan Gonzalo Morales[2]

ABSTRACT

Diseases and disorders are one of the main limitations of avocado crops for export and national markets. However, they are poorly studied in tropical countries such as Colombia. The objective of this research was to evaluate a polyphasic approach for the diagnosis of pathologies and disorders associated with avocado cv. Hass in nurseries and crop fields located in Antioquia, Colombia. Results allowed the identification of several diseases and disorders present on different tissues in all stages of plant development. The root rot disease was associated with 10 microorganisms and two abiotic disorders. Different organs and tissues of avocado plants were affected by pathogens such as *Lasiodiplodia theobromae*, *Colletotrichum gloeosporioides sensu lato,* and *Phytophthora palmivora*. Pathogens that had not been previously reported in Colombia were identified, and among them *P. palmivora*, *Fusarium oxysporum sensu lato*, *Phytopythium vexans*, *Phomopsis* sp., and *Pythium cucurbitacearum* stand out. Abiotic disorders such as hypoxia-anoxia, root atrophy, fruit sunburn, and hailstorm damage were also identified. The etiology of peduncle ringing was not identified. Polyphasic diagnosis of pathologies and disorders is an appropriate approach as part of an integrated disease management program in avocado cv. Hass crop. This work is a reference tool on basic aspects associated with the detection of disorders and pathologies in avocado and the taxonomy of the microorganisms involved.

Key words: root rot, abiotic and biotic causal agents, diseases in nurseries and field, detection techniques.

RESUMEN

Las enfermedades y los trastornos son una de las principales limitaciones de los cultivos de aguacate para la exportación y el mercado nacional. Sin embargo, estos factores limitantes son poco estudiados en países tropicales como Colombia. El objetivo de este trabajo fue evaluar un enfoque polifásico para el diagnóstico de patologías y trastornos asociados al aguacate cv. Hass en viveros y lotes en Antioquia, Colombia. Los resultados permitieron la identificación de varias enfermedades y trastornos presentes en diferentes tejidos en todas las etapas de desarrollo del cultivo. La enfermedad de la pudrición de la raíz se asoció a 10 microorganismos y dos trastornos abióticos. Diferentes órganos y tejidos de las plantas de aguacate fueron afectados por patógenos como *Lasiodiplodia theobromae*, *Colletotrichum gloeosporioides sensu lato* y *Phytophthora palmivora*. Se identificaron patógenos no reportados previamente en Colombia como *P. palmivora*, *Fusarium oxysporum sensu lato*, *Phytopythium vexans*, *Phomopsis* sp. y *Pythium cucurbitacearum*. Por otra parte, se caracterizaron trastornos abióticos como hipoxia-anoxia, la atrofia de la raíz, las quemaduras en fruta por el sol y el daño por granizo. No se identificó la etiología del anillamiento del pedúnculo. El diagnóstico polifásico de patologías y trastornos es un enfoque apropiado como parte de un programa de manejo integrado de enfermedades en el cultivo de aguacate cv. Hass. Este trabajo es una herramienta de consulta en aspectos básicos asociados a la detección de desórdenes y patologías en aguacate y la taxonomía de los microorganismos implicados.

Palabras clave: pudrición de raíces, agentes causales abióticos y bióticos, enfermedades en vivero y campo, técnicas de detección.

Introduction

The export of avocado fruit (*Persea americana* Mill) generates important commodities to several countries such as Mexico, Indonesia, The United States of America, Dominican Republic, Colombia, Chile, and Peru (FAO, 2018). Avocado crops exhibit several limiting factors when producing high quality fruit to satisfy a steady growing demand from international markets. In Colombia, a fast growth in the planted area has been observed in recent years leading to an increase on the economic profit at the agricultural sector. However, consistent issues derived from the lack of technical and scientific knowledge by some farmers related to the correct implementation of

Received for publication: 17 March, 2019. Accepted for publication: 8 August, 2019 Doi: 10.15446/agron.colomb.v37n3.78528

[1] Departamento de Agronomía, Facultad de Ciencias Agrarias, Universidad Nacional de Colombia, Bogota (Colombia).
[2] Departamento de Agronomía, Facultad de Ciencias Agrarias, Universidad Nacional de Colombia, Medellin (Colombia).
* Corresponding author: jgramireg@unal.edu.co

agronomical practices have been identified, along with the increase in the planted area. These problems include planting in flat areas susceptible to flooding and, hence, to diseases, bad phytosanitary quality of seedlings, lack of knowledge about environment, temporal and spatial factors involved in disease development, little training for correct identification and management of diseases and disorders, and shortage of certified laboratories to perform diagnostics (Tamayo, 2007; Ramírez-Gil *et al*., 2014; Ramírez-Gil *et al*., 2017; Ramírez-Gil, 2018).

Diseases are considered as important challenges to crop management because if not detected accurately and treated promptly, they may cause large economic losses depending on the cultivar and edapho-climatic conditions where the crop is grown. In addition, phytosanitary measures implemented for disease control are accompanied by adverse effects to human health and the environment (Zentmyer, 1980; Menge and Ploetz, 2003; Tamayo, 2007; Ramírez-Gil *et al*., 2014, 2017).

Root rot caused by *Phytophthora cinnamomi* Rands is considered the most important disease of avocado plantations in the world. Root rot is highly influenced by the abiotic factor hypoxia-anoxia caused by poor soil aeration, and usually by rain flooding (Stolzy *et al*., 1967; Zentmyer, 1984; Ramírez-Gil *et al*., 2014, 2017; Hardham and Blackman, 2018). In addition, other microorganisms, such as *Phytophthora heveae* Thompson., *Phytophthora parasitica* Dastur., *Verticillium* sp. Nees, *Armillaria mellea* (Vahl) P. Kumm., *Cylindrocladium* sp. Morgan, *Rosellinia* sp. De Not, *Fusarium solani* (Mart) Sacc., *Fusarium oxysporum* Schlecht., *Fusarium* sp. Link ex Grey, *Rhizoctonia* sp. DC, *Phymatotrichum omnivurum* (Duggar) Hennebert., *Cylindrocladiella* sp. Boesew, *Cylindrocarpon* sp. Wollenw, *Pythium* sp. Pringsheim, *Gliocladiopsis* sp., and the nematodes *Helicotylenchus* sp. Steiner, *Rotylenchulus* sp. Linford and Oliveira, and *Pratylenchus* sp. Filipjev, have been reported as causal agents of avocado root rot. However, their frequency and importance depend on many factors associated with the environmental and edaphic conditions of the planted areas and the agronomical management of the productive system (Tamayo, 2007; Vitale *et al*., 2012; Ramírez-Gil and Morales-Osorio, 2013; Ramírez-Gil *et al*., 2014, 2017; Parkinson *et al*., 2017).

After root rot, the most frequently identified pathologies in avocado crops are scab (*Elsinoe perseae*, (Jenkins) Rossman & W.C. Allen, synonymy = *Sphaceloma perseae*, Jenkins), anthracnose of fruits and dieback of buds and branches (*Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. Teleomorph = *Glomerella cingulata* (Stoneman) Spauld. & H. Schrenk), leaf and fruit spot (*Pseudocercospora purpurea* (Cooke) Deighton. = *Cercospora purpurea*), fruit rot (*Rhizopus stolonifer* Ehrenb. Fr Vuill. and *Dothiorella* sp.), graft rot (*Lasiodiplodia theobromae* Pat. Griffon & Maubl. = *Botryodiplodia theobromae*), algal leaf spot (*Cephaleuros virescens* Kunze), and sooty mold (*Capnodium* sp., and *Asteridiella perseae* F. Stevens Hansf.) (Zentmyer, 1984; Menge and Ploetz, 2003; Tamayo, 2007; APS, 2017; Sharma *et al*., 2017; Giblin *et al*., 2018).

In Colombia and other tropical countries, information about diagnosis of pathologies and disorders is scarce; therefore, an accurate and prompt disease identification system is not fully implemented in all growing areas. Avocado diseases have become complex to identify and difficult to manage, not only because of the economic losses they generate, but also because of the symptoms caused by the different pathogenic agents (both biotic and abiotic) that can be easily confused at plant diagnostic laboratories by technical assistants and farmers (Ramírez-Gil *et al*., 2017; Tamayo, 2007; Ramírez-Gil, 2018). Incorrect diagnostics usually lead to inappropriate management practices; for example, most root rot diseased trees are diagnosed as infected by *P. cinnamomi* and controlled accordingly. However, such management practices can be effective if that is truly the causal agent; otherwise, they not only fail to solve the problem, but the sanitary conditions of the crop worsen resulting in a rapid decay and death of trees (Ramírez-Gil *et al*., 2017; Ramírez-Gil, 2018).

Accurate and prompt identification of the causal agent of a disease is the first and more important step for an appropriate management program. Thus, effective control measures can be established optimizing resources and reducing negative effects to human health and the natural environment (Agrios, 2005). Nowadays, different techniques and aspects should be included to implement a correct process of diagnosis of plant diseases and disorders, such as field symptomatology, pathogen isolation, macroscopic and microscopic microbial morphology, pathogenicity tests, Koch postulates, and molecular sequencing; this combination of methods is named polyphasic diagnosis (Taylor *et al*., 2000; Alvarez, 2004).

The objective of this research was to establish a polyphasic approach for the identification of avocado diseases caused by biotic causal agents or abiotic disorders under standard

growing conditions of avocado cv. Hass crops in Antioquia, Colombia.

Materials and methods

Localization and sample processing

Ten commercial plots and six nurseries for production of avocado cv. Hass seedlings, located in the North, East and Southwest regions of Antioquia, Colombia, were surveyed during eight years for disease identification (2009-2016). Plot and nursery locations and their edapho-climatic conditions are reported in Supplementary material 1 and 2. Plants showing disease symptoms were recorded and photographed, and tissue samples were collected and processed for causal agent or disorder identification as described below. Each avocado plot was managed according to local agronomical practices without further technical intervention. Presence of symptoms was evaluated in nurseries in seeds, roots, stems, and foliage; in crop plots, symptoms were evaluated in roots, stems, foliage, flowers, and fruits. Sample processing and pathogenicity tests were performed at the Laboratorio de Fitotecnia Tropical at Universidad Nacional de Colombia, Medellin campus.

Polyphasic diagnosis of avocado cv. Hass diseases and disorders

Symptom description

Plants showing any disease symptoms in nurseries and in crop fields were photographed and registered for eight years. Each symptom associated with a particular disease was described during the complete duration of the pathology or disorder.

Isolation and morphological characterization of microorganisms associated with symptomatic plants

Samples were collected from each plant showing visible symptoms potentially associated with pathogens. Afterwards, samples were covered with paper towels, placed in zip-pack plastic bags in Styrofoam containers and transported to the laboratory for further analyses. Once in the laboratory, samples were rinsed with tap water and non-ionic detergent for 1 min (30% of tween 20 in sterile distilled water (SDW)) and dried in paper towels at room temperature (22-25°C). A portion of sample tissues was incubated in humid chambers at 20°C and >90% relative humidity to corroborate if the microorganism found in the diseased tissue corresponded to the isolated on culture media. The remaining portion of the sample was sectioned in pieces (~1 cm, depending on the tissue) in sterile laminar flow cabinet and surface-disinfested in 70% ethanol for 30 s followed by rinsing in SDW for 30 s. Samples were then submerged in sodium hypochlorite (3% in SDW) for 30 s and rinsed in SDW for 30 s. Finally, samples were dried out on sterile paper towels and placed on semi-selective media culture.

For *Phytophthora* spp. vegetable juice V8-agar (V8-AACB) (V8-180 ml L^{-1} and 24 g L^{-1} of agar Difco, USA) amended with ampicillin (200 µg L^{-1}), chloramphenicol (20 µg L^{-1}) and benomyl (100 µg L^{-1}) was used. For fungi, PDA acidified with lactic acid (PDA-A) (Difco, USA) and vegetable juice V8-agar (V8-AS) (Difco, USA) amended with streptomycin (100 µg L^{-1}) were used. For bacteria, nutrient agar (NAB) (Difco, USA) supplemented with benomyl (50 µg L^{-1}) and yeast-dextrose-calcium carbonate (YDCB) (Difco, USA), supplemented with benomyl (50 µg L^{-1}) were used (Shaad *et al*., 2001). All media plates with samples were incubated at 25°C for 15 d under a photoperiod of 12 h of light and 12 h of darkness.

Colony growth habit, consistency, and color were registered for each isolate obtained. Micro-mounting of mycelia and spores was performed from purified isolates and plant tissues incubated in the humid chamber and observed under light microscopy coupled with differential interference contrast (DIC) (Nikon Eclipse E200). Species identification was performed following the taxonomic keys and guidelines by Barnett and Hunter (1972) and Seifert *et al*. (2011) for fungi; Erwin and Ribeiro (1996) for *Phytophthora* spp., and Mai and Mullin (1996) for phytoparasitic nematodes. For bacteria, standard biochemical tests and guidelines were followed as proposed by Shaad *et al*. (2001). For nematode extraction, soil samples were passed through a series of sieves of 250, 53 and 38 µm. Soil suspension collected in the last sieve was centrifuged for 3 min at 3800 rpm and re-suspended in a solution of SDW and sucrose (50%) and centrifuged again as described. The obtained supernatant was passed through the 38 µm sieve (Jenkins, 1964). In addition, root samples obtained from trees in-field conditions were washed in SDW and air-dried; then fine sectioning and histological mountings were performed for microscope observation based on the standard method used at the laboratory of Fitotecnia Tropical.

Pathogenicity tests

Biotic causal agents

In order to identify if the microorganism isolated from diseased tissues was associated with the symptoms observed under field conditions, pathogenicity tests were performed on avocado plants to fulfill the Koch postulates. Seeds from

avocado fruits of cv. Hass of similar size and high phytosanitary conditions (based on visual inspection of absence of symptoms) were collected from plots planted with avocado (Supplementary material 1). Under laboratory conditions, seeds were surface-disinfected in sodium hypochlorite solution (3% in SDW), and then rinsed in SDW. In addition, pre-germination treatments were applied (seeds were cut in the upper, lateral and basal sides). Finally, seeds were then sown in previously autoclaved quartz sand (0.1 MPa and 121°C, per two cycles of 1 h each) (Ramírez-Gil *et al*., 2014).

Plants were maintained under net-house conditions at an average relative humidity of 90% and a temperature between 18 and 24°C. When plants had five fully expanded leaves and well-developed secondary root system, isolated microorganisms were inoculated on the same organs of avocado plants from which they were initially isolated (i.e. roots, stems, leaves). Pathogenicity tests of isolates associated with flowers and fruits were carried out on these same tissues collected from healthy plants in commercial plots, which were inoculated and incubated in humid chambers at 20°C and >90% relative humidity based on the standard method used at the laboratorio de Fitotecnia Tropical.

In order to process microorganisms isolated from roots, 200 ml of inoculum solution (PDA-SDW) at a concentration of $1*10^3$ - $1*10^6$ infective propagules ml^{-1} were inoculated following the method reported by Ramírez-Gil *et al*. (2014). A similar solution (200 ml of inoculum at $1*10^3$ infective propagules ml^{-1}) plus 2 g l^{-1} of agar (Difco, USA) for adhesion improvement was prepared for inoculation of microorganisms isolated from stems, foliage, fruits, and flowers, and was further sprayed over the surface of the corresponding tissue.

Inoculum of microorganisms that were not possible to isolate in the used media culture (i.e. *Capnodium* sp. and *Cephaleuros virescens*) was obtained by washing and scrapping infected tissues with SDW and a scalpel. Inoculum suspension was adjusted to $1 * 10^{-6}$ infective units ml^{-1} and inoculated as previously described.

Causal agents of biotic disorders

Experiments were conducted to reproduce symptoms induced by each potential abiotic factor of disorder observed under field conditions. Scion-rootstock compatibility in cv. Hass grafting was tested. Scions from avocado cv. Hass trees were grafted onto plants grown from seeds from the same cv. Hass rootstock and on plants grown from seeds of West Indian race rootstock collected from three avocado genotypes selected in San Pedro de Uraba, Antioquia, Colombia (9°46'49.6" N, 75°16'52.3" E, 239 m a.s.l). For hypoxia-anoxia conditions in roots, the guidelines reported by Ramírez-Gil *et al*. (2014) were followed. Root atrophy was tested by monitoring avocado seedlings growth in small plastic bags (5 cm height and 3 cm of diameter) for more than eight months. In addition, root shape and horizontal and vertical lengths were measured. Herbicide toxicity was reproduced by spraying foliage and fruits with *N*-(phosphonomethyl) glycine (glyphosate) in SDW at doses used by farmers. Applications were performed with a manual agricultural sprayer (0.03 MPa) coupled with hollow cone nozzle to produce a droplet size of 50 µm of volume mean diameter (VMD). Sunburn damage was tested by exposing fruits selected on the filling stage to direct sunlight during two hours per day for a week. Hail damage on fruits was simulated by impacting frozen balls of water (3.65 g of weight and 1 cm of radius on average) on fruit epidermis, launched from one meter of distance, with a terminal velocity of 14.15 m s^{-1} and impact energy of 4.29 Jules using a toy gun.

All pathogenicity tests were performed for a time period of 90 d, except fruits that were carried out only for 30 d. The variable measured was the incubation period (Ramírez-Gil *et al*., 2014). In pathogenicity tests, microorganisms were re-isolated in the same media culture as described. Pathogenicity tests were carried out under net-house conditions at a temperature of 18-22°C, 75-95% relative humidity and a photosynthetically active radiation (PAR) of 650-1920 µmol photons m^2 s^{-1}.

Molecular identification of pathogenic microorganisms

All microorganisms that were positive in the pathogenicity tests were further analyzed by sequencing the genomic ITS region as a complement of morphological identification. DNA was purified from mycelia using the DNeasy Plant Mini (QIAGEN®) following the manufacturer's instructions. DNA quality and quantity were measured using a Nanodrop spectrophotometer and separation by agarose gel electrophoresis (1%) in TBE 1X at 90 v stained with EZvision following the manufacturer's instructions and visualized and photographed under UV light in a transilluminator (Biometra, Göttingen, Germany).

Purified DNA was used as template for amplification of the genomic ITS region by the polymerase chain reaction (PCR) in a Thermal cycler (T3 Biometra, Göttingen, Germany), using amplification conditions and primer2 combinations ITS´4-ITS´5 (ITS 4: 5´- GGA AGT AAA AGT CGT AAC AAG G -3´; ITS 5: 5´- TCC TCC GCT TAT TGA TAT GC -3´) and ITS´1-ITS´4 (ITS 1: 5´- CTT

GGT CAT TTA GAG GAA GTA A-3′; ITS 4: 5′- GGA AGT AAA AGT CGT AAC AAG G -3′) reported by White *et al*. (1990). Amplified DNA fragments were separated and visualized by agarose gel electrophoresis (1.5%) with 2µl of SYBR green (10 mg ml^{-1}) following the manufacturer's recommendations and the size of the amplified products was determined by comparison to a Generuler 100 pb DNA ladder (Fermentas). PCR products were further purified with the QIAquick PCR Purification Kit (QIAGEN®) following the manufacturer's instructions.

Purified PCR products were sent for sequencing to Macrogen (Republic of Korea) following the company's guidelines (http://foreign.macrogen.com/eng/business/ngs_overview.html). Sequences were manually cleaned and edited using the software Bioedit 6.0.6 and Chromas 1.45. Then, sequences were aligned using Clustal W algorithm implemented in Bioedit software. Sequence identity was obtained by comparison with sequences in databases available at NCBI using the algorithm Blast implemented in the web page (http://www.ncbi.nlm.nih.gov/BLAST/Blast.cgi).

Results and discussion

Description of causal agents and disorders associated with root rot and stem rot

Biotic causal agents

Phytophthora cinnamomi Rands

This oomycete was identified in all stages of plant growth and development under field conditions. Symptoms associated with *P. cinnamomi* were foliar yellowing, tissue flaccidity, growth retardation, excessive flowering and fructification in adult trees, total defoliation, dieback, secondary and tertiary root rot, and plant death. Isolates showed rosette growth on PDA and cottony and white mycelia. Under light microscopy, mycelia were coralloid and with rounded nodules; sporangia were non-papillated, ellipsoid and no caduceus; chlamydospores were absent and there was presence of non-branched sporangiophores (Tab. 1 and Fig. 1 A-F). Genomic sequences showed the highest percentage of homology with those registered as *P. cinnamomi* in the NCBI database. Morphology and genomic sequences corresponded to *P. cinnamomi* (Erwin and Ribeiro, 1996; Ramírez-Gil *et al*., 2014) (Tab. 1). The incubation period was 20 d (Tab. 2).

Verticillium sp. Nees

A fungus was isolated from plants showing wilt, foliar yellowing, and stunted growth. Through time, these avocado trees exhibited hemilateral branch death and total death in seedlings. As a typical feature, infected leaves kept adhered to the plant. Transversal cut in the stems showed brown coloration in the vascular bundles. Rot was observed in part of the primary, secondary and tertiary roots. Isolates showed hyaline, floccose and septate mycelia. Under light microscopy, branched verticilate conidophores were observed, with unicellular hyaline and ovoid conidia (Tab. 1 and Fig. 1 G-J). All characteristics along with genomic sequences corresponded to *Verticillium* sp. and specific species *Verticillium albo-altrum* and *Verticillium dahliae* (Barnett and Hunter, 1972; Seifert *et al*., 2011; Ramírez-Gil *et al*., 2014). The incubation period was about 17.9 d (Tabs. 1 and 2).

Cylindrocarpon sp. Wollenweber

A fungus was isolated from seedlings at the nursery stage or recently transferred to field conditions. Plants exhibited generalized yellowing, stunted growth, flaccidity, defoliation and dieback symptoms. In media culture, velvety aerial mycelium with color variation from beige to creamy brown was observed. Under light microscopy, plain macroconidia, straight or curved, cylindrical-fusoid in shape, with a maximum of three septa were observed. Few microconidia were observed. They were plain, ellipsoid, cylindrical, straight or curved, with or without septa and scarce chlamydospores (Tab. 1 and Fig. 1 K-P). Morphology and genomic sequences corresponded to *Cylindrocarpon destructans* and *Cylindrocarpon* sp. (Barnett and Hunter, 1972; Seifert *et al*., 2011; Ramírez-Gil *et al*., 2014) (Tab. 1). The incubation period was 28.5 d (Tab. 2).

Phytophthora palmivora Butler

Most isolates of *P. palmivora* were obtained from roots and from the base of the stem from seedlings at the nursery stage. They were also obtained from plants recently transferred to field conditions and in a lower percentage from adult avocado trees. Foliar yellowing, stunted growth, cankers at the base of the stem, and root rot were symptoms associated to *P. palmivora*. Isolates in PDA exhibited stellate, hyaline and cottony mycelia, with variable sporangia of ovoid, ellipsoid, obpyriform, spherical, cenocytic and prominent papillae, with spherical chlamydospores present (Tab. 1 and Fig. 2 Q-X). Morphology and molecular sequences coincided with *P. palmivora* (Erwin and Ribeiro, 1996). The incubation period was 33.1 d (Tab. 2).

Lasiodiplodia theobromae (Pat.) Griffon & Maubl.

Generalized yellowing, stunted growth, foliar yellowing and pith rot characterized avocado trees infected with this microorganism. Grayish fast-growing colonies becoming

dark-black through time, without reproductive structures were observed in PDA media. When mycelia were placed on avocado fruits and stems, hyaline and pigmented conidia was observed, with or without septa, with spherical or ellipsoid shape, thick cell wall and septate paraphyses (Tab. 1 and Fig. 1 Y-E*). Morphology and genomic sequences indicated that isolates were *L. theobromae* (Barnett and Hunter, 1972; Seifert *et al*., 2011). The incubation period measured in the pathogenicity tests corresponded to 22.4 d. (Tab. 2).

Pythium sp. Pringsheim

Oomycete isolates were obtained from seedlings at the nursery stage and plants recently transferred to field conditions. In these plants, foliar yellowing, stunted growth and generalized wilt was observed, including small cankers in the base of the stem and pervasive root rot. Isolates exhibited abundant aerial mycelia in PDA media, with cottony texture, white and extended or rosette shape. Under light microscopy, coenocytic hyphae with thickenings were observed (Tab. 1 and Fig. 1 F*-H*). The characteristic described and genomic sequences allowed the identification of two different and one non-identified species (Erwin and Ribeiro, 1996) named *Pythium cucurbitacearum*, *Phytopythium vexans* and *Pythium* sp. (Tab. 1). The three oomycete species reproduced similar symptomatology with incubation periods between 35.4 and 38.1 d (Tab. 2).

Fusarium oxysporum sensu lato Schlect. emend. Snyd. & Hans.

This fungus was isolated from avocado plants in all growth and development stages under field conditions. In seedlings, *F. oxysporum* caused root rot and damping off; under field conditions it induced pervasive wilting in leaves and fruits, which remained adhered to the plant. Cross sections of the stem revealed necrosis in the vascular bundles. Fast growing colonies in PDA were observed, with cottony

TABLE 1. Morphological structures and DNA ITS sequences of microorganisms associated with avocado cv. Hass diseases.

Causal agent	Types of structures	Dimension[1]		Genetic sequence[2]
Phytophthora cinnamomi	Sporangia Sporangiophore	34.2 ±15.8[a] 2.9 ±1.1[c]	22.4±9.3[b] N.A.	+
Verticilllium sp.	Conidia	5.8 ±1.9	2.5±0.9	+
Cylindrocarpon destructans	Macroconidia Microconidia Chlamydospores	32.5 ±1.9 10.5± 1.5 10.2 ±1.5[c]	6.1±1.2 2.9±1 N.A.	+
Phytophthora palmivora	Sporangia Chlamydospores	50 ±23.8 25.1 ±4.5	27.9±9.8	+
Lasiodiplodia theobromae	Conidia	23.8± 2.3	18.9±1.3	+
Fusarium oxysporum sensu lato	Macroconidia Chlamydospores	4.5± 1.3 8.5± 3.5[c]	1.9±0.5	+
Rosellinia sp.	N.A.	N.A.	N.A.	-
Pythium sp.	N.A.	N.A.	N.A.	+
Pythium cucurbitacearum	N.A.	N.A.	N.A.	+
Pythium vexans	N.A.	N.A.	N.A.	+
Colletotrichum gloeosporioides sensu lato	Conidia	10.2 ±3.5	2.7 ±1.5	+
Capnodium sp.	Peritecia Asci Ascospore	135 ±15.1[c] 93.5 ±8.3 12.3 ±2.3	N.A. 8.8±1.9 4.5±1.3	-
Pestalotia sp.	Conidia	8.5 ±1.5	3.1±0.5	+
Cephaleuros virescens	Sporangia	40 ±8.5	34±6.3	-
Pseudocercospora purpurea	Conidia	55.8 ±15.3	6.3 ±1.8	-
Phomopsis sp.	Pycnidias Conidia alpha (α) Conidia beta (β)	160± 25.1[c] 6.3 ±1.3 18.3 ±1.3	N.A. 1.9 ±0.3 2.1 ±0.5	-
Penicillium sp.	Phialides Conidia	7.5 ±1.5 35 ±2.3	2.5 ±0.4 1.9 ±0.3	+
Sphaceloma perseae	N.A.	N.A.	N.A.	-

[a]Length; [b]width; [c]for these structures, the diameter was the mean of 20 measures. N.A.: not apply. [1] data in μm observed under 10 and 40 X (see Fig. 1 and 3). [2] +: sequence was obtained; -: sequence was not obtained respectively.

texture and variable color. Hyaline, almost-straight or semi-curved macroconidia with three to five septa were observed under light microscopy along with abundant microconidia with oval or ellipsoid shape and hyaline and spherical chlamydospores (Tab. 1 and Fig. 1 I*-P*). All characteristics coincided with *Fusarium oxysporum sensu lato* (Barnett and Hunter, 1972; Fourie *et al*., 2011; Seifert *et al*., 2011). Phytopathological tests showed an incubation period of 26.5 d (Tab. 2).

Rosellinia sp. (Fr.) De Not.

This fungus was isolated from plants in all stages of development showing general wilt and mild yellowing. Internal necrosis and superficial cracks accompanied by an abundant mass of white mycelia were identified in the root system of affected plants. Under light microscopy, septate and branched mycelia of brown color forming an abundant reddish stroma were visualized (Tab. 1 and Fig. 1 Q-V). All results pointed to *Rosellinia* sp. as the fungal species involved in diseased tissues (Barnett and Hunter, 1972; Seifert *et al*., 2011; Ramírez-Gil *et al*., 2014). The incubation period measured during pathogenicity tests was 38.9 d (Tab. 2).

Abiotic disorders

Root atrophy

Symptoms, such as foliar yellowing, stunting, and sparse foliage, were identified in avocado plants at nursery and field stages of development. In these plants, root atrophy was observed as a short, thick and forked main root, with reduced presence of secondary or tertiary roots. No microorganisms were isolated from plants affected with this pathology. Under laboratory conditions, symptoms were reproduced by sowing plants in small bags (25 cm of diameter and 36 cm of height). Reproduction of symptoms took 135 d (Tab. 2 and Fig. 2 A-E).

TABLE 2. Pathogenicity tests for potential causal agents of diseases and disorders on *Persea americana* cv. Hass.

Causal agent	Disease name	Incubation period				Re-isolated
Phytophthora cinnamomi	Wilt complex	15.4±1.3[a]	[b]	[c]	[d]	+
Verticilllium sp.	Wilt complex	17.9±1.3				+
Cylindrocarpon destructans	Wilt complex	28.5±1.8				+
Phytophthora palmivora	Wilt complex- Sudden burn	33.1±1.9		28.3±3.8		+
Lasiodiplodia theobromae	Wilt complex- Graft decay and dieback	22.4±1.3	25.2±2.9	45.7±5.3		+
Fusarium oxysporum sensu lato	Wilt complex	26.5±2				+
Rosellinia sp.	Wilt complex	38.9±2.5				+
Pythium sp.	Wilt complex	35.1±2.4				+
Pythium cucurbitacearum	Wilt complex	33.8±2.6				+
Pythium vexans	Wilt complex	38.1±3				+
Hypoxia and anoxia	Wilt complex	15.4±1.3				N.A.
Root atrophy	Wilt complex	135±5.9				N.A.
Colletotrichum gloeosporioides sensu lato	Leaf spot, necrosis of flowers, fruit anthracnose, and dieback		42.3±3.9	21.2±1.9	25.4±2.1-12.4±1.1[e] -15.3±1.8[f]	+
Capnodium sp.	Sooty mold		65.4±5.9	50.9±6.3	N.A.	-
Pestalotia sp.	Leaf spot and depressed peduncle lesion			65.7±5.9	85.3	+
Cephaleuros virescens	Leaf spot			71.8±8.3		-
Pseudocercospora purpurea	Leaf spot and fruit raised lesion			49.8±4.9		+
Phomopsis sp.	Leaf white spot			63.9±5.8		+
Sphaceloma perseae	Leaf curl and fruit scab			45.1±3.8	51.3±6.2	
Penicillium sp.	Cotyledon rot	15.6±1.1[f]				
Herbicide	Phytotoxicity			23±1.9		N.A.
Sunburn	Sunburn		62.4±4.9		19.5±2.1	N.A.
Hail damage	Defoliation and fruit lesion		5.5±0.5		3±0.8	N.A.
Incompatibility	Graft incompatibility		120±6.8			N.A.
Unknown	Peduncle ringing	-	-	-	-	N.A.

[a]Inoculation on roots, [b]inoculation on the stems, [c]inoculation on leaves, [d]inoculation on fruits, [e]inoculation on leaves and flowers, [f]inoculation on seeds. N.A. Not apply.

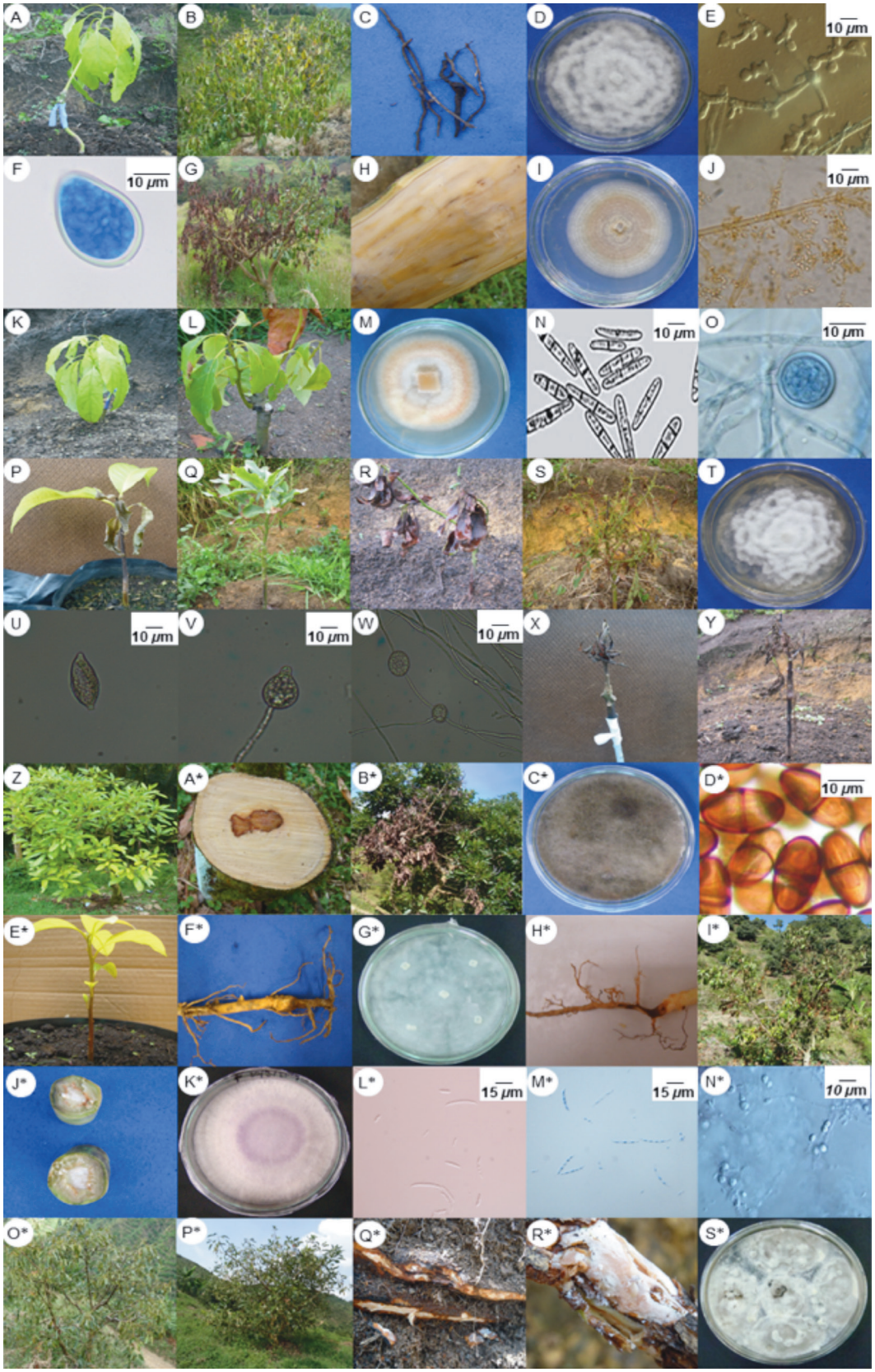


FIGURE 1. Symptomatology and structures of microorganisms associated with biotic causal agents of pre-harvest diseases in avocado cv. Hass. A to F: *Phytophthora cinnamomi*. G to J: *Verticillium* sp. K to O: *Cylindrocarpon* sp. P to Y: *Phytophthora palmivora*. Z to D*: *Lasiodiplodia theobromae.* E* to G*: *Pythium* sp. H* to N: *Fusarium oxysporum sensu lato*. O* to S*: *Rosellinia* sp.

FIGURE 2. Symptomatology associated with abiotic disorders of pre-harvest diseases in avocado cv. Hass. A to E: Root atrophy. F to H: Hypoxia-anoxia. I to K: Herbicide phytotoxicity. L to N: sunburn. O and P**:** rootstock-scion incompatibility. Q and R: hail stone damage. S: Peduncle ringing.

Hypoxia-anoxia

Leaf flaccidity, mild yellowing, necrosis of the leaf apex, stunted growth, total defoliation and death at the final stage depicted this pathology that was identified in all stages of plant development. Root rot was observed in main and secondary root systems, which was characterized by blue-greyish coloration visualized at dissection. No microorganisms associated to this pathology were isolated and symptoms were reproduced using plants grown in autoclaved soils with high humidity. Reproduction of symptoms took 15.4 d (Tab 2 and Fig. 2 F-H). Symptomatology corresponded to disorders caused by low oxygen levels in the soil known as hypoxia-anoxia (Stolzy *et al*., 1967; Ramírez-Gil *et al*., 2014, 2017).

Biotic causal agents of diseases and disorders affecting stems, leaves, flowers, fruits and seeds on avocado cv. Hass

Sudden leaf burn caused by *Phytophthora palmivora*

This pathology was observed mainly on plants at the nursery stage or recently transferred to field conditions. Plants exhibited leaves with burned edges of dark brown color leading to wrinkling toward the adaxial side of the leaf blade.

In advanced stages of infection, necrosis and defoliation were observed. Isolated and identified microorganisms and genomic sequences corresponded to *P. palmivora* (Tab. 1 and Fig. 1 R to X). The incubation period in pathogenicity tests was 28.3 d. (Tab. 2).

Graft rot and dieback caused by *Lasiodiplodia theobromae*

Stem rot in the grafting zone between the scion-rootstock was observed on plants at the nursery stage or recently transferred to field conditions. Symptoms advanced rapidly leading to defoliation and dieback. In adult trees, this microorganism caused dieback. Leaves became brown and remained adhered and the stem showed necrosis with deep black color. *L. theobromae* was consistently isolated from these diseased tissues and it was confirmed by genomic sequences as previously described (Tab. 1 and Fig. 1 Y, Z, C* and E*). Symptoms were reproduced in the grafting zone and leaf apex in seedlings, with incubation periods of 25.2 and 45.7 d, respectively (Tab. 2).

Seed rot, foliar spot, flower necrosis, anthracnose in fruits and dieback caused by *Colletotrichum gloeosporioides sensu lato*

The pathology caused by this fungus was identified during all stages of plant development. Symptomatology included necrosis in plumule and radicle, stem rot in the grafting zone between the scion-rootstock, tiny black foliar spots with necrotic center, branch dieback, and tissue rot from the apical zone to the base of the stem. Flowers showed black necrosis. Mummification was observed during all developmental stages in fruits that remained adhered to the plant. In developed fruits, small black spots on the epidermis were visualized, and a pink mass was observed under high humidity.

Colonies in PDA with a highly variable in color, white, greyish or salmon, of fast growth with spongy and dense mycelia were obtained from the different affected tissues. Conidia were hyaline, unicellular, cylindrical, oval, and ellipsoid-fusiform with one side tapered and the other rounded (Tab. 1 and Fig. 3 A to I). Morphology and genomic sequences corresponded to *Colletotrichum* sp. (Barnett and Hunter, 1972; Seifert *et al*., 2011; Sharma *et al*., 2017; Giblin *et al*., 2018). This group has been recently referred to as a species complex known as *Colletotrichum gloeosporioides sensu lato*, and their accurate identification at the species level requires the use of other methods beyond the scope of the present research (Weir *et al*., 2012; Sharma *et al*., 2017; Giblin *et al*., 2018). The incubation periods of pathogenicity tests carried out in leaves, stems, flowers, and fruits were 21.2, 42.3, 25.4 and 12.4 d, respectively (Tab. 2).

Sooty mold by *Capnodium* sp.

Symptomatology was characterized by a black layer of mycelia on the adaxial or upper side of the leaf blade, mainly observed in adult trees and in a lower percentage in nursery seedlings. Mycelia were easily removable from affected leaves, stems, and fruits. In advanced stages it caused leaf fall. It was not possible to isolate any microorganism in media culture. Under light microscopy, the structures observed from diseased leaves revealed dark-brown mycelia with short septa, globose perithecia, almost pedunculate, bitunicated ascus and ascospores (Tab. 1 and Fig. 3 J-M). Characteristics corresponded to *Capnodium* sp. (Barnett and Hunter, 1972; Seifert *et al*., 2011). An incubation period of 50.9 d was measured in the pathogenicity tests (Tab. 2).

Foliar spots and depressed lesion on the peduncle caused by *Pestalotia* sp.

This disease was identified affecting leaves and fruit peduncle in all stages of plant development. In leaves, a brown irregular spot of variable size preferably located in the leaf apex was observed. In the fruit peduncle, this pathogen caused a black depressed lesion with black dots over it, which corresponded to pathogen structures in advanced stages. In PDA, a fungus of white color, cottony texture, and with deep black oily protrusions was isolated. Under light microscopy, abundant fusoid conidia with 2-3 septa, of brown color in the center but hyaline with appendices in the apices were observed (Tab. 1 and Fig. 3 O-S). The associated microorganism was identified as *Pestalotia* sp. (Barnett and Hunter, 1972; Seifert *et al*., 2011). The incubation periods during pathogenicity tests in leaves and fruit peduncles were 65.7 and 85.3 d, respectively (Tab. 2).

White spot by *Phomopsis* sp.

This disease was only identified in leaves of adult trees, characterized by small spots (<2mm) with necrotic borders and white-greyish center, which in advanced stages coalesced to generate large necrotic areas. From diseased tissues, a fast-growing microorganism was isolated in PDA, of white color evolving to grey, with unilocular and globular pycnidia, inside of which hyaline, unicellular α conidia constricted in the center with blunt base were observed. Longer, filiform, flexuous or curved β conidia without septa were also observed (Tab. 1 and Fig. 3 I-X). Characteristics observed corresponded to *Phomopsis* sp. (Barnett and Hunter, 1972; Seifert *et al*., 2011). The incubation period in the pathogenicity tests was 63.9 d (Tab. 2).

Foliar spot caused by *Cephaleuros virescens*

This pathology was observed in all stages of plant development causing green, yellow or orange, rounded and raised

spots of velvety appearance on the leaf blade surface. No microorganisms were isolated from lesions with media used in the present work. Under light microscopy, coenocytic mycelia with sporangiophores showing oval sporangia were observed (Tab. 1 and Fig. 3 Y-Z). Data obtained corresponded to *C. virescens* (Barnett and Hunter, 1972). Symptoms were reproduced after an incubation period of 71.8 d in the pathogenicity tests (Tab. 2).

Foliar spot and raised lesion in avocado fruits by *Pseudocercospora purpurea*

This pathology preferably appeared in adult trees affecting leaves and fruits but it did not occur commonly in the present study. Small foliar spots (<5 mm), brown in color, showing necrotic margins and irregular shape were observed. In fruits, superficial cracks in the epidermis that evolved to raised lesions were identified. In PDA, isolates exhibited slow growth, cottony colonies that were white at the beginning but became gray and dark through time. Under light microscopy, basal stroma, from which straight or slightly curved conidiophores emerged, was observed. Conidia were cylindrical in shape, elongated with several septa (Tab. 1 and Fig. 3 A*-E*). Characteristics indicated that isolates corresponded to *P. purpurea* (Barnett and Hunter, 1972; Seifert *et al*., 2011). An incubation period of 49.8 d was measured in the pathogenicity tests (Tab. 2).

Leaf curl and fruit scab by *Sphaceloma perseae*

Although rarely seen during the present study, this pathology presented affected leaves and fruits inducing small spots (<10 mm) of irregular shape and light brown color that occasionally induced leaf curling. In fruits, irregular, corking, depressed or raised lesions were observed. A microorganism from white to grey color was isolated in PDA which showed cylindrical or elliptical, lunate or falcate conidiophores of variable size (Tab. 1 and Fig. 3 F*-I*). *S. perseae* was identified from findings reproducing symptoms in the pathogenicity tests (Barnett and Hunter, 1972; Seifert *et al*., 2011) (Tab. 2).

Cotyledon rot by *Penicillium* sp.

Brown irregular lesions that grew covering most of the cotyledon surface were observed. In PDA, a fast-growing fungus was isolated, with light yellow color that became dark through time, cottony texture with abundant conidia, hyaline and septate hyphae, with simple or branched conidiophores, 3-7 phialides and globular conidia in short or divergent chains (Tab. 1 and Fig. 3 J*-O). Morphological characteristics and DNA sequences coincided with *Penicillium* sp. (Barnett and Hunter, 1972; Seifert *et al*., 2011). Pathogenicity tests were positive for the isolated microorganism (Tab. 2).

In this study, no pathogenic nematodes or bacteria were found based on the methodology used (Mai and Mullin, 1996; Shaad *et al*., 2001).

Abiotic causal agents of diseases and disorders affecting stems, leaves, flowers, fruits and seeds on avocado cv. Hass

Herbicide phytotoxicity on leaves and fruits

Leaves affected by herbicides became light brown and detached; total defoliation and plant death may be observed when large areas were exposed. Spots were usually scattered in an irregular manner through foliage. In fruits, herbicides produced irregular black spots that remained unaltered for long periods. No microorganisms were isolated from those lesions; symptoms were reproduced at 13 d after plants were sprayed with herbicide (Tab. 2 and Fig. 2 I to K).

Fruit and stem sunburn

Fruits showed mild yellowing with reddish hues or a rounded lesion of dark brown color when exposed to direct sunlight because of partial defoliation or large fruiting. In stems, green-yellow coloration that became brown or black was identified when directly exposed to strong sunlight. No microorganisms were found associated to this disorder and the pathogenicity tests reproduced symptoms when tissues were continuously exposed to direct sunlight. Reproduction of symptoms was observed at 19.5 and 62.4 d for fruits and stems, respectively, after sun exposure (Tab. 2 and Fig. 2 L-O).

Rootstock-scion incompatibility

This abnormality was observed in all stages of plant development from nursery to adult trees. The disorder was characterized by thickening in the grafting zone between the rootstock and the scion. More frequently, scion showed a bigger diameter in the stem than the rootstock, causing stunted growth and sometimes plant death. Symptoms of stunted development were reproduced in plants when compared to compatible grafts (Tab. 2 and Fig. 2 P-Q).

Defoliation and lesions caused by hailstorm

Hail caused defoliation to a degree that depends on the hailstorm intensity, but that may lead to total defoliation. On fruits, hail caused deep (5 mm) and small rounded lesions (<4 mm) that became necrotic spots through time. Over the lesions, a corking tissue developed that remained until harvest causing damage to fruit quality. The worst damage was caused in the first stages of fruit development. The reproduction of symptoms was of 3 days in the performed pathogenicity tests (Tab. 2 and Fig. 2 R-S).

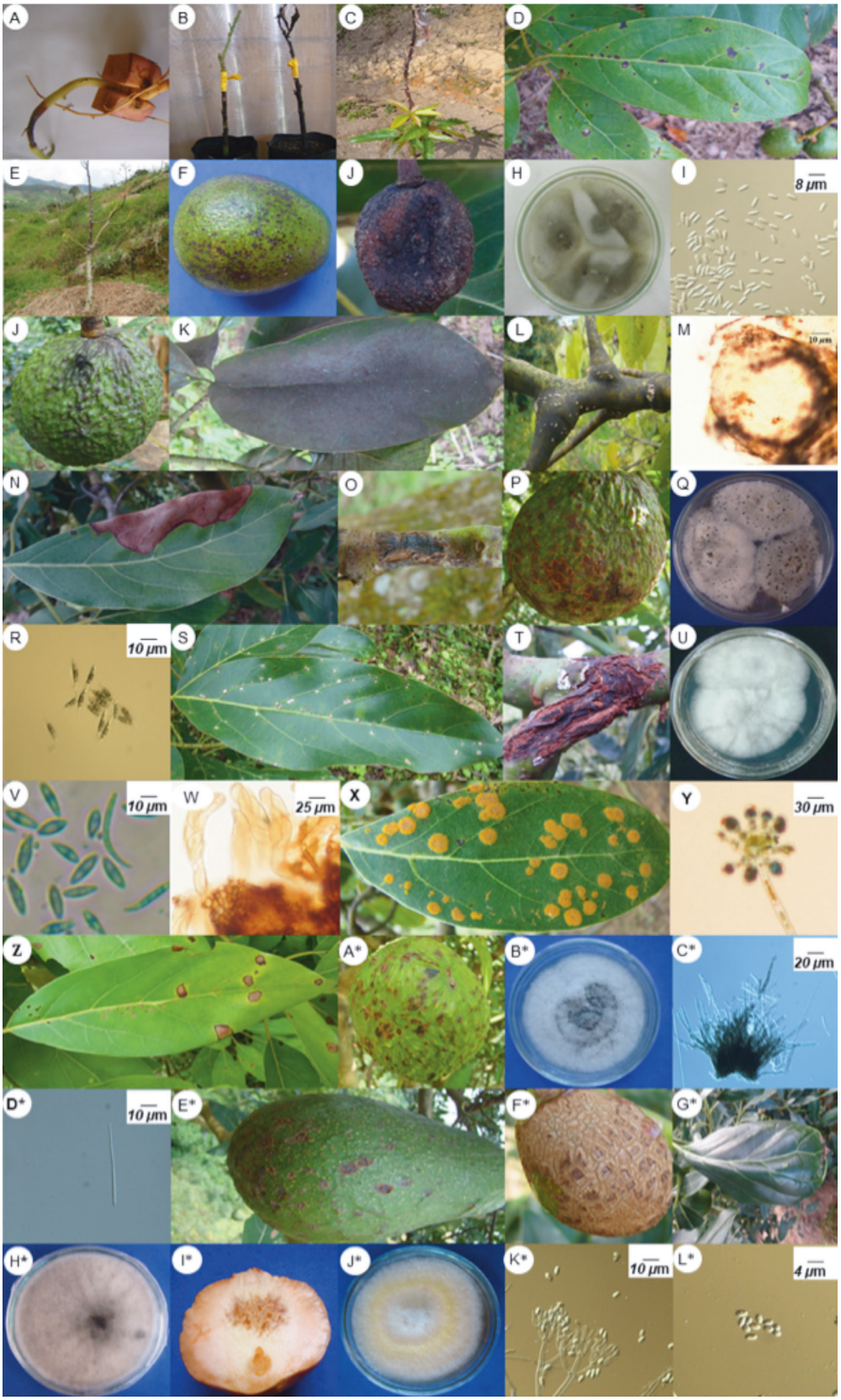


FIGURE 3. Symptomatology and structures of microorganisms associated to causal agents of pre-harvest diseases on avocado cv. Hass. A to I: *Colletotrichum gloeosporioides sensu lato.* J to M: *Capnodium* sp. N to R: *Pestalotia* sp. S to W: *Phomosis* sp. (teleomorph *Botryosphaeria* sp.). X and Y: *Cephaleuros virescens.* Z* to D*: *Pseudocercospora purpurea.* E* to H*: *Sphaceloma perseae.* I* to L*: *Penicillium* sp.

Peduncle ringing

This disorder was more frequently observed on small fruits (<10 mm in diameter) and during the development stage known as fruit filling. It was expressed as a ring in the fruit peduncle, which acquired round shape and purple hue from the peduncle to the apex, which may remain adhered to the plant showing progressive dehydration until mummified or detached. No microorganisms were isolated from diseased plants and it was not possible to replicate the symptoms in the pathogenicity tests; therefore, this disease remains of unknown etiology (Fig. 2 T).

Avocado root rot was associated with ten biotic and two abiotic causal agents inducing similar symptoms. As this pathology has multi-agent etiology, accurate and prompt diagnosis is difficult and frequently leads to the application of wrong control methods that may cause large losses (Zentmyer, 1984; Machado *et al*., 2008; Vitale *et al*., 2012; Ramírez-Gil *et al*., 2014, 2017; Parkinson *et al*., 2017). *Phytophthora palmivora, L. theobromae, Fusarium oxysporum sensu lato, Phytopythium vexans*, *Pythium cucurbitacearum,* and *Pythium* sp. were identified as associated with root rot causal agents in our research; in addition, they have not been previously reported in Colombia (Tamayo, 2007; Ramírez-Gil *et al*., 2014, 2017). *Pythium cucurbitacearum* had not been reported before in the avocado crop, being this the first report worldwide (APS, 2017). Similarly, the abiotic factor root atrophy had not been previously reported as a root rot causal agent in Colombia and around the word (Tamayo, 2007; Ramírez-Gil *et al*., 2014, 2017).

Colletotrichum gloeosporioides sensu lato and *L. theobromae* excelled as pathogens associated to stems, leaves, fruits and seeds, and *P. palmivora* was mainly involved in diseases affecting roots, the base of the stem and leaves. As these pathogens may affect several avocado tissues, their importance should not be neglected in an integrated disease management program. Disorders of abiotic origin associated with symptoms in stems, leaves, flowers and fruits identified in the present work have been reported before with slight variation in symptom expression. This may be due to the local edaphoclimatic conditions in the regions studied which may influence symptomatology (Zentmyer, 1984; Menge and Ploetz, 2003; Sanders and Korsten, 2003; Tamayo, 2007; Machado *et al*., 2008; APS, 2017). Diseases produced by *Phomopsis* sp., hail damage and phytotoxicity by herbicide had not been reported before for Colombia.

Despite the fact that avocado diseases have been studied from a long time, some pathologies remain of unknown etiology such as the peduncle ringing. Potential causal agents have been proposed including humidity deficit during fruit filling, warm and dry winds, high nitrogen and potassium or low magnesium levels (Toerien, 1979) and secondary microorganisms. Rootstock-scion incompatibility has been attributed to phylogenetically distant genotypes; however, it has been also observed in closely related genotypes of *Persea* sp. This problem may cause large losses in advanced stages of plant development when trees are in full production. Further research is needed to clearly elucidate the main factors predisposing to this disorder and how to prevent them (Frolich *et al*., 1958).

Correct and prompt diagnosis of avocado diseases is of paramount importance for successful crop production. Polyphasic approaches for disease identification have proven to be useful for appropriate integrated crop management because misleading identification of problems may cause large losses and plant death. Disorders caused by abiotic factors are usually accompanied by secondary microbial colonization mainly due to dead tissue. This fact may induce a wrong causal agent identification and incorrect control measures, leading to the aforementioned economic and plant losses. As many diseases were identified in the present research, permanent monitoring and epidemiological studies should be implemented to promptly identify and control new or expanding pathologies in tropical environments where avocado cv. Hass has been growing in Colombia. Nationwide studies should also be considered to detect prevalent or emerging diseases at local areas different from the ones studied here.

This work is a comprehensive contribution for the detection and diagnosis of the most frequent pathologies of avocado production systems under the evaluated conditions. Here, we display a multistep approach of disease identification including morphological characterization of associated microorganisms, detailed characterization of the symptoms associated with each of the pathologies and disorders and different factors that may be involved in disorders that affect this crop. The present research may be a guide to be consulted by avocado producers, technical assistants, professors, students, government agencies and other actors involved in the avocado industry. On the other hand, it is necessary to continue with studies to look for alternative technologies for the early, sensitive and accurate detection and diagnosis of diseases to maintain and increase sustainability for this crop.

Conclusion

The polyphasic approach developed in this work for diagnosis of cv. Hass avocado pre-harvest pathologies and disorders was an alternative that allowed the appropriate

identification of microorganisms and causal agents of the most frequent diseases and disorders in roots, stems, leaves, flowers, and fruits of avocado crops in tropical conditions in Antioquia, Colombia. Additionally, our research may be used as a guide for the detection of different types of phytosanitary problems in avocado.

Acknowledgments

The authors would like to thank Universidad Nacional de Colombia Medellin campus for partial funding, Colciencias for the Ph.D. scholarship and the avocado producers for valuable information and help during research. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Literature cited

Agrios, G. 2005. Plant pathology. Elsevier Academic Press, Dana Dreibelbis, London.

Alvarez, A. 2004. Integrated approaches for detection of plant pathogenic bacteria and diagnosis of bacterial diseases. Annu. Rev. Phytopathol. 42, 339-366. Doi: 10.1146/annurev.phyto.42.040803.140329

APS. 2017. Diseases of Avocado (*Persea americana* Miller). URL: https://www.apsnet.org/publications/commonnames/Pages/Avocado.aspx (accessed 9 August 2017).

Barnett, H.L. and B.B. Hunter. 1972. Illustrated genera of imperfect fungi. Burgess Publishing Company, Minnesota, USA.

Erwin, D.C. and O.K. Ribeiro. 1996. Phytophthora diseases worldwide. American Phytopathological Society, St. Paul, Minnesota, USA.

FAO. 2018. FAOSTAT. URL: http://www.fao.org/faostat/es/#home (accessed 9 January 2019).

Fourie, G., E.T. Steenkamp, R.C. Ploetz, T.R. Gordon, and A. Viljoen. 2011. Current status of the taxonomic position of *Fusarium oxysporum formae specialis cubense* within the *Fusarium oxysporum* complex. Infect. Genet. Evol. 11(3), 533-542. Doi: 10.1016/j.meegid.2011.01.012

Frolich, E., C. Schroeder, and G. Zentmyer. 1958. Graft compatibility in the genus *Persea*. California Avocado Soc. Yearbook 42, 102-105.

Giblin, F. R., Y.P. Tan, R. Mitchell, L.M. Coates, J.A.G. Irwin, and R.G. Shivas. 2018. *Colletotrichum* species associated with pre- and post-harvest diseases of avocado and mango in eastern Australia. Australasian Plant Pathol. 47(3), 269-276. Doi: 10.1007/s13313-018-0553-0

Hardham, A.R. and L.M. Blackman. 2018. *Phytophthora cinnamomi*. Mol. Plant Pathol. 19(2), 260-285. Doi: 10.1111/mpp.12568

Machado, M., M. Peña, O. Coto, M.O. López, V. Zamora, R.I. Cabrera, and M. Aranguren. 2008. Caracterización morfológica y fisiológica de *Phytophthora* spp. aisladas de plantas de aguacatero con síntomas de marchitez. CitriFrut 25(2), 49-56.

Mai, W. and P. Mullin.1996. Plant-parasitic nematodes: a pictorial key to genera. Cornell University Press, Ithaca, USA.

Menge, J.A. and R.C. Ploetz. 2003. Diseases of avocado. In: R.C. Ploetz (ed.). Diseases of tropical fruit crops. CABI, Wallingford, USA.

Parkinson, L.E., R.G. Shivas, and E.K. Dann. 2017. Pathogenicity of Nectriaceous fungi on avocado in Australia. Phytopath. 107(12), 1479-1485. Doi: 10.1094/PHYTO-03-17-0084-R

Ramírez-Gil, J.G., E. Gilchrist-Ramelli, and J.G. Morales-Osorio. 2017. Economic impact of the avocado (cv. Hass) wilt disease complex in Antioquia, Colombia, crops under different technological management levels. Crop Prot. 101, 103-115. Doi: 10.1016/j.cropro.2017.07.023

Ramírez-Gil, J. and J.G. Morales-Osorio. 2013. Primer informe de *Cylindrocarpon destructans* (Zinss) Scholten afectando plántulas de aguacate (*Persea americana* Mill) en Colombia. Revista de Protección Vegetal 28(1), 27-35.

Ramírez-Gil, J.G., D.A. Castañeda, and J.G. Morales-Osorio. 2014. Estudios etiológicos de la marchitez del aguacate en Antioquia-Colombia. Revista Ceres 61(1), 50-61. Doi: 10.1590/S0034-737X2014000100007

Ramírez-Gil, J.G. 2018. Avocado wilt complex disease, implications and management in Colombia. Revista Facultad Nacional Agronomía Medellín 71(2), 8525-8541. Doi: 10.15446/rfna.v71n2.66465

Sanders, G.M. and L. Korsten. 2003. Comparison of cross inoculation potential of South African avocado and mango isolates of *Colletotrichum gloeosporioides*. Microbiol Res. 158(2), 143-150. Doi: 10.1078/0944-5013-00186

Seifert, K., G. Morgan-Jones, W. Gams, and B. Kendrick. 2011. The Genera of Hyphomycetes. CBS-KNAW Fungal Biodiversity Centre, Utrecht, Netherlands.

Shaad, N., J. Jones, and W. Chun. 2001. Laboratory guide for identification of plant pathogenic bacteria. American Phytopathological Society. St. Paul, USA.

Sharma, G., M. Maymon, and S. Freeman. 2017. Epidemiology, pathology and identification of *Colletotrichum* including a novel species associated with avocado (*Persea americana*) anthracnose in Israel. Sci. Rep. 7(1), 15839. Doi: 10.1038/s41598-017-15946-w

Stolzy, L., G. Zentmyer, L. Klotz, and C. Labanauskas. 1967. Oxygen diffusion, water, and *Phytophthora cinnamomi* in root decay and nutrition of avocados. J. Am. Soc. Hortic. Sc. 90, 67-76.

Tamayo, P. 2007. Enfermedades del aguacate. Rev. Politecnica 4, 51-70.

Taylor, J.W., D. Jacobson, S. Kroken, T. Kasuga, D. Geiser, D. Hibbett, and M. Fisher. 2000. Phylogenetic species recognition and species concepts in fungi. Fun. Gen. Biol. 31, 21-32. Doi: 10.1006/fgbi.2000.1228

Toerien, J. 1979. Seasonal incidence of ring-neck on avocados and possible causes. South African Avocado Growers 3, 49-51.

Vitale, A., D. Aiello, V. Guarnaccia, G. Perrone, G. Stea, and G. Polizzi. 2012. First report of root rot caused by *Ilyonectria* (= *Neonectria*) *macrodidyma* on avocado (*Persea americana*) in Italy. J. Phytopathol. 160(3), 156-159. Doi: 10.1111/j.1439-0434.2011.01869.x

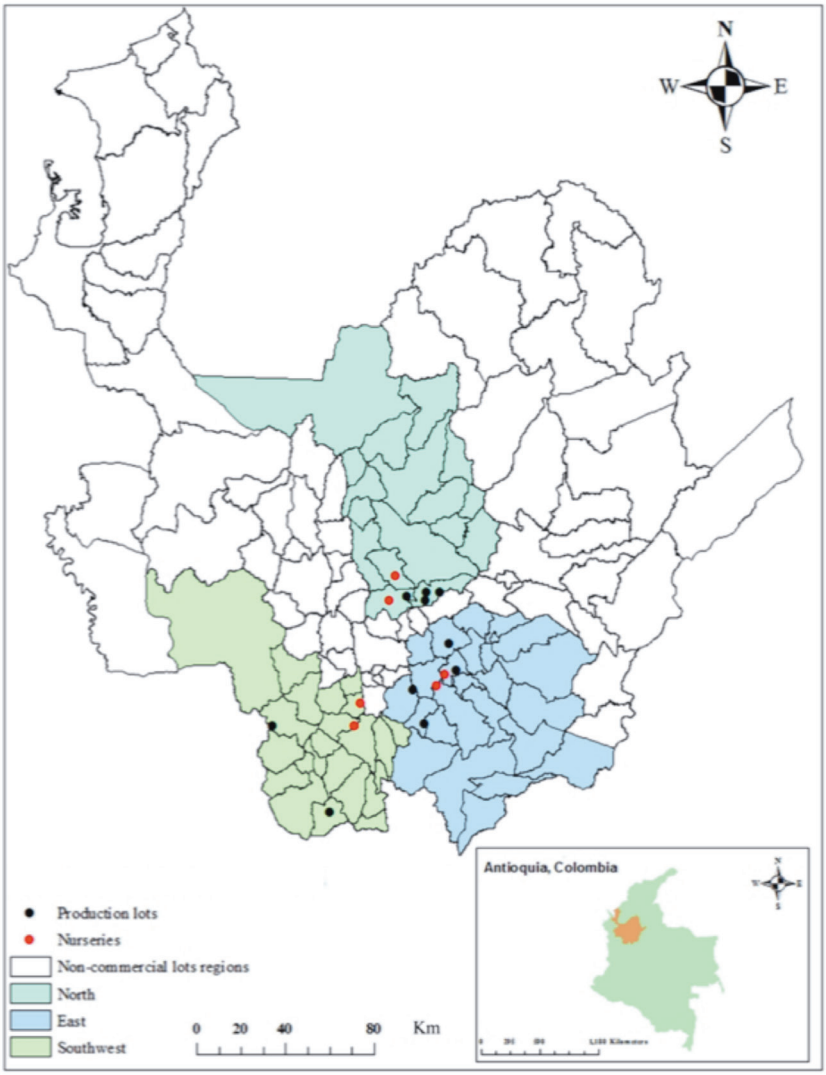
Weir, B.S., P.R. Johnston, and U. Damm. 2012. The *Colletotrichum gloeosporioides* species complex. Stud. Mycol. 73(1), 115-180. Doi: 10.3114/sim0011

White, T., T. Bruns, S. Lee, and L. Taylor. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: PCR Protocols: A guide to methods and applications. Academic Press, San Diego, USA. Doi: 10.1016/b978-0-12-372180-8.50042-1

Zentmyer, G. 1984. Avocado diseases. Trop. Pest Manage. 30, 677-682. Doi: 10.1080/09670878409370915

Zentmyer, G. 1980. *Phytophthora cinnamomi* and diseases it causes. American Phytopathological Society, St. Paul, USA.

SUPPLEMENTARY MATERIAL 1. Location of the avocado nurseries and plots evaluated.



Regions where avocado plots were tested exhibited an annual mean temperature of 14-20°C, 1800-2600 mm of annual precipitation, 75-95% relative humidity and 1850-2400 m a.s.l. These regions correspond to tropical life zones of lower montane humid forest (lm-HF) and lower montane very humid forest (lm-VMB) (Holdridge, 1967). All trees sampled were avocado cv. Hass grafted on West Indian rootstock, at planting distances of 7 x 7, 6 x 5, and 6 x 6 m.

SUPPLEMENTARY MATERIAL 2. General edaphic properties in the soil of the avocado nurseries and plots evaluated.

Soil/region	Sn[1]	Si[1]	Cla[1]	pH	OM[2]	Al[3]	Ca[3]	Mg[3]	K[3]	P[4]	S[3]	Cu[5]	Zn[5]	Mg[5]	B[5]
North	52	28	20	5.2	12.3	0.6	2.55	1.1	0.45	21.3	5.1	0.8	2.3	1.9	0.3
East	60	22	18	5.3	14.8	0.8	3.5	1.3	0.55	24.4	5.6	0.9	1.9	2.5	0.5
Southwest	58	23	19	5.5	13.1	1.6	1.9	0.9	0.35	15.9	5.9	0.9	2.4	2.1	0.35
Nurseries	62	15	23	5.1	6.0	0.9	1.1	0.9	0.4	12.3	4.8	0.6	1.7	1.9	0.2

Effect of thermal and *in vitro* fungicide treatments on pathogens of the genus *Fusarium* associated with maize seeds

Efecto de tratamientos térmicos y de fungicidas *in vitro* sobre patógenos del género *Fusarium* asociados a semillas de maíz

Natalia Piñeros-Guerrero[1]*, Germán Maldonado-Archila[1], and Sandra Gómez-Caro[1]

ABSTRACT

Stalk rot in maize plants is commonly associated with many species of the genus *Fusarium*. This disease affects the seedbed and the establishment of maize crops because of seeds contaminated with different pathogens of this genus. Maize crops in the Ubate Valley, in the province of Cundinamarca, are currently infected by this disease, which reduces the yield and final quality of the maize seeds. This research evaluated the effects of thermal and fungicide treatments on pathogens of the genus *Fusarium* associated with maize seeds. Seeds were treated at 50°C, 55°C and 60°C with dry heat and hot water. Mycelial colonization of seeds, germination percentage, seedling length, and fresh weight were evaluated as variables. In *in vitro* tests, the fungicides fludioxonil + metalaxyl-M, tebuconazole + trifloxystrobin, prochloraz + difenoconazole and carboxin + captan were evaluated at 0.5, 1.0 and 1.5 the commercial dose on the radial growth and conidial germination of *Fusarium subglutinans* and *Fusarium graminearum* isolates. The most effective heat treatments on *Fusarium* colonization of maize seeds were obtained with the two heat sources at 55°C without a significant reduction in the percentage of germination and seedling length and fresh weight. Commercial doses of the evaluated fungicides completely inhibited the radial growth of *F. graminearum* and only commercial doses of carboxin + captan and prochloraz + difenoconazole completely inhibited *F. subglutinans* growth. Germination inhibition of *F. subglutinans* and *F. graminearum* conidia was found with fludioxonil + metalaxyl-M at the three evaluated doses.

Key words: maize seed pathogens, physical control, chemical control, *Fusarium graminearum*, *Fusarium subglutinans*, stalk rot.

RESUMEN

El volcamiento en plantas de maíz es causado por la pudrición del tallo comúnmente asociado a varias especies del género *Fusarium*. Esta enfermedad afecta el semillero y el establecimiento de cultivos de maíz por el uso de semilla contaminada con diferentes patógenos de este género. Actualmente los cultivos de maíz en el Valle de Ubaté, Cundinamarca están siendo afectados por esta enfermedad, reduciendo el rendimiento y la calidad final de las semillas. Por esto, este estudio evaluó el efecto de tratamientos térmicos y con fungicidas sobre patógenos del género *Fusarium* asociados a semillas de maíz. Las semillas fueron tratadas a 50°C, 55°C y 60°C con calor en seco y agua caliente. Como variables se evaluaron la colonización micelial de la semilla, el porcentaje de germinación y la longitud y el peso fresco de las plántulas. En pruebas *in vitro*, se evaluaron los fungicidas fludioxonil + metalaxyl-M, tebuconazole + trifloxystrobin, prochloraz + difenoconazol y carboxin + captan en 0.5, 1.0 y 1.5 la dosis comercial sobre la inhibición del crecimiento radial y germinación de conidias de aislamientos de *Fusarium subglutinans* y *Fusarium graminearum*. Los tratamientos térmicos más efectivos sobre la colonización de *Fusarium* en semillas de maíz se presentaron con las dos fuentes de calor a 55°C sin una reducción significativa en el porcentaje de germinación, la longitud y el peso fresco de plántulas. Las dosis comerciales de los fungicidas evaluados inhibieron completamente el crecimiento radial de *F. graminearum* y solo las dosis comerciales de carboxin + captan y prochloraz + difenoconazol inhibieron completamente el crecimiento de *F. subglutinans*. Inhibición de germinación de conidias de *F. subglutinans y F. graminearum* se encontró con fludioxonil + metalaxyl-M a las tres dosis evaluadas.

Palabras clave: patógenos de semilla de maíz, control físico, control químico, *Fusarium graminearum, Fusarium subglutinans,* pudrición de tallo.

Introduction

Maize (*Zea mays* L.) currently has a world production of 1,078,080,000 t per year. The United States is the principal producing country followed by China. In South America, Brazil is the main maize producer followed by Argentina and Bolivia (USDA, 2019). Colombia occupies fourth place with yellow maize production of 939,677 t in 2017,

Received for publication: 11 June, 2019. Accepted for publication: 5 December, 2019 Doi: 10.15446/agron.colomb.v37n3.80302

[1] Facultad de Ciencias Agrarias, Universidad Nacional de Colombia, Bogota (Colombia).
* Corresponding author: npinerosg@unal.edu.co

constituting the second largest cereal crop in the country (DANE, 2019). In Colombia, yellow maize crops represent about 326.326 ha nationwide (DANE, 2019). The province of Cundinamarca has 26,953 ha planted with yellow maize, and the Ubate Valley occupies the fifth place of planted areas (DANE, 2016).

The production of cereals like maize currently shows yield limitations due to various factors, such as environmental deterioration and climate change that increase pests and diseases in crops and threaten global food security (FAO, 2016). An important number of diseases in maize are caused by *Fusarium* species. Stalk rot is one of the diseases caused by *Fusarium* spp., and it is present in maize crops around the world (Fumero *et al*., 2015). Species of the genus *Fusarium*, among other organisms (that include fungi, bacteria and oomycetes) are related to this alteration and cause significant damage during the establishment stage of a maize crop (Shin *et al*., 2014). The symptoms of this disease are associated with affected internal stem tissue, characterized by brown streaks in the internodes at early stages and a pink discoloration in mature plants (Shin *et al*., 2014). Several *Fusarium* species, which include *F. graminearum*, *F. temperatum*, *F. subglutinans*, *F. proliferatum* and *F. verticillioides*, have been reported as associated with maize plants with symptoms of this disease (Shin *et al*., 2014; Gromadzka *et al*., 2016; Zhang *et al*., 2016; Gromadzka *et al*., 2018). Some of these fungal species can also produce mycotoxins and may be seed-transmitted, leading to significant yield losses and a decrease in seed quality (Leslie and Summerell, 2006).

The management of seed pathogens in maize can be achieved using several methods. Among these procedures, treatment with fungicides prior to planting is used to protect seeds from fungal infection as well as to limit fungal growth originating from seeds (Munkvold and O'mara, 2002). However, the control of fungal infection in cereal seeds has been managed by physical means such as thermal seed treatments (Coutinho *et al*., 2007).

There are many published studies on treatments for the control of seed pathogens. Much research around the world has been carried out on thermal seed treatments resulting in superior control of the incidence of pathogens (Daniels, 1983; Clear *et al*., 2002; Coutinho *et al*., 2007; Bennett and Colyer, 2010). Clear *et al.* (2002) evaluate the incidence of *F. graminearum* and the viability of wheat and barley seeds after treatment with dry heat at 50°C, 70°C, 60°C and 80°C. The authors find that dry heat treatments can eliminate *F. graminearum* completely from wheat seeds without a significant reduction of seed germination under treatments at 70°C and 80°C. In addition, *F. graminearum* is eliminated in barley seeds showing a significant reduction of germination under treatments of 10 d at 80°C. Coutinho *et al*. (2007) evaluated the sanitary and physiological quality of maize seeds subjected to thermotherapy with hot water at 60°C for 5, 10 and 20 min. The authors report that heat treatments for 10 min and 20 min significantly reduced the percentage of occurrence of *F. verticillioides* in the seeds. Nevertheless, the longest thermal treatments (10 min and 20 min) significantly reduce the seed germination rate compared to treatments of 5 min. Bennett and Colyer (2010) evaluated treatments with dry heat at 60°C, 70°C and 80°C from 2 to 14 d and treatments with hot water at 90°C for 45 s to 180 s for disinfection of cotton seeds infected with *F. oxysporum*. The results show that as the temperature increases with dry heat, seed infection diminishes more quickly. However, treatments at 80°C result in significant alterations of seed germination and vigor. All treatments with hot water show a significant reduction in the incidence of infected seeds compared to untreated seeds. Treatments with water at 90°C for periods of less than 120 s and 150 s do not significantly reduce the vigor of seeds, but the treatments do reduce the incidence of infected seeds from 50 to 10%. These cited studies demonstrate that thermal treatment of seeds could be a promising alternative for controlling maize seed pathogens.

Regarding the use of chemical products for the control of pathogens of the genus *Fusarium*, Broders *et al*. (2007) evaluated under *in vitro* conditions the sensitivity of *F. graminearum* strains isolated from maize seeds to the fungicides azoxystrobin, captan, trifloxystrobin, and fludioxonil. In this study, fludioxonil at 1.0 mg L^{-1} completely controls the radial growth of *F. graminearum* colonies, while azoxystrobin and trifloxystrobin do not exert control even at higher doses (100 mg L^{-1}). These authors also report that captan exerts greater control than strobirulins at a dose of 200 mg L^{-1} without generating a complete inhibition of the radial growth of this species.

Ivić *et al*. (2011) report prochloraz (1.0 mg L^{-1}) as the most effective fungicide among carbendazim, tebuconazole, flutriafol, and metconazole for the inhibition of *F. graminearum* mycelia growth when *F. graminearum*, *F. avenaceum* and *F. verticillioides* isolates from wheat seeds are evaluated.

Solorzano and Malvick (2011) find that treatments of maize seeds with active ingredients fludioxonil and azoxystrobin improve the germination, emergence and yield of maize

plants. Germination values of 81% are obtained under fludioxonil and azoxystrobin compared to values of 74% in control treatments. Emergency values of 89.6% and 85.4% are achieved under seed treatments with azoxystrobin and fludioxonil + mefenoxam respectively, compared to untreated seeds (79.6%). The highest yield values are obtained under treatments with azoxystrobin and fludioxonil compared to control treatments.

Shin *et al*. (2014) evaluated the *in vitro* efficacy of fungicides tebuconazole, difenoconazole, fluquinconazole, azoxystrobin, prochloraz and kresoxim-methyl for the control of *F. temperatum* and *F. subglutinans* associated with stem rot in maize plants. As a result, the authors find that fungicides of the azole group tebuconazole and prochloraz completely inhibit *F. subglutinans* and *F. temperatum* mycelial growth at a dose of 10 µg ml^{-1}. On the other hand, the fungicide kresoxim-methyl limits *F. subglutinans* and *F. temperatum* colony formation at concentrations of 0.1 and 0.01 µg ml^{-1}, respectively. The four azole fungicides completely inhibit the formation of *F. subglutinans* colonies at higher doses (1.0 µg ml^{-1}).

Some of the maize diseases in Colombia caused by fungi such as *Fusarium* have an impact on both crop yield and the quality of the seed used by farmers (Varón and Sarria, 2007). Diseases caused by *Fusarium* species have been reported affecting maize plants in the provinces of Antioquia, Cordoba, Cundinamarca, Santander, Tolima and Valle, showing symptoms associated with stalk and ear rot (Buriticá 1999; Arrieta *et al.,* 2007; Varón and Sarria, 2007).

Nowadays, maize crops in the Ubate Valley in Cundinamarca show symptoms of stalk rot, causing low crop yields. Observations performed in the area in 2017 showed that the incidence of this disease can vary between 9% and 44%, and its impact on production may vary depending on the agronomic management of the crop. Information on the phytosanitary status of the seeds used by producers in this region is scarce and guidelines for seed management have not been defined despite the importance of maize seeds on the dissemination of *Fusarium* species (Duncan and Howard, 2010). Therefore, it is necessary to develop, evaluate and implement sustainable management practices for maize crops in producer areas. Due to the importance of the disease and the lack of management strategies regarding seed treatment, the objective of this study was to determine the effect of thermal treatments on Simijaca variety maize seeds in the presence of pathogens of the genus *Fusarium*. Additionally, the effect of thermal treatments on seedling growth and development was assessed. Finally, the *in vitro* response of *F. graminearum* and *F. subglutinans* isolates from maize plants was evaluated at different doses of commercial fungicides.

Materials and methods

The study was conducted at the Malherbology, Plant Clinic and Phytopathology laboratories of the Faculty of Agricultural Sciences at the Universidad Nacional de Colombia, Bogota campus, during the second and first semester of 2017 and 2018.

Evaluation of the effect of thermal seed treatments on the presence of pathogens of the genus *Fusarium* on seedling growth and development

Naturally infected maize (*Zea mays* L.) seeds of the regional variety Simijaca provided by farmers from the Ubate Valley in Cundinamarca were used as plant material. Prior to the treatments, the seeds were soaked in water for 4 h to facilitate heat conduction in the seed tissues (Daniels, 1983; Coutinho *et al*., 2007). The seeds were then subjected to thermal treatments with two heat sources, hot water and dry heat at temperatures of 50°C, 55°C and 60°C to control for the presence of *Fusarium* in maize seeds, following the methodologies of Bennett and Colyer (2010). An evaporator (Water B-480, BÜCHI Labortechnik AG, Switzerland) was used for seed treatment with hot water. In this case, the seeds were wrapped in gauze and immersed in water at the desired temperatures for 5 min (Coutinho *et al*., 2007). For treatments with dry heat, the seeds were placed in a heat chamber (Model FD 23, BINDER, Germany) at the three temperatures to be evaluated for 10 min (Bennett and Colyer, 2010). Once the heat treatment was carried out, treated and untreated seeds (controls) were disinfected with 5% sodium hypochlorite for 1.0 min, 70% ethanol for 3 min, and sterile distilled water for 2 min following the methodology proposed by Murillo and Munkvold (2008). Seeds were then incubated in Blotter chambers consisting of plastic boxes with absorbent paper moistened with sterile distilled water kept at room temperature (± 20°C) for 10 d (Warham *et al*., 2008). Each chamber contained 40 seeds, and two chambers were used per treatment. A total of seven treatments were evaluated using untreated seeds as controls. The experiment was replicated three times.

The methodologies of Bennett and Colyer (2010) were adapted for the evaluation of the effect of thermal seed treatments on maize seedlings growth and development. The variables of germination percentage, seedling length

and fresh weight of 80 seedlings were evaluated 10 days after treatment (dat). The germination percentage was calculated according to Khodarahmpour (2012). Seedling length was measured from the coleoptile to the longest root with a caliper (Lopes *et al*., 2018), and fresh weight of 80 seedlings was estimated with a precision balance scale (EWB 220-2M, Kern, Germany).

The presence of pathogens of the genus *Fusarium* in maize seeds was evaluated by the severity index in terms of mycelial colonization of the seed, using a diagrammatic severity scale of five grades (Machado *et al*., 2013). Visual assessments were performed at 10 dat in search of symptoms and signs of the pathogen. Those seeds that had the classic *"Starburst"* symptom, which consists of white striae in the pericarp as a product of cell disintegration accompanied by white or pink mycelium colonizing the grain, were considered as affected (Murillo and Munkvold, 2008; Duncan and Howard, 2010). Finally, to corroborate the presence of fungi of the genus *Fusarium* in the treated and untreated (control) maize seeds, seeds were sown in a selective medium of Potato Dextrose Agar (PDA) + benzoxazolin-2(3H)-one (BOA), reported for maize pathogens of the species *F. graminearum, F. subglutinans,* and *F. verticillioides* due to their capacity to degrade BOA and grow on the surface of the amended agar (Vilich *et al*., 1999; Glenn *et al*., 2001; Leslie and Summerell, 2006). Subsequently, for the confirmation of the *Fusarium* species, hyphal tipping was performed from mycelium grown in the PDA+BOA media for the evaluation of coloration and structures formed in PDA and Carnation Leaf Agar medium (CLA) following Leslie and Summerell (2006) and Warham *et al*. (2008).

In vitro evaluation of the effect of commercial fungicides on isolates of pathogens of the genus *Fusarium*

Four monosporic isolates were used (143A and 26B of *F. graminearum,* and 45D and 186A of *F. subglutinans*), which were previously obtained from maize plants showing symptoms of stalk rot in commercial and semi-commercial crops of the Ubate Valley in 2017. These isolates were used to evaluate the effect of different commercial fungicides on the inhibition of radial growth at 20 days after sowing (das) and the inhibition of conidial germination at 24 h. The active ingredients and commercial doses of the study were as follows: fludioxonil + metalaxyl-M (100 ml/100 kg of seed), tebuconazole + trifloxystrobin (16 ml/100 kg of seed), prochloraz + difenoconazole (1.5 ml/100 kg of seed) and carboxin + captan (125 g/100 kg of seed). These active ingredients are used in maize crops and some are registered for seed treatment (ICA, 2019). Three doses were evaluated: 0.5, 1.0 and 1.5 the commercial doses (CD) of each of the fungicides of interest. The methodologies used by Nisa *et al*. (2011) were adapted for the evaluation of radial growth inhibition of the colonies. For this purpose, the fungicides were added after the PDA medium was autoclaved and cooled down to 50°C. Then, disks of 5 mm diameter were extracted from 10-day colonies of each of the isolates to be evaluated and placed in the center of Petri dishes with PDA medium (CM0139, Oxoid®) amended with the concentrations of each of the four fungicides for evaluation (Nisa *et al*., 2011). Petri dishes with PDA medium without fungicides were used as controls. Three plates were used per treatment and placed in an incubator (MLR-351H, SANYO, Japan) at 24°C ± 2.0°C. The experiments were replicated three times. The radial growth of the colonies was measured every 48 h during 20 d to calculate the percentage of inhibition by Equation 1 after Plascencia *et al*. (2003):

$$\% \text{ Ix} = \frac{Xc - Xi}{Xc} \times 100 \qquad (1)$$

where:

% Ix: percentage of radial inhibition.

X_c: mean radius (mm) of the control colony.

X_i: mean radius (mm) of colonies in media with fungicide.

The methodology for the evaluation of conidial germination inhibition was adapted from Steinkellner *et al*. (2005). Sterile 12-well culture plates (Nest® Cat. No. 712001, China) were used. In each well, 1000 µl of the fungicide solutions were placed in a mixture with 200 µl of the conidial suspension, maintaining the respective doses to be evaluated. Macroconidia for the suspensions of each of the isolates were taken from 8-day cultures in the CLA medium (Retana *et al*., 2018) incubated at 22°C ± 2.0°C (MLR-351H, SANYO, Japan). The suspension was adjusted to $5x10^5$ macroconidia ml^{-1} sterile distilled water using a haemocytometer (Steinkellner *et al*., 2005). Three wells were used per treatment and the plates were incubated at 24°C ± 2.0°C in the dark under permanent agitation at 200 rpm (Inkubator 1000 - Unimax 1010, Heidolph, Germany) (Steinkellner *et al*., 2005). Conidial germination was determined after 24 h by randomly evaluating 100 conidia per well of each treatment under a microscope (CX 31, Olympus, Japan). Conidia were considered germinated if the germ tube length was as long as the spore (Steinkellner *et al*., 2005). Subsequently, the percentage of conidial germination inhibition of each treatment was determined to estimate the efficacy of

fungicides. This percentage was estimated using Equation 2 taken from Plascencia *et al.* (2003):

$$\% \text{ Is} = \frac{Sc - \%St}{\% Sc} \times 100 \qquad (2)$$

where:

% Is: percentage of inhibition of conidial germination.

% Sc: percentage of germinated conidia in control at 24 h.

% St: percentage of germinated conidia in media with fungicide at 24 h.

Statistical analysis

The evaluation of the thermal seed treatments was performed under a completely randomized factorial design with seven treatments and three replicates per treatment. For *in vitro* tests with commercial fungicides, a completely randomized factorial design was proposed with four treatments and three replicates per treatment. The results obtained were subjected to an ANOVA for all the trials with a value of $P \leq 0.05$. The differences between the mean results were calculated with the Tukey test ($P \leq 0.05$). The Levene test was performed to check the homoscedasticity of the obtained data and thus be able to perform an analysis of variance. Data were analyzed with the statistical package R Studio® version 3.5.1.

Results and discussion

Effect of thermal treatments of maize seeds on pathogens of the genus *Fusarium*

The symptoms and signs that were observed and evaluated for the determination of the severity index are presented in Figure 1. The presence of fungi of the genus *Fusarium* in treated and untreated maize seeds was corroborated by sowing seeds in PDA + benzoxazolin-2(3H)-one (BOA) medium (Fig. 2). As shown in Figure 2, in all the media amended with BOA no growth of yeasts, bacteria, and filamentous fungi was observed, showing the exclusive growth of *Fusarium* species. These fungi could correspond to the species *F. graminearum, F. subglutinans,* and *F. verticillioides* because of their growth on the BOA amended agar medium (Vilich *et al.*, 1999; Glenn *et al.*, 2001).

FIGURE 1. Mycelial colonization in maize seeds of the regional variety Simijaca 10 days after treatment under Blotter chamber conditions. A) detail of the "starburst" symptom or white striae in the pericarp as a product of cell disintegration with pink coloration on the pericarp. A, B and C) white and pink mycelium colonizing the maize seed.

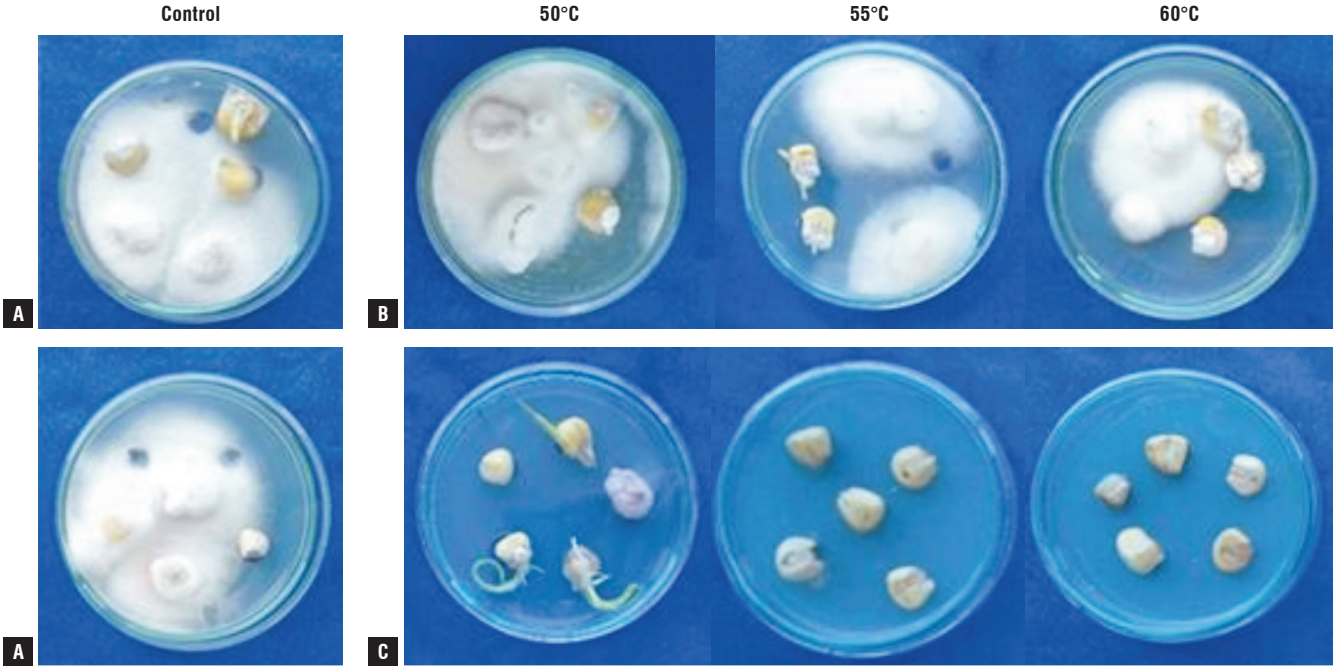


FIGURE 2. Maize seeds of the regional variety Simijaca subjected to heat treatments by two methods at temperatures 50°C, 55°C, and 60°C. A) control. B) dry heat treatment. C) hot water treatment. The figure shows colony growth of pathogens of the genus *Fusarium* in Potato Dextrose Agar (PDA) + benzoxazolin-2(3H)-one (BOA) medium 15 days after sowing (das).

In addition, cultures of these colonies in PDA medium initially exhibited abundant white mycelium that later changed to orange or purple colorations with dark violet close to black pigmentations in the agar. Orange sporodochia and macroconidia with three to four septa, curved apical cells and poorly developed basal cells (as described for *F. subglutinans*) were observed in CLA medium (Leslie and Summerell, 2006). Other colonies showed the growth of yellow or orange mycelium with a feathery appearance that eventually formed reddish pigments on the agar. The presence of pale orange sporodochia containing macroconidia with five to six septa moderately curved, with the ventral parts straight and the dorsal parts arched, the basal cells in the shape of a foot and the straight apical cells corresponded to species of *F. graminearum* were observed in CLA medium as reported by Leslie and Summerell (2006).

The statistical analysis showed significant differences between the heat sources and the different temperatures evaluated with interaction between these two factors ($P<0.05$) for the variable severity index ($P=0.00323$). The effect of thermal treatments on the seed severity index in terms of the percentage of area colonized by *Fusarium* fungi is shown in Figure 3. According to the results, the severity index 10 dat with hot water compared to dry heat corresponded to 3.2% and 16.8% at 50°C, 2.0% and 9.9% at 55°C and 1.4% and 8.0% at 60°C, respectively, in contrast to values of 15.6% in the control treatment (Fig. 3). The severity index was significantly lower in seeds treated with hot water at the three evaluated temperatures and in seeds treated with dry heat at 60°C compared to untreated seeds (control).

The results show that the severity index in seeds decreases as the treatment temperature increases under both methods. From the heat treatments evaluated in this study, the treatment with hot water at the three evaluated temperatures was the most effective in limiting the presence of *Fusarium* in naturally infected maize seeds. These results agree with those reported by Bennett and Colyer (2010) who found that infection in seeds decreased more rapidly with increases in the incubation temperature. A significant reduction in the incidence of infected seeds (10%) compared to non-treated seeds (50%) was obtained with hot water for 105 s. Similar results were also obtained by Rahman and Emran (2008) who report that the lowest incidence of *F. moniliforme* (4%) was found in seeds treated with hot water at 52°C for all the evaluated maize varieties. Coutinho *et al.* (2007) also report that treatments with hot water at 60°C can control *F. verticillioides* in maize seeds. In this study, as the time of heat treatment increased, the incidence of the fungus decreased especially for 10 min and 20 min of treatment.

Clear *et al.* (2002) found that treatments with dry heat for 15 d at 60°C, 5 d at 70°C and 2 d at 80°C eliminated *F. graminearum* completely from wheat seeds without a significant reduction of germination at 70°C and 80°C. Additionally, *F. graminearum* was completely eliminated in barley seeds only after 21 d at 60°C, 9 d at 70°C or 5 d at 80°C, showing a significant reduction of germination after 10 d at 80°C. These results show that to achieve complete control of some *Fusarium* species using dry heat treatments, it would be necessary to treat seeds for several days.

The observed decrease in the severity index of seeds in the hot water treatments could be due to the fact that the temperature acted against the contaminating fungi present in the seed (Rahman and Emran, 2008). Fungal death inside the seed can be generated due to thermal stress or desiccation (Clear *et al.*, 2002). As the temperature increases, it manages to penetrate the seed more deeply eliminating the pathogens that have colonized its interior (Rahman and Emran, 2008).

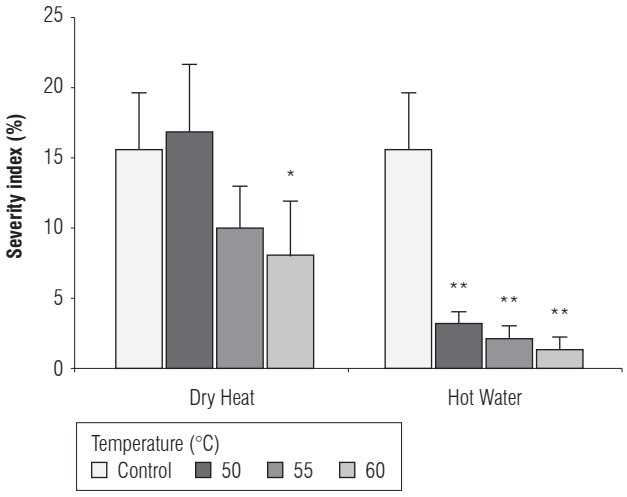


FIGURE 3. Effect of thermal treatments applied by two methods, dry heat and hot water, at temperatures 50°C, 55°C, and 60°C on the severity index of maize seeds of the regional variety Simijaca in terms of percentage of mycelial colonization under laboratory conditions 10 days after treatment (dat). Significant differences between treatments and control are either denoted as * ($P<0.05$) or ** ($P<0.01$). Bars represent the standard error.

Effect of thermal treatments of maize seeds on the variables of seedling growth and development.

The statistical analysis showed significant differences between the heat sources and the different temperatures evaluated, with interaction between these two factors ($P<0.05$) for the variables of seedling length ($P=1.35e-11$) and fresh weight ($P=0.0435$).

Seed germination percentages were significantly higher under dry heat treatments (71.7%) compared to hot water treatments (49.4%). Treatments at 60°C showed significantly lower values (41.6%) compared to those obtained at 50°C, 55°C and control (61.9%, 67.9% and 69.3% respectively) (Fig. 4A). The length of maize seedlings was significantly higher in dry heat treatments compared to hot water treatments, with 13.8 cm and 11.7 cm at 50°C, 14.1 cm and 12.1 cm at 55°C, and 13.4 cm and 7.0 cm at 60°C respectively (Fig. 4B). Regarding fresh weight, the highest values were obtained under dry heat treatments compared to hot water treatments with values of 110.4 g and 92.4 g at 50°C, 107.6 g and 93.1 g at 55°C, and 112.0 g and 86.7 g at 60°C, respectively (Fig. 4C). Fresh weight was significantly lower under hot water treatment at 60°C (Fig. 4C). Similar results were reported by Nega *et al.* (2003) and Rahman and Emran (2008), who underlined that treatments with hot water at 50°C for 20 min to 30 min and up to 53°C for 10 min may result in a significant decrease in infection of seed-borne pathogens without affecting seed germination. However, higher temperatures can generate a decrease in the percentage of germination of sensitive crops. Nega *et al.* (2003) reported that seedlings of treated seeds were smaller than seedlings of untreated seeds. This is, possibly, due to the loss of nutrients during the treatment, resulting in a delay in the normal development of the seedling that can affect the seedling length and fresh weight. Coutinho *et al.* (2007) reported that thermal treatments at 60°C can significantly reduce the germination rate of maize seeds by 30% when subjected to treatment for more than 5 min. These authors reported an alteration in the electrophoretic pattern of the enzymes esterase and malate dehydrogenase, demonstrating a loss of integrity of the membrane system such as the mitochondrial membrane, caused by exposing seeds to a high temperature. The reduction in germination percentages obtained in this study could be due to a loss of the integrity of the seed membrane system with the increase of temperatures. This allows a higher leaching of exudates, which include essential metabolites for the processes of germination and subsequent growth of seedlings (Coutinho *et al.*, 2007).

In this study, treatments with hot water and dry heat at 55°C were the most effective in reducing the colonization of maize seeds by pathogens of the genus *Fusarium* without significantly affecting seedling germination and length. These results were based on the severity indexes obtained and the growth and developmental variables of maize seedlings. Dry heat treatments require higher temperatures and exposure times for generating a significant decrease in seed infection similar to that obtained with hot water (Fig. 3), hindering their implementation in large-scale commercial operations (Bennett and Colyer, 2010).

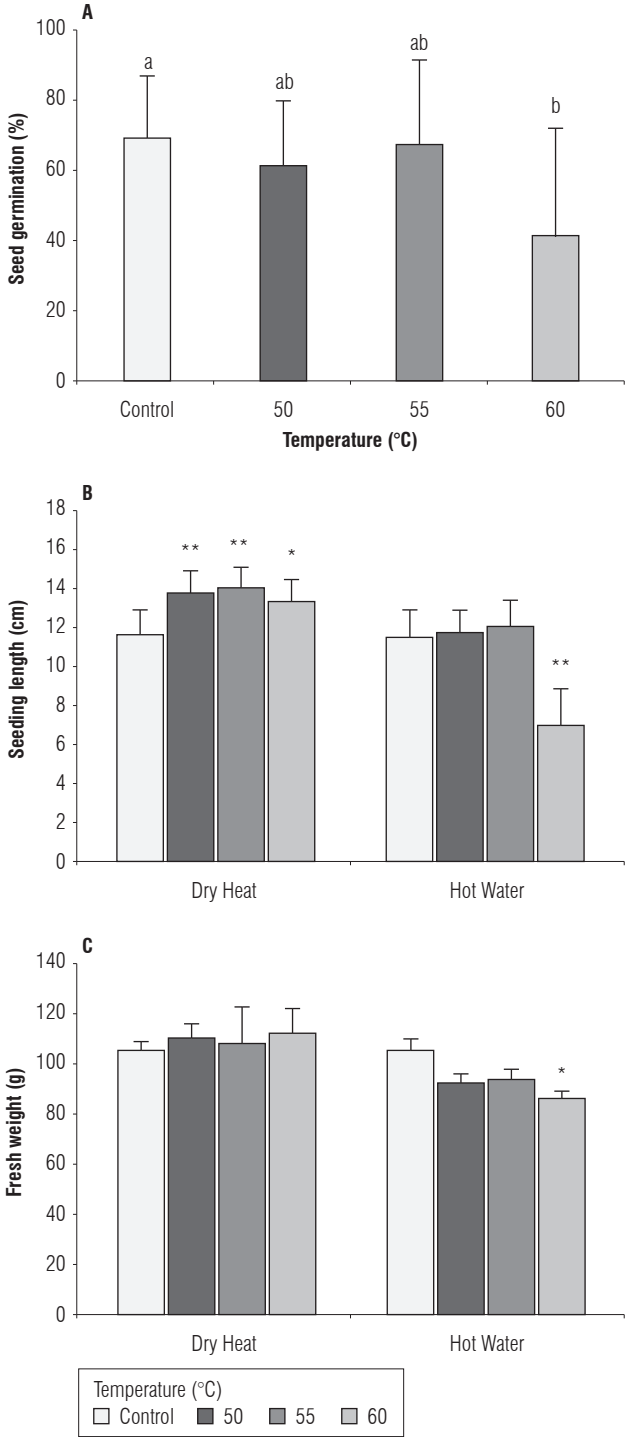


FIGURE 4. Effect of heat treatments applied by two methods, dry heat and hot water at temperatures 50°C, 55°C, and 60°C on A) germination percentage, B) seedling length, and C) fresh weight of maize seedlings of the regional variety Simijaca under laboratory conditions 10 days after treatment (dat). Significant differences between treatments and control are either denoted as * ($P< 0.05$) or ** ($P<0.01$). Bars represent the standard error.

In vitro effect of fungicides on radial growth and germination of conidia of fungi of the genus *Fusarium* associated with stalk rot in maize

The inhibition of the radial growth of *F. subglutinans* and *F. graminearum* colonies showed an interaction between the different evaluated factors. The isolates 45D and 186A of *F. subglutinans* showed 100% inhibition of radial growth under prochloraz + difenoconazole and carboxin + captan at the three evaluated doses. Additionally, isolate 45D of *F. subglutinans* showed 100% inhibition with tebuconazole + trifloxystrobin at the three evaluated doses (Tab.1). The results coincide with that reported by Shin *et al*. (2014), who stated that the most effective fungicides against the mycelial growth of *F. subglutinans* were prochloraz and difenoconazole, which belong to the azoles group.

F. graminearum isolates 143A and 26B showed 100% inhibition of radial growth under the treatments prochloraz + difenoconazole, carboxin + captan, and tebuconazole + trifloxystrobin at the three evaluated doses. Complete inhibition of these two isolates was also observed with fludioxonil + metalaxyl-M at doses of 1.0 and 1.5 CD (Tab.1).

Prochloraz + difenoconazole, carboxin + captan and tebuconazole + trifloxystrobin at the three evaluated doses were also the most effective in inhibiting the radial growth of the evaluated isolates of *F. graminearum*. In contrast, a complete radial inhibition of this species was found with fludioxonil + metalaxyl-M at doses of 1.0 and 1.5 CD. The obtained results match the ones reported by Jones (2000), Broders *et al*. (2007), Ivić *et al*. (2011) and Shin *et al*. (2014).

Jones (2000) found that 1.0 mg L^{-1} of fludioxonil inhibits *F. graminearum* mycelial growth by 100%, as did tebuconazole at a dose of 100 mg L^{-1}. Broders *et al*. (2007) reported that fludioxonil is the most effective fungicide in inhibiting *F. graminearum* mycelial growth with complete inhibition at 1.0 mg L^{-1} compared to azoxystrobin and trifloxystrobin (25 and 35%, respectively). Captan at a dose of 100 mg L^{-1} showed inhibition of up to 20% of *F. graminearum* mycelial growth, which is higher than treatments with strobirulins at the same dose. However, these last two active ingredients do not provide a complete inhibition of this species.

Ivić *et al*. (2011) reported that prochloraz is the most effective fungicide at controlling *F. graminearum, F. avenaceum* and *F. verticillioides,* inhibiting growth by 50% at a dose of 0.1 mg L^{-1}, while flutirafol is the least effective even with doses higher than 8.51 mg L^{-1}. Metconazole is more efficient compared to carbendazim and tebuconazole, inhibiting growth by 50% at a dose of 1.66 for *F. graminearum*. Carbendazim inhibits fungal growth by 50% at a dose of 1.41 mg L^{-1}, and tebuconazole at 2.57 mg L^{-1} for *F. graminearum*.

Shin *et al*. (2014) found that the fungicides of the azole group tebuconazole, difenoconazole, fluquinconazole and prochloraz are the most effective in inhibiting *F. subglutinans* mycelial growth at a dose of 10 µg ml^{-1}. These fungicides were more effective than strobilurins (kresoxim-methyl and azoxystrobin) at the same dose, with 100% inhibition with azoles and 60% with strobilurins. At the same dose, the most effective fungicides to inhibit 100% of *F. temperatum* mycelial growth were tebuconazole and prochloraz.

The counting of conidia germinated in fungicide-amended media is one of the most widely used methods for evaluating fungicide efficacy (Matheron and Porchas, 2000; Shin *et al*., 2014). In this evaluation with the different isolates, the results showed interaction between the evaluated factors. *F. subglutinans* isolates 45D and 186A showed significantly higher values of conidial germination inhibition under

TABLE 1. *In vitro* effect of fungicides on the inhibition of radial growth (%) of *F. subglutinans* (45D and 186A) and *F. graminearum* (26B and 143A) colonies with commercial fungicides fludioxonil + metalaxyl-M, tebuconazole + trifloxystrobin, prochloraz + difenoconazole and carboxin + captan at doses 0.5, 1.0 and 1.5 CD (commercial dose). Mean values in the same column followed by different letters are significantly different according to the Tukey test ($P<0.05$).

	Inhibition of radial growth (%)											
	Fusarium subglutinans						*Fusarium graminearum*					
Isolate	45D			186A			26B			143A		
Fungicide/ Dose (CD)	0.5	1	1.5	0.5	1	1.5	0.5	1	1.5	0.5	1	1.5
Fludioxonil + metalaxyl-M	52.1 b	60.2 b	70.1 b	59.8 c	66.6 c	71.0 c	100 a	100 a	100 a	97.2 b	100 a	100 a
Tebuconazole + trifloxystrobin	100 a	100 a	100 a	87.7 b	88.8 b	91.3 b	100 a	100 a	100 a	100 a	100 a	100 a
Prochloraz + difenoconazole	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a
Carboxin + captan	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a
Control	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

TABLE 2. *In vitro* effect of fungicides on the inhibition of conidial germination (%) of *F. subglutinans* (45D and 186A) and *F. graminearum* (26B and 143A) with the active ingredients fludioxonil + metalaxyl-M, tebuconazole + trifloxystrobin, prochloraz + difenoconazole and carboxin + captan at doses 0.5, 1.0 and 1.5 CD (commercial dose). Mean values in the same column followed by different letters are significantly different according to the Tukey test ($P<0.05$).

	Inhibition of conidial germination (%)											
	Fusarium subglutinans						*Fusarium graminearum*					
Isolate	**45D**			**186A**			**26B**			**143A**		
Fungicide/Dose (CD)	0.5	1	1.5	0.5	1	1.5	0.5	1	1.5	0.5	1	1.5
Fludioxonil + metalaxyl-M	100 a	100 a	100 a	100 a	100a	100a	100 a	100 a	100 a	98.1 a	100 a	100 a
Tebuconazole + trifloxystrobin	96.2 bc	98.9 a	99.6 a	98.2 ab	99.3 a	99.3 a	100 a	100 a	100 a	100 a	100 a	99.2 a
Prochloraz + difenoconazole	95.5 c	96.5 b	96.2 b	97.5 b	97.9 a	98.9 a	100 a	100 a	100 a	95.2 b	93.2 b	95.1 b
Carboxin + captan	97.5 b	98.2 ab	99.3 a	97.2 b	99.3 a	99.3 a	100 a	100 a	100 a	94.0 b	94.3 b	94.3 b
Control	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

fludioxonil + metalaxyl-M at the three evaluated doses (Tab. 2) compared to the dose 0.5 CD of prochloraz + difenoconazole and carboxin + captan in the two isolates, and 0.5 CD of tebuconazole + trifloxystrobin in isolate 45D. For *F. graminearum* isolate 143A, the inhibition values were significantly higher under fludioxonil + metalaxyl-M and tebuconazole + trifloxystrobin at the three evaluated doses compared to prochloraz + difenoconazole and carboxin + captan at the three evaluated doses. For *F. graminearum* isolate 26B, there were no significant differences between the evaluated treatments, showing 100% inhibition of conidial germination (Tab. 2).

These results agree with the ones reported by Munkvold and O'mara (2002), who observed that fludioxonil and difenoconazole are significantly more effective in the control of *F. subglutinans* and *F. graminearum* species compared to fungicides such as captan. However, fludioxonil is significantly more effective than difenoconazole in inhibiting *F. graminearum* isolates. Shin *et al*. (2014) reported that the fungicide kresoxim-methyl (strobilurin) completely inhibits *F. subglutinans* and *F. temperatum* colony formation at concentrations of 0.1 and 0.01 μg ml^{-1} respectively. The four azole fungicides tested (difenoconazole, fluquinconazole, prochloraz and tebuconazole) only completely inhibit the formation of *F. subglutinans* colonies at higher doses (1.0 μg ml^{-1}). The inhibition of conidial germination with fungicides is essential for reaching chemical control of pathogenic fungi in plants since the spread of the disease and colonization of plants is mainly mediated by these reproductive structures of the pathogens (Shin *et al*., 2014).

The different responses to the same active ingredient between *Fusarium* species and even between isolates of the same species are phenotypic characteristics that vary between fungal populations (Müllenborn *et al*., 2008). Differences between isolates of the same species exposed to the same chemical compound could imply a decrease in the sensitivity to the active ingredient or resistance between the evaluated isolates (Ivić *et al*., 2011). In this study, fludioxonil, from the phenyl-pyroles group, caused greater inhibition of conidial germination of the species *F. gramiearum* and *F. subglutinans* possibly due to its action mechanism. This active ingredient alters MAP proteins in the transduction of osmotic signals causing inappropriate activation of the Hog1 MAPK, which is a protein involved in the reproduction of filamentous fungi (Degani, 2015).

Demethylation inhibitors (DMI fungicides), such as prochloraz, have become one of the most utilized fungicide groups in agriculture, being effective in controlling diseases in wheat and soybeans. They are also used in seed treatments, especially for the control of *Fusarium* spp. (Ivić *et al*., 2011). In this research, prochloraz at the three evaluated doses was also effective in controlling the assessed *Fusarium* species associated with maize seeds.

Although the results obtained are a contribution to the management of stalk rot in cold-climate maize producing areas, similar studies with a higher number of isolates of these pathogens are necessary. Additionally, *in vivo* studies to determine the effect of thermal seed treatments and research on fungicides at the commercial crop level are required. If the best treatments found in this study generate a lower yield loss due to stalk rot, they could be implemented in combination with cultural practices that may contribute to the sustainability of maize crops in areas affected by this problem.

Conclusion

Treatments with hot water (5 min) and dry heat at 55°C (10 min) were the most effective in controlling *Fusarium* spp. colonization of maize seeds of the regional variety

Simijaca, without generating a significant reduction of the germination percentage, length and fresh weight of seedlings. The highest values of seed germination, length and fresh weight of maize seedlings were obtained under dry heat treatments. Commercial doses of the evaluated fungicides generated 100% inhibition of radial growth of *F. graminearum* isolates. Commercial doses of prochloraz + difenoconazole and carboxin + captan caused 100% inhibition of *F. subglutinans* isolates. Prochloraz + difenoconazole and carboxin + captan at the three evaluated doses were the most effective in inhibiting the radial growth of the isolates of the two evaluated *Fusarium* species, with no less than 90% effect on conidial germination inhibition. Fludioxonil + metalaxyl-M at the three evaluated doses generated a complete inhibition of conidial germination of all the assessed isolates. Based on the results of this study, active ingredients such as prochloraz + difenoconazole and carboxin + captan at commercial doses could be promising treatments of maize seeds of regional variety Simijaca for the control of pathogens of the genus *Fusarium* associated with stalk rot.

Acknowledgments

The authors would like to thank the Corredor Tecnológico Agroindustrial, the farmers of the Ubate Valley, Cundinamarca and Germán González Strusberg for his contribution to the data analysis of these results.

Literature cited

Arrieta, K., C. Salazar, R.O. Campo, and N. Villareal. 2007. Enfermedades patogénicas en los híbridos de maíz (*Zea mays*) en el medio y bajo Sinú del Departamento de Córdoba. Temas Agrarios 12(1), 58-69. Doi: 10.21897/rta.v12i1.651

Bennett, R.S. and P.D. Colyer. 2010. Dry heat and hot water treatments for disinfecting cottonseed of *Fusarium oxysporum* f. sp. *vasinfectum*. Plant Dis. 94, 1469-1475. Doi: 10.1094/PDIS-01-10-0052

Broders, K.D., P.E. Lipps, P.A. Paul, and A.E. Dorrance. 2007. Evaluation of *Fusarium graminearum* associated with corn and soybean seed and seedling disease in Ohio. Plant Dis. 91, 1155-1160. Doi: 10.1094/PDIS-91-9-1155

Buriticá, C.P. 1999. Directorio de patógenos y enfermedades de las plantas de importancia económica en Colombia. ICA -Universidad Nacional de Colombia. Medellin, Colombia.

Clear, R.M., S.K. Patrick, R. Wallis, and T.K. Turkington. 2002. Effect of dry heat treatment on seed-borne *Fusarium graminearum* and other cereal pathogens. Plant Pathol. 24, 489-498. Doi: 10.1080/07060660209507038

Coutinho, W., R. Silva, M. Vieira, C. Machado, and J. Machado. 2007. Qualidade sanitária e fisiológica de sementes de milho submetidas a termoterapia e condicionamento fisiológico. Fitopatol. Bras. 32, 458-464. Doi: 10.1590/S0100-41582007000600002

DANE, Departamento Administrativo Nacional de Estadística. 2016. 3° Censo nacional agropecuario. URL: https://www.dane.gov.co/files/images/foros/foro-de-entrega-de-resultados-y-cierre-3-censo-nacional-agropecuario/CNATomo2-Resultados.pdf (accessed May 2018).

DANE, Departamento Administrativo Nacional de Estadística. 2019. Boletín técnico comunicación informativa. Encuesta nacional agropecuaria ENA-2017. URL: https://www.dane.gov.co/files/investigaciones/agropecuario/enda/ena/2017/boletin_ena_2017.pdf (accessed November 2019).

Daniels, B.A. 1983. Elimination of *Fusarium moniliforme* from corn seed. Plant Dis. 67, 609-611. Doi: 10.1094/PD-67-609

Degani, O. 2015. Mediation of fludioxonil fungicide activity and resistance through *Cochliobolus heterostrophus* G-protein and MAPK signaling pathways. Phytoparasitica 43(2), 215-228. Doi: 10.1007/s12600-014-0434-1

Duncan, K.E. and R.J. Howard. 2010. Biology of maize kernel infection by *Fusarium verticillioides*. Mol. Plant Microbe In. 23(1), 6-16. Doi: 10.1094/MPMI-23-1-0006

FAO, Organización de la Naciones Unidas para la Alimentación y la Agricultura. 2016. Ahorrar para crecer en la práctica maíz-arroz-trigo. Guía para la producción sostenible de cereales. Rome.

Fumero, M.V., M.M. Reynoso, and S. Chulze. 2015. *Fusarium temperatum* and *Fusarium subglutinans* isolated from maize in Argentina. Int. J. Food Microbiol. 199, 86-92. Doi: 10.1016/j.ijfoodmicro.2015.01.011

Glenn, A.E., D.M. Hinton, I.E. Yates, and C.W. Bacon. 2001. Detoxification of corn antimicrobial compounds as the basis for isolating *Fusarium verticillioides* and some other *Fusarium* species from corn. Appl. Environ. Microbiol. 67(7), 2973-2981. Doi: 10.1128/AEM.67.7.2973-2981.2001

Gromadzka, K., J. Chełkowski, A. Basińska-Barczak, and J. Lalak -Kańczugowska. 2018. Diversity and mycotoxin production by *Fusarium temperatum* and *Fusarium subglutinans* as causal agents of pre-harvest *Fusarium* maize ear rot in Poland. J. Appl. Gen. 60(1), 113-121. Doi: 10.1007/s13353-018-0478-x

Gromadzka, K., K. Górna, J. Chełkowski. and A. Waśkiewicz. 2016. Mycotoxins and related *Fusarium* species in preharvest maize ear rot in Poland. Plant Soil Environ. 62(8), 348-354. Doi: 10.17221/119/2016-PSE

ICA, Instituto Colombiano Agropecuario. 2019. Registros Nacionales, septiembre 17, 2019 (accessed November 2019).

Ivić, D., Z. Sever, and B. Kuzmanovska. 2011. *In vitro* sensitivity of *Fusarium graminearum, F. avenaceum* and *F. verticillioides* to carbendazim, tebuconazole, flutriafol, metconazole and prochloraz. Pestic. Phytomed. 26(1), 35-42. Doi: 10.2298/PIF1101035I

Jones, R.K. 2000. Assessments of *Fusarium* head blight of wheat and barley in response to fungicide treatment. Plant Dis. 84(9), 1021-1030. Doi: 10.1094/PDIS.2000.84.9.1021

Khodarahmpour, Z. 2012. Evaluation of maize *(Zea mays* L.) hybrids, seed germination and seedling characters in water stress conditions. Afr. J. Agric. Res. 7(45), 6049-6053. Doi: 10.5897/AJAR12.2113

Leslie, J.F. and B.A. Summerell. 2006. The *Fusarium* laboratory manual. Blackwell publishing, Iowa, USA. Doi: 10.1002/9780470278376

Lopes, C.A., M.L.M.D. Carvalho, M.C. Vasconcelos, A.M.P. Ribeiro, and H.O.D. Santos. 2018. Primary leaf growth and coleoptile length in maize seedlings. Acta Sci. Agron. 40, 2-8. Doi: 10.4025/actasciagron.v40i1.35366

Machado, J.D.C., A. Machado, E.A. Pozza, C.F. Machado, and W. Zancan. 2013. Inoculum potential of *Fusarium verticillioides* and performance of maize seeds. Trop. Plant Pathol. 38(3), 213-217. Doi: 10.1590/S1982-56762013000300005

Matheron, M.E. and M. Porchas. 2000. Impact of azoxystrobin, dimethomorph, fluazinam, fosetyl-Al, and metalaxyl on growth, sporulation, and zoospore cyst germination of three *Phytophthora* spp. Plant Dis. 84(4), 454-458. Doi: 10.1094/PDIS.2000.84.4.454

Müllenborn, C., U. Steiner, M. Ludwig, and E. Oerke. 2008. Effect of fungicides on the complex of *Fusarium* species and saprophytic fungi colonizing wheat kernels. Eur. J. Plant Pathol. 120, 157-166. Doi: 10.1007/s10658-007-9204-y

Munkvold, G.P. and J.K. O'mara. 2002. Laboratory and growth chamber evaluation of fungicidal seed treatments for maize seedling blight caused by *Fusarium* species. Plant Dis. 86(2), 143-150. Doi: 10.1094/PDIS.2002.86.2.143

Murillo, A. and G.P. Munkvold. 2008. Systemic infection by *Fusarium verticillioides* in maize plants grown under three temperature regimes. Plant Dis. 92(12), 1695-1700. Doi: 10.1094/PDIS-92-12-1695

Nega, E., R. Ulrich, S. Werner, and M. Jahan. 2003. Hot water treatment of vegetable seed an alternative seed treatment method to control seed-borne pathogens in organic farming. J. Plant Dis. Protect. 110(3), 220-234.

Nisa, T., M.Y. Bhat, S.A. Pala, and R.A. Mir. 2011. *In vitro* inhibitory effect of fungicides and botanicals on mycelial growth and spore germination of *Fusarium oxysporum*. J. Biopest. 4(1), 53-56.

Plascencia, M., G. Viniegra, R. Olayo, M. Castillo, and K. Shirai. 2003. Effect of chitosan and temperature on spore germination of *Aspergillus niger.* Macromol. Biosci. 3(10), 582-586. Doi: 10.1002/mabi.200350024

Rahman, M. and A. Emran. 2008. Hot water thermal treatment for controlling seed-borne mycoflora of maize. Int. J. Sustain. Crop Prod. 3(5), 5-9.

Retana, K., J.A.R. Coché, O. Castro, and M. Blanco. 2018. Caracterización morfológica y molecular de *Fusarium oxysporum* f.sp. *Apii* asociado a la marchitez del apio en Costa Rica. Agron. Costarricense 42(1), 115-126. Doi: 10.15517/rac.v42i1.32199

Shin, J., J. Han, J. Lee, and K. Kim. 2014. Characterization of the maize stalk rot pathogens *Fusarium subglutinans* and *F. temperatum* and the effect of fungicides on their mycelial growth and colony formation. Plant Pathol. J. 30(4), 397-406. Doi: 10.5423/PPJ.OA.08.2014.0078

Solorzano, C.D. and D.K. Malvick. 2011. Effects of fungicide seed treatments on germination, population, and yield of maize grown from seed infected with fungal pathogens. Field Crop Res. 122(3), 173-178. Doi: 10.1016/j.fcr.2011.02.011

Steinkellner, S., R. Mammerler, and H. Vierheilig. 2005. Microconidia germination of the tomato pathogen *Fusarium oxysporum* in the presence of root exudates. J. Plant Interact. 1(1), 23-30. Doi: 10.1080/17429140500134334

USDA, United States Department of Agriculture. 2019. World Agricultural Production. Circular Series WAP 10-19 (accessed November 2019).

Varón, F. and G. Sarria. 2007. Enfermedades del maíz y su manejo. Instituto Colombiano Agropecuario. Produmedios, Palmira, Colombia.

Vilich, V., B. Löhndorf, R.A. Sikora, and A. Friebe. 1999. Metabolism of benzoxazolinone allelochemicals of *Zea mays* by *Fusarium subglutinans.* Mycol. Res. 103(12), 1529-1532. Doi: 10.1017/S0953756299008862

Warham, E.J., L.D. Butler, and R.C. Sutton. 2008. Ensayos para la semilla de maíz y de trigo: manual de laboratorio. CIMMYT, Mexico.

Zhang, H., B. Brankovics, W. Luo, J. Xu, J.S. Xu, C. Guo, C. Waalwijk. 2016. Crops are a main driver for species diversity and the toxigenic potential of *Fusarium* isolates in maize ears in China. World Mycotoxin J. 9(5), 701-715. Doi:10.3920/wmj2015.2004

Diversity of arbuscular mycorrhizal fungi communities associated with cape gooseberry (*Physalis peruviana* L.) crops

Diversidad de comunidades de hongos formadores de micorrizas arbusculares asociados a los cultivos de uchuva (*Physalis peruviana* L.)

Margarita Ramírez-Gómez[1]*, Urley Pérez-Moncada[1], Diana Serralde-Ordoñez[1], Andrea Peñaranda-Rolón[1], Gabriel Roveda-Hoyos[2], and Alia Rodriguez[3]

ABSTRACT

The diversity of arbuscular mycorrhizal fungi (AMF) communities in agricultural systems depends on biotic and abiotic factors as well as on cultural practices. This research aimed to evaluate the diversity of AMF present in an altitudinal transect cultivated with cape gooseberry (*Physalis peruviana* L.). A set of 13 soil samples from cape gooseberry plantations located in the Colombian Andean mountains in the provinces of Cundinamarca and Boyaca were collected during dry (0-20 mm/month) and rainy (150-330 mm/month) seasons between 1500 and 3000 m a.s.l., in order to establish the relationship between the altitudinal characteristics and AMF diversity. The evaluation of the abundance of spores and species and diversity indexes showed the presence of 46 AMF species in the dry season and 31 in the rainy season. This shows the high diversity of AMF in the tropical Andes with spore abundance between 20 and 120 spores 10 g^{-1} of soil in the rainy season and between 127 and 1531 spores 10 g^{-1} of soil in the dry season.

Key words: diversity, richness, Colombian Andes, Glomeromycota.

RESUMEN

La diversidad de las comunidades hongos formadores de micorrizas (HFMA) en sistemas agrícolas depende de factores bióticos y abióticos, así como de prácticas culturales. La investigación tuvo como propósito evaluar la diversidad de los HFMA presentes en un transecto altitudinal (1500 a 3000 msnm) cultivado con uchuva (*Physalis peruviana* L.). Se recolectaron 13 muestras compuestas de suelo de plantaciones de uchuva localizadas en Los Andes colombianos de los Departamentos de Cundinamarca y Boyacá, durante las temporadas seca (0-20 mm/mes) y lluviosa (150-330 mm/mes), para establecer la relación entre las características altitudinales y la diversidad de HFMA. La evaluación de la abundancia de esporas y especies e índices de diversidad evidenció la presencia de 46 especies de HFMA en época seca y 31 en época de lluvias. Esto muestra la alta diversidad de HFMA en los Andes tropicales, con una abundancia entre 20 y 120 esporas 10 g^{-1} de suelo en temporada de lluvias y entre 127 y 1531 esporas 10 g^{-1} de suelo en época seca.

Palabras clave: diversidad, riqueza, Andes Colombianos, Glomeromycota.

Introduction

One of the symbiotic associations with the greatest geographic and botanical distributions is the interaction between arbuscular mycorrhizal fungi (AMF), which covers more than 80% of plant species and is found in a great diversity of ecosystems (Brachmann and Parniske, 2006; Bonfante and Genre, 2008). A bi-directional exchange of nutrients is the basis of this association (Breuninger and Requena, 2004; Genre *et al*., 2005, 2008), which favors plant nutrition and plant tolerance to biotic or abiotic stress (Van der Heijden and Sanders, 2002; Smith and Read, 2008; Smith and Smith, 2011). To understand this complex symbiotic association, it is necessary to know the environment in which it is developed and the factors that affect the establishment and functioning of AMF communities. Many factors affect the dynamics of this symbiosis, such as geophysical factors (i.e. altitude) or the different stages of plant development that influence the composition of AMF communities (Husband *et al*., 2002a, b; Oehl *et al*., 2006; Senés *et al*., 2014). Senés *et al*. (2014) evaluated the composition of AMF communities in the Peruvian Andes in potato crops at four different altitudes from 2,658 to 4,075 m a.s.l., and they found a direct relationship between altitude and the community composition of AMF species. Some factors that affect the structure, diversity and distribution of AMF

Received for publication: 3 August, 2018. Accepted for publication: 3 December, 2019 Doi: 10.15446/agron.colomb.v37n3.74008

[1] Corporación Colombiana de Investigación Agropecuaria, AGROSAVIA. Centro de investigación Tibaitatá, Mosquera, Cundinamarca (Colombia).

[2] Ingeniero Agrónomo. PhD.

[3] Departamento de Biología, Facultad de Ciencias, Universidad Nacional de Colombia, Bogota (Colombia).

* Corresponding author: mmramirez@agrosavia.co

communities are soil microorganism populations (Garbaye, 1994; Gehring and Whitham, 2002), agricultural practices, such as logging, burning, use of fertilizer and tillage (Jansa *et al*., 2003), and indirectly microclimate and topography (Johnson, *et al*., 1992; Kernaghan, 2005).

Cape gooseberry (*Physalis peruviana* L.) belongs to the Solanaceae family and is distributed in the wild highlands of the South American Andes (Pérez, 1996; Trillos *et al*., 2008), its place of origin (Morton, 1987; Bartholomäus *et al*., 1990; Medina, 1991; Criollo and Ibarra, 1992; Chia *et al*., 1997). In Colombia, the optimal conditions for its cultivation include altitudes between 2300 and 2800 m a.s.l., temperatures between 13 and 17°C, relative humidity between 70 and 80%, and precipitation between 600 and 1100 mm/year (Fischer, 2000; Espinal *et al*., 2005). The interest in working with this plant species is based on the fact that the plant is native to the Andes and has a wide range of edapho-climatic adaptations (Fischer, 2000) that may be related to its ability to associate with AMF.

The objective of this study was to evaluate AMF diversity in Andean soils cultivated with cape gooseberry to determine if the composition of AMF communities is modulated by altitude. The possible effect of altitude on the establishment of AMF communities is fundamental for understanding symbioses and finding behavioral patterns in AMF communities that would allow a better management of agroecosystems.

Materials and methods

Soil sampling

Sampling was performed on an altitudinal transect between 1500 and 3000 m a.s.l. Composite samples of cape gooseberry rhizospheric soils were collected at 13 sites. At each site 4 kg (15 subsamples) of soils were collected at a depth of 0-20 cm, in duplicate, for physicochemical and AMF analysis (Tab. 1). From each sample, 200 g were taken for analysis of the abundance and diversity of AMF spores, in duplicate. The remaining soil was stored to be used as inoculum or as a source of spores for a plant tramp assay. Two samplings were carried out: one in the rainy season (150-330 mm/month) and the other in the dry season (0-20 mm/month).

Isolation and identification of AMF spores

For each sample, the number of spores 10 g^{-1} of soil was determined according to the methodology described by Gerdermann and Nicholson (1963), with modifications. The percentage of AMF colonization was estimated using the Trypan Blue differential staining methodology by Phillips and Hayman (1970) and Giovannetti and Mosse (1980) with modifications. The taxonomic classification of the AMF was performed at the species level based on the morphology of the spores. The spores were isolated and arranged in sheets with polyvinyl lactic acid-glycerin (PVLG) (Koske and Tessier, 1983) and, in some cases, with a mixture (1:1 v/v) of PVLG with Melzer (Brundrett *et al*.,

TABLE 1. Sampling sites, altitude, soil taxonomy, soil pH, organic matter (OM) and phosphorus (P) content in Cundinamarca and Boyaca.

	Location	Nomenclature	Taxonomic classification	Altitude (m a.s.l.)	pH		OM (%)		P (mg kg^{-1})	
				Sampling season						
				R	D	R	D	R	D	
Cundinamarca	Zipacon	Z1	Andic Dystrudepts	2675	5.9	5.1	16.0	11.15	24.3	49.0
		Z2	Andic Dystrudepts	2627	6.0	5.1	17.8	13.15	13.8	39.0
	Granada	G1	Andic Dystrudepts	2380	5.9	5.18	16.6	13.63	32.6	63.1
		G2	Dystric Eutrudepts	2302	5.5	5.18	22.5	14.34	54.0	50.4
		G3	Dystric Eutrudepts	2250	5.5	5.15	14.7	12.68	12.0	30.3
		G4	Dystric Eutrudepts	2000	5.4	5.00	8.3	9.34	5.0	30.4
	Mosquera	M1	Aeric Epiaquents	2560	5.6	5.10	6.1	14.22	35.0	53.0
	Alban	A	Dystric Eutrudepts	1639	6.0	5.14	12.8	12.92	3.6	70.1
Boyaca	Combita	C1	Typic Humitropept	2869	4.9	5.3	7.1	14.40	32.7	53.1
		C2	Typic Humitropept	2930	5.0	5.21	8.6	14.60	62.4	62.2
		C3	Typic Humitropept	2750	5.2	5.2	10.7	14.46	15.8	36.8
	Arcabuco	A1	Oxic Humitropept	2575	5.6	5.07	8.3	12.58	9.4	81.8
		A2	Oxic Humitropept	2636	5.0	5.11	9.4	11.97	19.6	68.3

R: Rainy, D: Dry.

1994). The isolated and identified spores corresponded to the two sampling periods, dry season (<20 mm/month) and rainy season (150-350 mm/month). The classification codes of Schenck and Pérez (1990) and INVAM (International Culture Collection of Vesicular Arbuscular Mycorrhizal Fungi (http://invam.caf.wvu.edu/cultures/cultsearch.htm)) were used, along with scientific publications as support for the genus or species classification (Morton and Benny, 1990; Blazkowski, 1991; Morton and Redecker, 2001; Schüßler *et al*., 2001; Oehl and Sieverding, 2004; Walker and Schüßler, 2004; Blaskowski *et al*., 2006, 2008; Sieverding and Oehl, 2006; Palenzuela *et al*., 2008; Alves Da Silva *et al*., 2009; Oehl *et al*., 2008, 2010, 2011 a, b, c, d; Goto, *et al*., 2011; Redecker *et al*., 2013).

Diversity index

Density (DE), richness (R), relative abundance (RA), isolation frequency (IF), Shanon-Wiener diversity index (H´), uniformity index (E), Simpson dominance index (D) and Simpson-Gini diversity index (Y) were used to determine AMF diversity in each sample and between all 13 samples collected. Indices were applied at the species level (Franke-Snyder *et al*., 2001; Zhang *et al*., 2004; Rodríguez *et al*., 2005; Zhao and Zhao, 2007; Kwasna *et al*., 2008; Chifflot *et al*., 2009).

Statistical analysis

Correlations were performed between spore diversity, abundance and richness of species. Multiple regressions were used for the diversity indexes and abundance and richness variables, as well as correlations between diversity variables, using the SAS program, version 10.

Results and discussion

AMF Communities

The presence of plant-AMF symbiotic associations was measured as percentages of colonization of cape gooseberry roots and the total number of spores present in the rhizosphere of the plant to verify the interaction of these AMF communities with the plant.

The results showed that the highest percentages of colonization occurred during the rainy season for most municipalities (Fig. 1), except for G1, A2 and A, with values between 7.4 and 68.5%. Municipalities G4, A1, C3 and G2 were noted for having a higher percentage of colonization, and lower values were seen in G1, A2 and Z2. In the dry season, the colonization range was between 2 and 22%. The higher values were recorded in G1, G4, A and A2, and the lowest were seen in G3, C1 and Z2 (Fig.1). In all samples evaluated, the presence of AMF associated with roots of cape gooseberry plants was registered, independent of colonization rates, demonstrating that it is a mycotrophic species.

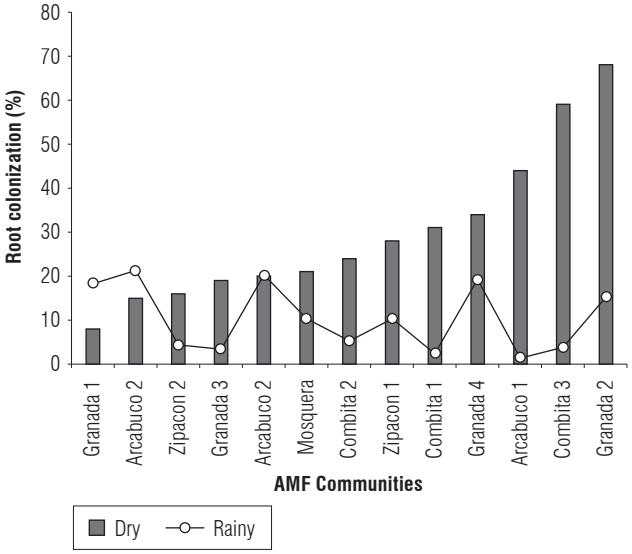


FIGURE 1. Natural colonization levels (%) of cape gooseberry roots by AMF at two sampling seasons, dry and rainy.

The number of spores varied between 20 and 120 spores 10 g^{-1} of soil during the rainy season. The higher spore number values were seen in municipalities A and C1, and the smallest amount was recorded in Z1. However, the dry season presented the highest spore values, between 170 and 1531 spores 10 g^{-1} of soil, in G3 and G4, respectively (Fig. 2).

During the rainy season, a negative correlation was observed in the number of spores, since the highest number of spores was recorded at the lowest altitudes. During the dry season, the correlation was positive, since a greater number of spores was observed at higher altitudes. These results agree with publications that show how, under water stress conditions, AMF sporulate by increasing the production of spores g^{-1} of soil (Caproni *et al*., 2003; Roveda *et al*., 2012; Pagano *et al*., 2013).

Taxonomic identification of AMF

A total of 46 species, grouped in 16 genera, 11 families and 5 orders, were taxonomically identified, illustrating the great diversity of AMF found in the Colombian Andean soils. The distribution of species and genera of AMF identified in each of the evaluated locations during dry and rainy seasons can be seen in Table 2. The results show 23 species for Alban, 12 for Mosquera, 25 for Zipacon and 35 for Granada, the latter presenting the greatest diversity of AMF species. Three types of spores that had not previously been described were found: two of them corresponded to the genus *Glomus* and the other was found from the genus

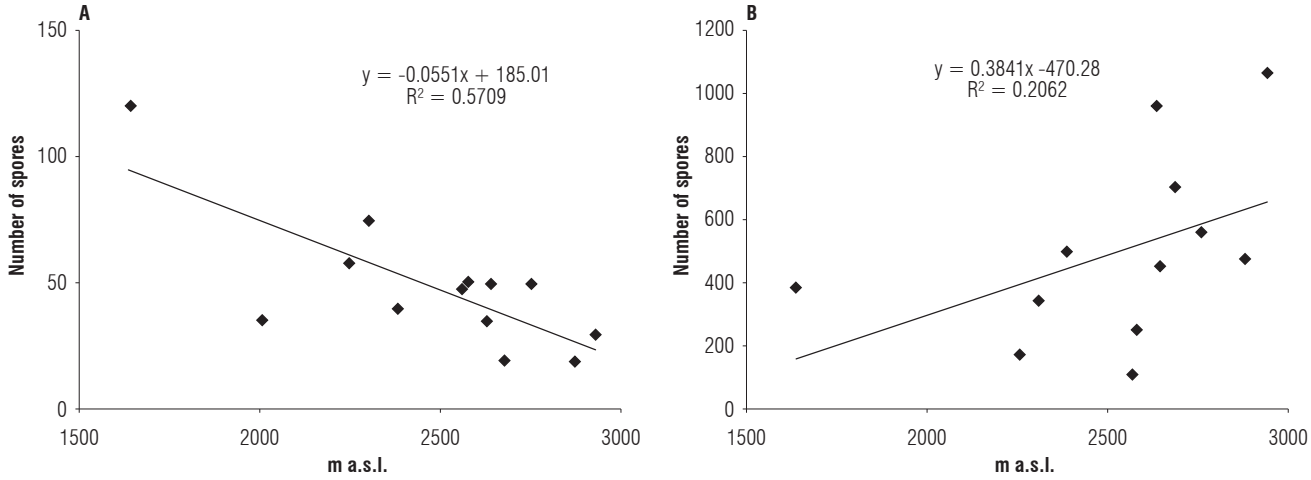


FIGURE 2. Relationship between altitude and number of AMF spores grown with cape gooseberry in the provinces of Cundinamarca and Boyaca. a) Rainy season and b) dry season.

Acaulospora (Personal communication from F. Oehl and E. Sieverding), which were isolated from the soils of Granada, Zipacon and Combita. In the altitudinal transect (1636-2675 m a.s.l.) of the province of Cundinamarca, between 4 and 18 species were found in the soils during the rainy season and between 18 and 33 were found in the dry season in consolidated zones of cape gooseberry production (more than 20 years). For the altitudinal transect of the province of Boyaca (2572 to 2869 m a.s.l.), between 6 and 13 species were identified in the rainy season and between 11 and 17 in the dry season, in a zone that is considered as new for cape gooseberry cultivation (between 5 and 7 years).

It is important to point out that five AMF species were not identified in soils of the consolidated zones of cape gooseberry production in Cundinamarca: *Acaulospora* sp2, *A. scrobiculata*, *A. rehmii*, *A. colombiana* and *Paraglomus laccatum*. In a recent crop production in the province of Boyaca, 13 AMF species were not identified: *Glomus* sp1, *Rhizoglomus fasciculatum, R. proliferum, Funneliformis geosporum, F. coronatus, F. monosporum*, *Septoglomus constrictum*, *Claroideoglomus walkerii*, *Acaulospora longula*, *A. morrowiae, Acaulospora* sp1, *Intraspora* sp. and *P. occultum*. Mahdai *et al.* (2017) reported a higher density of AMF spores associated with a coffee crop (256 spores 100 g^{-1} soil) at higher altitudes (1400 m a.s.l.) as compared to lower altitudes (700 m a.s.l.) in the mountains of Saudi Arabia.

AMF species

The total number of species in the rainy season was 31, while in the dry season it was 46. Regardless of the sampling time, the highest number of species was observed in sample G4, followed by A, while the lowest values were observed in G1 and G3.

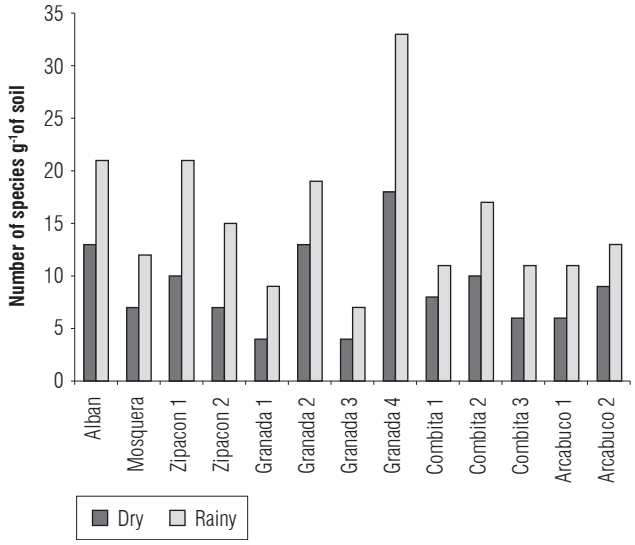


FIGURE 3. Number of AMF species (10 g^{-1} of soil) identified in the cape gooseberry crop in Cundinamarca and Boyaca at two sampling seasons, dry and rainy.

At the seasonal level, a high diversity in AMF communities was also observed, which was expressed as a higher number of spores, richness and relative abundance of species in the dry season compared to the wet season in which higher levels of root colonization were detected. Similar results have been obtained by several authors in the dry season (Pagano *et al*., 2013; Guadarrama *et al*., 2014; Rabelo *et al*., 2014) as well as in the wet season (Guadarrama and Álvarez-Sánchez, 1999). The seasonal variation of the communities was evident by the differences found in the number of species between the dry (46) and rainy (31) seasons, of which 32.6% of the species were not isolated in the rainy season. These results are in agreement with previous reports on seasonal variations of AMF communities

TABLE 2. Distribution of AMF genus and species in soil samples cultivated with cape gooseberry in an altitudinal transect between 1636 and 2869 m a.s.l. (R: Rainy, D: Dry).

Location	Cundinamarca																Boyaca									
	ALBAN		MOSQUERA		ZIPACON				GRANADA								COMBITA						ARCABUCO			
	A		M		Z1		Z2		G1		G2		G3		G4		C1		C2		C3		A1		A2	
Sample season	R	D	R	D	R	D	R	D	R	D	R	D	R	D	R	D	R	D	R	D	R	D	R	D	R	D
	Species																									
Glomus macrocarpum	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Glomus brohutii		*			*	*			*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Rhizoglomus intraradices	*	*	*	*	*	*	*	*			*	*			*	*				*			*	*	*	*
Rhizoglomus aggregatum																*					*			*		
Rhizoglomus irregulare		*				*																		*		
Glomus sinuosum		*																		*						
Glomus microcarpus		*		*		*	*	*	*	*			*	*	*	*	*	*		*	*	*				*
Glomus sp1					*	*	*	*			*	*			*	*										
Glomus sp 2						*						*				*				*						
Rhizoglomus fasciculatum						*						*				*										
Rhizoglomus proliferus	*	*									*	*			*											
Funneliformis mosseae	*	*	*	*				*			*	*			*	*		*	*	*					*	*
Funneliformis geosporus	*			*	*	*										*										
Funneliformis coronatus						*										*										
Funneliformis monosporus		*				*										*										
Simioglomus hoii		*																				*		*		
Septoglomus constricum																*										
Clareidoglomus claroideum	*	*	*	*		*	*	*		*	*	*		*	*	*	*	*	*	*	*	*			*	*
Clareidoglomus etunicatum		*				*		*		*	*	*		*	*	*	*	*	*	*		*			*	*
Clareidoglomus drummondii				*													*	*								
Claroideoglomus luteum				*			*	*			*	*		*	*	*				*						
Claroideoglomus walkeri												*														
Diversispora celata	*															*								*	*
Diversispora versiformis																*										*
Entrophospora infrequens					*	*		*		*	*	*			*	*			*	*		*		*	*	*
Entrophospora nevadensis						*		*				*				*				*		*		*		*
Acaulospora longula											*				*	*										
Acaulospora morrowiae					*	*	*	*								*										
Acaulospora sp 1		*										*				*										
Acaulospora sp 2																									*	*
Acaulospora sp 3								*				*			*	*	*	*		*						
Acaulospora scrobiculata	*	*		*													*	*								
Acaulospora rehmii	*	*																							*	
Acaulospora spinosa																*						*	*	*	*	
Acaulospora denticulata									*	*			*	*		*								*	*	
Kuklospora colombiana																						*	*	*	*	
Pacispora sp	*	*	*	*		*		*							*	*		*	*	*						
Scutellospora nodosa	*	*						*		*	*	*			*	*		*	*	*						

CONTINUATION TABLE 2. Distribution of AMF genus and species in soil samples cultivated with cape gooseberry in an altitudinal transect between 1636 and 2869 m a.s.l. (R: Rainy, D: Dry).

Location	Cundinamarca																Boyaca									
	ALBAN		MOSQUERA		ZIPACON				GRANADA								COMBITA						ARCABUCO			
	A		M		Z1		Z2		G1		G2		G3		G4		C1		C2		C3		A1		A2	
Sample season	R	D	R	D	R	D	R	D	R	D	R	D	R	D	R	D	R	D	R	D	R	D	R	D	R	D
Species																										
Racocetra tropicana		*	*	*							*	*			*	*						*				
Cetrospora pellucida	*	*								*		*			*	*				*						
Intraspora sp		*	*	*	*	*									*	*						*				
Archeospora trappei					*	*													*							
Ambispora sp	*	*														*										*
Ambispora appendicula					*	*													*							
Paraglomus occultum						*		*								*										
Paraglomus laccatum																				*						
Species	13	21	7	12	10	21	7	15	4	9	13	19	4	7	18	33	8	11	10	17	6	12	6	11	9	13

(Courty *et al*., 2008; Davey *et al*., 2012; Bonfim *et al*., 2013; Guadarrama *et al*., 2014). Some authors have reported similar effects of the rainy season on root colonization to those obtained in the present study (Rabatin, 1979; Allen, 1983; Lodge, 1989; Miller, 2000; Miller and Sharitz, 2000), while other authors observed no effect (Bryla and Duniway, 1997; Ming and Hui, 1999). The presence of spores and the different levels of root colonization showed the existence of an active interaction between AMF and cape gooseberry plants in the Andean soils. The dry season increased the number of spores.

In the present study, a high number of spores was found in 10 g of soil, both in the rainy season (20-120 spores g^{-1}) and dry season (170-1531 spores g^{-1}). These values were higher than those reported by Jayachandran and Shetty (2003) for the wetlands of the Everglades (18-124 spores g^{-1}) and by Lopes *et al*. (2013) in humid forests and pastures in Brazil (2.5 and 77.9 spores g^{-1}), where the number of species varied between 31 and 46 for the rainy and dry seasons, respectively. These values are similar to those reported in the Chilean Andes (39 species) (Castillo, 2005; Castillo *et al*., 2005), in the Amazonian-pasture forest in Brazil (36 species) (Lopes *et al*., 2013), and in the forests of Mexico (37 species) (Violi *et al*., 2008). A lower diversity of species has been reported in the dunes in Brazil (25 species) (Stümer *et al*., 2013), forests (13-29 species) and pasture lands (18 species) of Mexico (Gavito *et al*., 2008; Fernandes *et al*., 2009; Guadarrama *et al*., 2014), tropical humid forests of Colombia (18 species) (Peña-Venegas *et al*., 2007), forests in Brazil (Aidar *et al*., 2004; Zandavalli *et al*., 2008; Moreira *et al*., 2009; Bonfim *et al*., 2013; Rabelo *et al*., 2014) and in general in various studies that have demonstrated a range of 12-26 species 10 g^{-1} of AMF (Wilson *et al*., 1992; Wang *et al*., 2008).

The high number of spores identified in the present study, associated with high species diversity from the ecological point of view, is a reflection of the history of the establishment of communities in a specific environment. It can be considered a reserve bank that may contains AMF adapted to various environmental conditions with the potential to associate with different hosts at a particular moment in time, with different growth strategies and adaptive mechanisms to the dynamic changes of the environment (Hijri *et al*., 2006; Oehl *et al*., 2006; Moebius-Clune *et al*., 2013). From the agronomic point of view, the high diversity represents the high potential presented by the Andean ecosystems for the establishment of symbiotic associations. Although this was an analysis of agroecosystems with semi-intensive use, AMF diversity was high, contrary to that reported by different authors on the reduction of AMF diversity in agricultural systems (Mason *et al*., 1992; Munyanziza *et al*., 1997; Cowden and Peterson, 2009).This study verified the presence of "generalist" species (according to Oehl *et al*., 2003). These are AMF species that can be isolated under different soil and climatic conditions, in contrast to "specialist" species that only occur under specific soil or climatic conditions. Generalist species can be isolated under different edaphic conditions and at different altitudes, showing their high tolerance for diverse soil and climatic conditions, including: G. *macrocarpum; G. brouhtii; G. microcarpum, C. claroideum, C. luteum, E. infrequens and E. nevadensis*. The existence of "generalist"

species has been reported by different authors (Oehl *et al*., 2003, 2010; Castillo, 2005; Öpik *et al*., 2006; Guadarrama *et al*., 2007; Stümer and Siqueira, 2008). *Glomus macrocarpum* has been reported as a "generalist" species in several ecosystems and agroecosystems (Oehl *et al*., 2004; Castillo *et al*., 2005; Oliveira Freitas *et al*., 2014; Rabelo *et al*., 2014). Guadarrama *et al*. (2014) identified 10 species considered as generalist, while Rabelo *et al*. (2014) identified 4 generalist species among 40 identified species.

In the case of fungi of the genus *Glomus*, their predominance in diverse edaphoclimatic conditions was reported in several plant species (Gavito *et al*., 2008; Wang *et al*., 2008; Schnoor *et al*., 2011; Boonlue *et al*., 2012; Mahdhi *et al*., 2017). The high presence of species of the genus *Glomus* was probably associated with their high sporulation capacity favoring the colonization of roots in different environments, especially in environments with agronomic operations (Caprioni *et al*., 2003; Rabelo *et al*., 2014). This quality results in the species of this genus being more abundant in manmade systems, especially in agroecosystems like the one in the present study, where fungi of the order Glomerales and specifically species of the genus *Glomus* showed wide dispersion and high abundance in soils of the Colombian Andes.

Other species of fungal genera, such as *Acaulospora* and *Glomus*, predominate in the Chilean Andes (Castillo, 2005; Castillo, *et al*., 2005), showing similarity to those found in this study in Colombia, possibly because of the affinity of some of the edaphic characteristics, such as the presence of volcanic ash (allophane), soil acidity, low P contents in the soil and high organic matter (OM), although they differ in altitude and latitude. In contrast, in studies in the Peruvian Andes Sénes *et al*. (2014) found that *Funneliformis mossseae* was the most predominant species in both the soil and root of potato plants. This species is an early stage colonizer and seems to be adapted to frequent soil disturbances, such as contamination by hydrocarbons, fungicides, heavy metals, salinity, drought and cold climates (Abdel-Azeem *et al*., 2007; Huang *et al*., 2007; Zarei *et al*., 2010; Hassan *et al*., 2011; Krishnamoorthy *et al*., 2014).

The increment of the available phosphorus in the soil produced a reduction of AMF root colonization, which was previously reported by Jansa *et al*. (2009) and Selvam and Mahadevan (2002). Low levels of phosphorus in the soil favor and promote the establishment and development of symbiosis, and therefore, AMF multiplication. Phosphorus deficiency in the soil is one of the main activators of recognition signals between plants and HFMA (Ramírez and Rodriguez, 2010). It was found that 32% of AMF species, especially Glomerales, were favored by increasing the contents of this element in the dry season, while 17.4% were negatively affected in the wet season. This type of interaction has been previously reported (Jeffries *et al*., 1988; Sieverding, 1991; Oehl *et al*., 2003; Landis *et al*., 2004; Uhlmann, *et al*., 2004; Bashan *et al*., 2007), showing correlations between phosphorus contents and richness and abundance of AFM species. There are reports of the presence of HFMA in soils with high phosphorus contents (Davidson and Christensen, 1977; Allen and MacMahon, 1985), showing the great versatility of adaptation that AMFs have. The soils of the Andean region have high phosphorus fixation, so at relatively high phosphorus levels, but with low availability, the abundance of certain HFMA species can be favored.

The tolerance of some species to edaphoclimatic conditions is a desirable characteristic of species considered "generalist" since it allows species to be easily adapted to changes in the environment. This is a common situation in agroecosystems, where the edaphic environment is modified by cultural practices associated with crops. In addition, the identification and use of "generalist" species can facilitate the establishment of symbiosis under different conditions in cape gooseberry because of the high mobility of the crop as an escape mechanism for diseases. The high frequency of isolates along the altitudinal transect of some species showed the high adaptability of these species to conditions of biotic stress and strong changes in agroecosystems from practices such as fertilizer applications (Sturmer and Siqueira, 2008; Zangaro and Moreira, 2010). In this study, species such as *G. macrocarpum* were seen under rainfall conditions below 20 mm/month as well as with rainfall between 150 and 300 mm/month, while other species only occurred in the dry season.

In the case of AMF species considered as "specialists", because their presence is associated with specific conditions (climate, soil or both), it was found that *R. agregatum, R. irregulare, G. sinuosum*, two species of *Glomus* sp., *F. coronatus, F. monosporum, S. hoi, S. constrictum, C. walkeri, E. nevadensis, Acaulospora sp, P. occultum and P. laccatum* were exclusively associated with climate, while *S. constrictum, C. walkierii, Acaulospora* sp2 and *P. laccatum* were associated with the soil type, specifically soils cultivated with *Physalis peruviana*. These AMF characteristics, which present a "specialist" behavior for soil types, climate and moisture regimen, have been reported in AMF community analysis studies in tropical ecosystems, such as humid forests and semi-arid zones of Brazil and Africa, in tropical

savannas and the Swiss Alps (Landis *et al*., 2004; Uhlmann *et al*., 2004; Lekberg *et al*., 2007; Oehl, *et al*., 2010). Although they are not ecologically similar to the Colombian Andes, they showed a trend of AMF behavior. Rabelo *et al*. (2014) identified 26 intermediate and 19 exclusive species or specialists in communities composed of 40 species. The identification of "specialist" species allows species to adapt to specific stresses, which can occur both in space and time because of anthropic intervention or global or local phenomena, such as climate change and variation.

Relative abundance and species richness of AMF

The relative abundance exhibited higher values in the dry season than in the rainy season, related to the greater number of species collected and identified at that time. A relative abundance of more than 71% was obtained for the sample from G4, whereas for that same sample in the rainy season, only 60% was reached. The samples from G1 and G3 had the lowest relative abundances. It is evident that in C1, A2 and G2, the relative abundance of the species was higher in the rainy season, while in the other samples it was always higher in the dry season (Fig. 4).

Species richness varied between 1.57 and 8.30 in the dry season and between 0.87 and 4.95 in the rainy season, reflecting the differences between the two sampling periods. The highest species richness values were observed in the samples from A and G4, and the lowest values in G1 and G3.

Frequency of species isolates in soil samples

In the rainy season, the frequency of isolation ranged between 7.6% from isolated species in a sample to 100% of isolated species in all samples. According to Oehl *et al*. (2003), this analysis allows the determination of "generalist" species (15%), such as *G. macrocarpum, G. brohutii, C. claroide, C. etunicatum, G. microcarpum, G. intraradices* and *E. infrequens*, which were isolated from a high number of samples in rainy and dry seasons with a clear predominance of *Glomus* species in terms of isolation frequencies. The species that can be considered as "specialists" corresponded to 8.7% of the species: *Septoglomus constrictum, Claroideoglomus walkierii, Acaulospora* sp2 and *Paraglomus laccatum*, as they were isolated in a single sample and in one single season.

Of the total species 45.6% showed the highest frequency of isolation in the dry season (21), and only 4 showed higher frequency in the rainy season (*Rhizoglomus proliferum, A. longula, A. rehmii and Archeospora tropeii*). We observed that only 4 species (*G. macrocarpum, Glomus* sp2, *Diversispora celata* and *Kuklospora colombiana*) had the same frequency of isolation in the dry and rainy seasons, compared to the total species isolated in each season.

Only 32.6% of the species (15) were isolated in the dry season: *Rhizoglomus agregatum, R. irregulare, G. sinuosum*, two species of *Glomus* sp, *F. coronatus, F. monosporum, Simioglomus hoi, S. constrictum, C. walkeri, E. nevadensis, Acaulospora sp, P. occultum and P. laccatum.* These were "specialists" for the wet regime rather than for soil type or altitude. According to the scale proposed by Zhang *et al*. (2004), the dominant species in the rainy season were *G. macrocarpum, G. brohutii, G. intraradices and C. claroideum,* and in the dry season *G. macrocarpum, G. brohutii,*

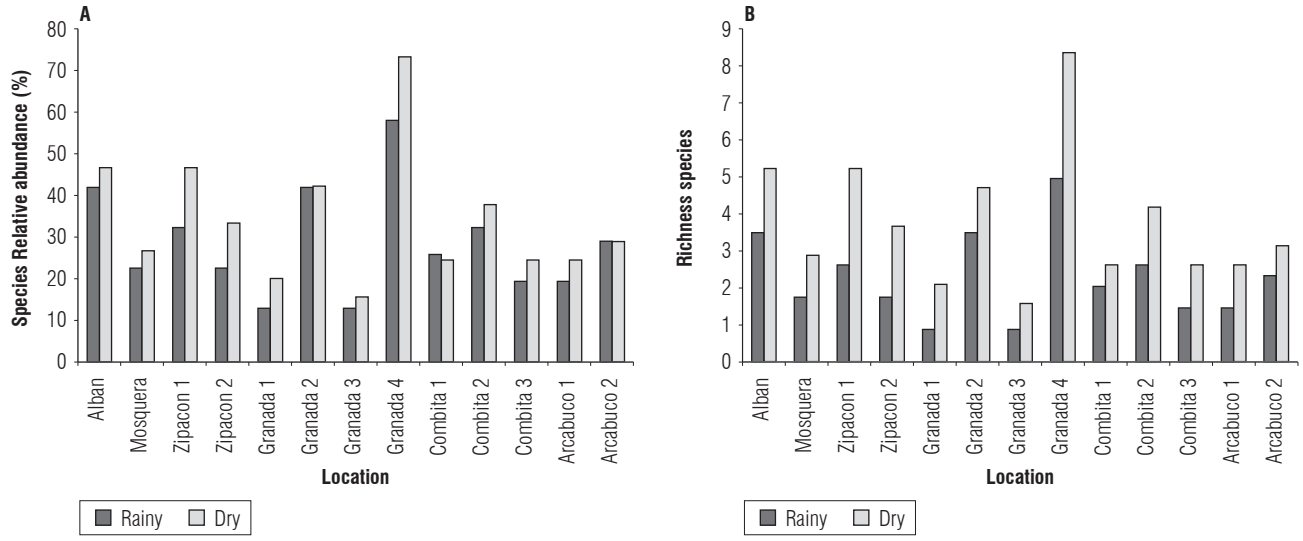
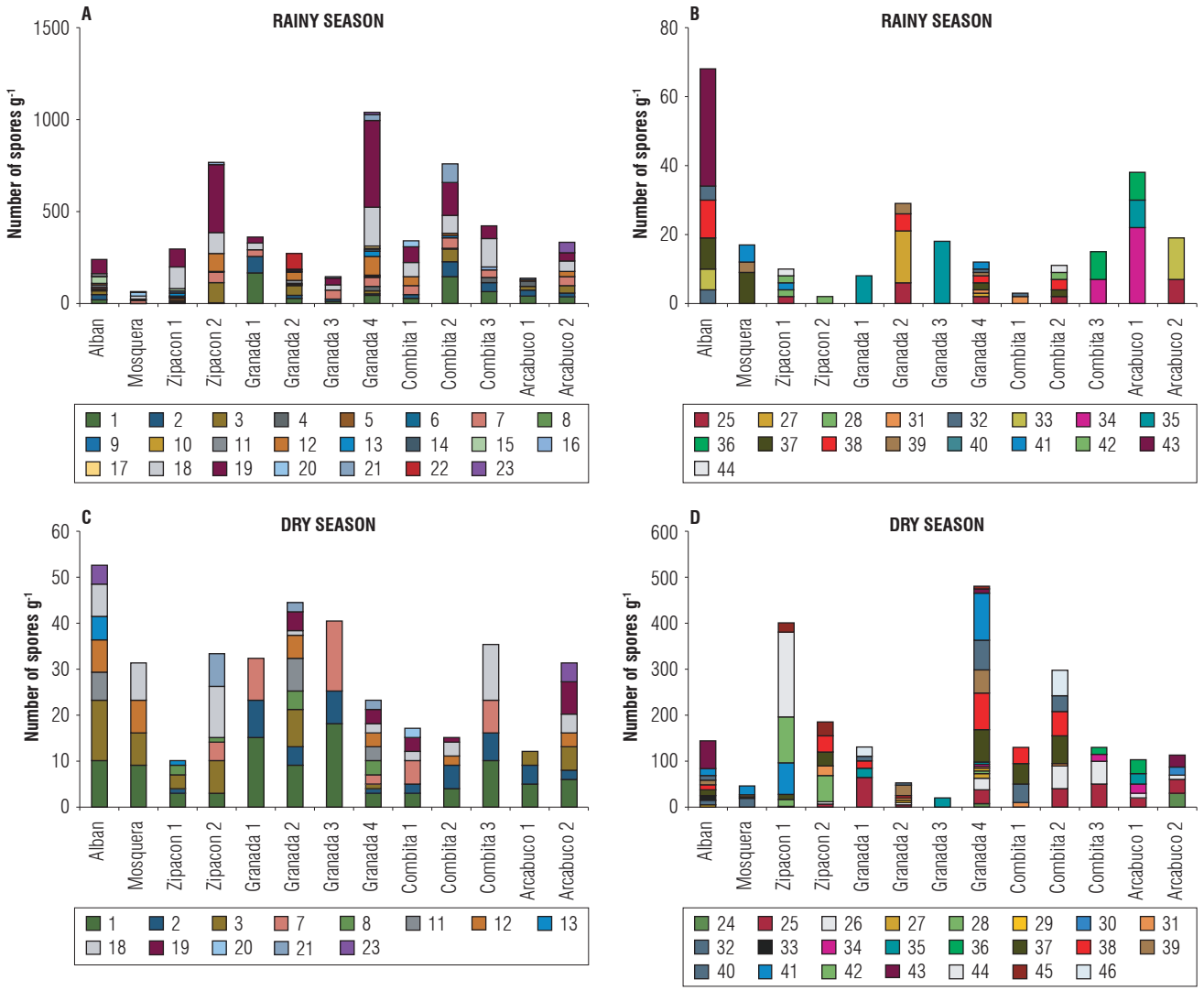


FIGURE 4. Relative abundance (a) and richness (b) of AMF species (g^{-1} of soil) in soils cultivated with cape gooseberry in Cundinamarca and Boyaca in rainy and dry seasons.

G. intraradices, G. microcarpus, F. mosseae, C. claraideum, C. etunicatum, E. infrequens, E.nevadensis, Pacispora sp. *and Scutellospora* sp. The rare species in the rainy season were *C. drummondii and Ambispora* sp., and for the dry season, they were *S.constrictum, C. walkeri, A. longula, Acaulospora* sp2, *A.rehmi, Archeospora troppei, Ambispora appendicula and P. laccatum*. Regardless of the season, *G. macrocarpum, G. brohutii, G. intraradices and C. claroideum* were dominant, and most of the rare species in the dry season were not isolated in the rainy season, except for *A. longula, A. rehmi, Archeospora troppei and Ambispora appendicula*.

Figure 5 shows the abundance of spores for each of the identified species in each community. We determined that although a species may be present in all evaluated communities, its abundance can vary widely.



1. *Glomus macrocarpum*
2. *Glomus brohutii*
3. *Rhizoglomus intraradices*
4. *Rhizoglomus aggregatum*
5. *Rhizoglomus irregulare*
6. *Glomus sinuosum*
7. *Glomus microcarpus*
8. *Glomus* sp1
9. *Glomus* sp 2
10. *Rhizoglomus fasciculatum*
11. *Rhizoglomus proliferus*
12. *Funneliformis mosseae*
13. *Funneliformis geosporus*
14. *Funneliformis coronatus*
15. *Funneliformis monosporus*
16. *Simioglomus hoii*
17. *Septoglomus constricum*
18. *Clareidoglomus claroideum*
19. *Clareidoglomus etunicatum*
20. *Clareidoglomus drummondii*
21. *Claroideoglomus luteum*
22. *Claroideoglomus walkeri*
23. *Diversispora celata*
24. *Diversispora versiformis*
25. *Entrophospora infrequens*
26. *Entrophospora nevadensis*
27. *Acaulospora longula*
28. *Acaulospora morrowiae*
29. *Acaulospora* sp 1
30. *Acaulospora* sp 2
31. *Acaulospora* sp 3
32. *Acaulospora scrobiculata*
33. *Acaulospora rehmii*
34. *Acaulospora spinosa*
35. *Acaulospora denticulata*
36. *Kuklospora colombiana*
37. *Pacispora* sp
38. *Scutellospora nodosa*
39. *Racocetra tropicana*
40. *Cetrospora pellucida*
41. *Intraspora* sp
42. *Archeospora trappei*
43. *Ambispora* sp
44. *Ambispora appendicula*
45. *Paraglomus occultum*
46. *Paraglomus laccatum*

FIGURE 5. Abundance of spores for each of the species identified in the rainy (a and b) and dry (c and d) seasons.

Shannon-Wiener Index (H ‘)

In the rainy season, Shannon-Wiener diversity indexes were found between 1.33 and 2.8, considered as mean values according to Gove *et al*. (1999). The locations G4, A, Z1, C2 and A2 had mean levels of diversity related to the number of species found in the samples (Fig. 6A). In the dry season, the values were similar, with variations between 1.12 and 2.5 with the highest levels of diversity in A2, A1, C2, C1, M, G2 and Z1. It is interesting to observe how in the same location but in a different sampling area the levels of diversity may vary widely either by soil type or agronomic management of the lots.

Uniformity index

This index had a range between 0 and 1, with 1 as the maximum value when all species are present in equal abundance and it decreases when the dominance extent of a species or morphotype occurs (Hurlbert, 1971).

Two measures of uniformity were considered: between samples and inside each sample. Results are presented in Figure 6 (B and C). In the first case, for the rainy season differences in uniformity between the samples were observed when the identified species had variations between 0.06 in Z1 and 0.82 in G4, representing values of low uniformity for Z1 and high uniformity for G4 in relation to the other sampling sites according to Hurlbert (1971). This indicates that Z1 had few species with high disparity with the other samples. In the dry season, there was greater homogeneity between the samples with values between 0.29 (G4) and 0.65 (A2), showing a more homogeneous distribution of the species. When measuring the uniformity per sample

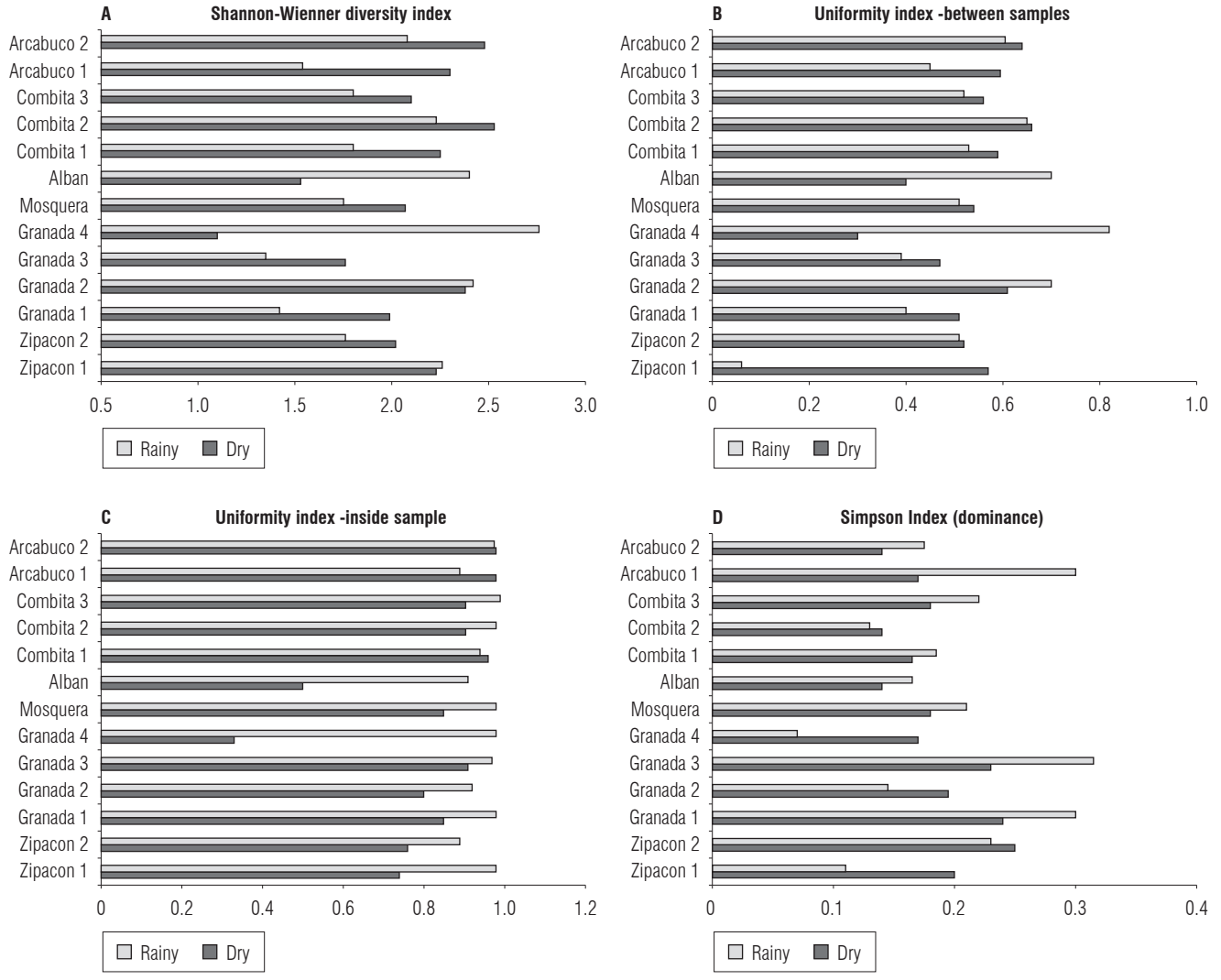


FIGURE 6. Indexes of diversity of AMF species isolated in cape gooseberry in the provinces of Cundinamarca and Boyaca during the rainy and dry seasons. a) Shannon-Wiener Diversity Index; b) Uniformity Index between samples; c) Uniformity Index inside samples and d) Simpson Index (dominance).

(ratio of number of spores to total spores in the sample), in the rainy season, there was a low number of spores the values were very close to 1 in all of the samples reflecting similar values of spores per species in each of the samples. In the dry season, the values were high but with greater variations, with a range between 0.32 in G4 and 0.91 in G3, showing wide variations in the number of spores of each species present in the sample.

Simpson's Dominance Index:

Simpson's Dominance Index shows the highest values for samples G1, G3 and A1 during the rainy season and for Z2, G1 and G3 in the dry season, signifying that these samples had dominant species (Bouza and Covarrubias, 2005). This is consistent with the results of the Uniformity Index since these were the same samples that showed lower uniformity values (Fig. 6D).

The estimated diversity indexes corroborated the hypothesis of high diversity of AMF in these systems, with low-average levels of uniformity between and within the analyzed samples and with species dominance in some of the analyzed communities, especially in those that had a low number of species. The Shannon-Wiener diversity index values recorded for cape gooseberry (1.1 to 2.8) showed that, while the host affects AMF diversity (Vandenkoornhuyse *et al*., 2002), edaphic conditions and altitude also play an important role. This is evident in the ranges of diversity found in the evaluated altitudinal transect where the variations were mainly environmental and not from the host. However, the host component can be evaluated by comparing the values of the present study with those obtained by Helgason *et al*. (1998) and Tanja *et al*. (2004), who reported ranges from 0.4 in agricultural soils to 2.3 in forests, with higher values in cape gooseberry. It is important to consider the characteristics of the cape gooseberry crop since it is a species that is cultivated in agricultural fields but is also in the process of domestication. Cape gooseberry originated from Andean ecosystems, where the diversity centers of the species are found and has been adapted to these ecosystems with restrictive soil-climatic conditions, possibly through co-evolution processes with AMF. Additionally, these results indicate that the tropical Andes of Colombia are a niche with broad AMF diversity.

The previous results confirm the existence of high AMF diversity in the ecosystems of the tropical Andes. Although only cape gooseberry soils were sampled, the number of AMF species was higher to that identified with a greater variety of hosts as mentioned previously. The identification of "generalist" or "specialist" species is very important for the establishment of the AMF-cape gooseberry association since this is a "nomadic" crop that changes with location, soil, climate and altitude. Due to this, AMF species with high adaptability to different climatic and altitude conditions may have a greater possibility of establishing symbiosis than those affected by edaphic changes. Knowing the factors that can affect the abundance of species allows the creation of practices that favor the presence of species of interest for an ecosystem or an agroecosystem.

Conclusions

This research contributed to our knowledge of AMF diversity in the cape gooseberry (*Physalis peruviana* L.) production system in the evaluated altitudinal transect (1500-3000 m a.s.l.). It also determined the relationship between diversity, abundance and composition of communities with the characteristics of the soils in which these communities of fungi associated with cape gooseberry plants are established. This is the first time this kind of research has been carried out in the Andes.

The presence of spores and different levels of root colonization showed the existence of an active interaction between AMF and cape gooseberry plants in Andean soils. This high diversity can be considered as a reserve bank of AMF species adapted to the conditions in the Colombian Andes, which will allow the establishment of symbiotic associations for sustainable and competitive agricultural systems.

Acknowledgments

The authors would like to thank Dr. F. Oehl and Dr. E. Sieverding for their assistance in the identification of the AMF, Dr. Ian Sanders for his recommendation for the PhD internship at the Agroscope Research Center in Zurich and the managers of Agroscope, Zurich for their acceptance of the doctoral internship under the direction of Dr. Fritz Oehl.

Literature cited

Abdel-Azeem, A.M., T.S. Abdel-Moneim, M.E. Ibrahim, M.A.A. Hassan, and M.Y. Saleh. 2007. Effects of long-term heavy metal contamination on diversity of terricolous fungi and nematodes in Egypt: a case study. Water Air Soil Pollut. 186, 233-254. Doi: 10.1007/s11270-007-9480-3

Aguilar-Fernández, M., V.J. Jaramillo, L. Varela-Fregoso, and M.E. Gavito. 2009. Short-term consequences of slash-and-burn practices on the arbuscular mycorrhizal fungi of a tropical dry forest. Mycorrhiza 19, 179-186. Doi: 10.1007/s00572-009-0229-2

Aidar, M.P.M., R. Carrenho, and C.A. Joly. 2004. Aspects of arbuscular mycorrhizal fungi in an atlantic forest chronosequence

in parque estadual turístico do Alto Ribeira (PETAR), SP. Biota Neotrop. 4, 1-15. Doi: 10.1590/S1676-06032004000200005

Allen, M.F. 1983. Formation of vesicular-arbuscular mycorrhizae in *Atriplex gardneri* (Chenopodiaceae): seasonal response in a cold desert. Mycologia 75, 773-776. Doi: 10.2307/3792769

Allen, M., and J.A. MacMahon. 1985. Importance of disturbance on cold desert fungi: comparative microscale dispersion patterns. Pedobiologia 28, 215-224.

Alves Da Silva, D., N. De Oliveira Freitas, G. Cuenca, M.L. Costa, and F. Oehl. 2008. *Scutellospora pernambucana*, a new fungal species in the Glomeromycetes with a diagnostic germination orb. Mycotaxon 106, 361-370.

Bashan, Y., T. Khaosaad, B.G. Salazar, J.A. Ocampo, A. Wiemken, F. Oehl, and V. Vierheilig. 2007. Mycorrhizal characterization of the boojum tree, *Fouquieria columnaris*, an endemic ancient tree from the Baja California Peninsula, Mexico. Trees 21, 329-335. Doi: 10.1007/s00468-007-0126-2

Blaszkowski, J. 1991. Polish Glomales VIII. *Scutellospora nodosa*, a new species with Knobby spores. Mycologia 83, 537-542. Doi: 10.2307/3760369

Blaszkowski, J., C. Renker, and F. Buscot. 2006. *Glomus drummondii* and *Glomus walkeri*, two new species of arbuscular mycorrhizal fungi (Glomeromycota). Mycol. Res. 110, 555-566. Doi: 10.1016/j.mycres.2006.02.006

Blaszkowski, J., B. Czerniawska, T. Wubet, T. Schafer, F. Buscot, and C. Renker. 2008. *Glomus irregulare* a new arbuscular mycorrhizal fungus in the Glomeromycota. Mycotaxon 106, 247-267.

Bonfante, P. and A. Genre. 2008. Plants and arbuscular mycorrhizal fungi: an evolutionary developmental perspective. Trends Plant Sci. 13, 492-498. Doi: 10.1016/j.tplants.2008.07.001

Bonfim, J.A., R.L.F. Vasconcellos, S.L. Stürmer, and E.J. Cardoso, 2013. Arbuscular mycorrhizal fungi in the Brazilian Atlantic forest: a gradient of environmental restoration. Appl. Soil Ecol. 71, 7-14. Doi: 10.1016/j.apsoil.2013.04.005

Boonlue, S., W. Surapat, C. Pukahuta, P. Suwanarit, A. Suwanarit, and T. Morinaga. 2012. Diversity and efficiency of arbuscular mycorrhizal fungi in soils from organic chili (*Capsicum frutescens*) farms. Mycoscience 53, 10-16. Doi: 10.1007/s10267-011-0131-6

Bouza, C. and D. Covarrubias. 2005. Estimación del índice de diversidad de Simpson en m sitios de muestreo. Revista Investigación Operacional 26, 187-197.

Brachmann, A. and M. Parniske. 2006. The most widespread symbiosis on earth. PLOS Biology 4, 239-240. Doi: 10.1371/journal.pbio.0040239

Breuninger, M. and N. Requena. 2004. Recognition events in AM symbiosis: analysis of fungal gene expression at the early appressorium stage. Fungal Genet. Biol. 41, 794-80. Doi: 10.1016/j.fgb.2004.04.002

Brundrett, M., L. Melville, and L. Peterson. 1994. Practical methods in mycorrhiza research. Mycologue Publications. University of Guelph, Guelph, Canada.

Bryla, D.R. and J.M. Duniway. 1997. Water uptake by safflower and wheat roots infected with arbuscular mycorrhizal fungi. New Phytol. 136, 591-601. Doi: 10.1046/j.1469-8137.1997.00781.x

Caproni, A.L., A.A. Franco, R.L.L. Berbara, S.B. Trufem, J.R. Granha, and A.B. Monteiro. 2003. Arbuscular mycorrhizal fungi occurrence in revegetated areas after bauxite mining at Porto Trombetas, Pará State, Brazil. Pesq. Agropec. Bras. 38, 1409-1418. Doi: 10.1590/S0100-204X2003001200007

Castillo, C. 2005. Biodiversidad y efectividad de hongos micorrízicos arbusculares en ecosistemas agro-forestales del Centro-Sur de Chile. PhD thesis, Universidad de La Frontera, Chile.

Castillo, C., F. Borie, R. Godoy, R. Rubio, and E. Sieverding. 2005. Diversity of mycorrhizal plant species and arbuscular mycorrhizal fungi in evergreen forest, deciduous forest and grassland ecosystems of Southern Chile. J. Appl. Bot. Food Qual. 80, 40-47.

Chia, C.L., M.S. Nishima, and D.O. Evans. 1997. Poha. CTAHR Fact Sheet. Horticultural Commodity No 3. University of Hawai, Manoa.

Chifflot, V., D. Rivest, A. Olivier, A. Cogliastro, and D. Khasa. 2009. Molecular analysis of arbuscular mycorrhizal community structure and spores distribution in tree-based intercropping and forest systems. Agri. Ecosys. Environ. 131, 32-39. Doi: 10.1016/j.agee.2008.11.010

Courty, P.E., A. Franc, and J. Garbaye. 2008. Temporal changes in the ectomycorrhizal community in two soil horizons of a temperate oak forest. Appl. Environ. Microbiol. 74, 5792-5801. Doi: 10.1128/AEM.01592-08

Cowden, C. and C. Peterson. 2009. A multi-mutualist simulation: applying biological market models to diverse mycorrhizal communities. Ecol. Model. 220, 1522-1533. Doi: 10.1016/j.ecolmodel.2009.03.028

Criollo, E.H. and C.V. Ibarra. 1992. Germinación de la uvilla (*Physalis peruviana*) bajo diferentes grados de madurez y tiempo de almacenamiento. Acta Hortic. 310, 183-187.

Davey, M.L., E. Heegaard, R. Halvorsen, M. Ohlson, and H. Kauserud. 2012. Seasonal trends in the biomass and structure of bryophyte associated fungal communities explored by 454 pyrosequencing. New Phytol. 195, 844-856. Doi: 10.1111/j.1469-8137.2012.04215.x

Davidson, D.E. and M. Christensen. 1977. Root-microfungal and mycorrhizal associations in a shortgrass prairie. pp. 279-287. In: Marshall, J.K. (ed.). The Belowground Eco systems: a synthesis of plant-associated processes. Colorado State University Press, Collins, USA.

De Carvalho, F., F. De Souza, R. Carrenho, F. De Souza Moreira, E. Da Conceicao, and G. Fernandes. 2012. The mosaic of habitats in the high-altitude Brazilian rupestrian fields is a hotspot for arbuscular mycorrhizal fungi. Appl. Soil Ecol. 52, 9-19. Doi: 10.1016/j.apsoil.2011.10.001

De Oliveira Freitas, R., E. Buscardo, L. Nagy, A.S. dos Santos Maciel, R. Carrenho, and R. Luizão. 2014. Arbuscular mycorrhizal fungal communities along a pedo-hydrological gradient in a Central Amazonian terra firme forest. Mycorrhiza 24, 21-32. Doi: 10.1007/s00572-013-0507-x

Espinal, C.F., H.J. Martínez, and Y. Peña. 2005. La cadena de los frutales de exportación en Colombia una mirada global de su estructura y dinámica 1991-2005. Work document no. 67. Ministerio de Agricultura y Desarrollo Rural, Observatorio

Agrocadenas, Bogota. URL: http://www.agrocadenas.gov.co/ (accessed 6 June 2017).

Fernández, M.A., V.J. Jaramillo, L. Varela-Fregoso, and M.E. Gavito. 2009. Short-term consequences of slash-and-burn practices on the arbuscular mycorrhizal fungi of a tropical dry forest. Mycorrhiza 19, 179-186. Doi: 10.1007/s00572-009-0229-2

Fischer, G. 2000. Crecimiento y desarrollo. In: Flórez, V.J, G. Fischer, and A.D. Sora (eds.). Producción, poscosecha y exportación de la uchuva *(Physalis peruviana* L.). Unibiblos, Universidad Nacional de Colombia, Bogota.

Franke-Snyder, M., D. Douds, L. Galvez, J. Phillips, P. Wagoner, L. Drinkwater, and J. Morton. 2001. Diversity of communities of arbuscular mycorrhizal (AM) fungi present in conventional versus low-input agricultural sites in eastern Pennsylvania, USA. Appl. Soil Ecol. 16, 35-48. Doi: 10.1016/S0929-1393(00)00100-1

Garbaye, J. 1994. Helper bacteria: a new dimension to the mycorrhizal symbiosis. New Phytol. 128, 197-210. Doi: 10.1111/j.1469-8137.1994.tb04003.x

Gavito, M.E., D. Pérez-Castillo, C.F. González-Monterrubio, T. Vieyra-Hernández, and M. Martínez-Trujillo. 2000. High compatibility between arbuscular mycorrhizal fungal communities and seedlings of different land use types in a tropical dry ecosystem. Mycorrhiza 19, 47-60. Doi: 10.1007/s00572-008-0203-4

Gehring, C.A. and T.G. Whitham. 2002. Mycorrhizae-herbivore interactions: population and community consequences. pp. 295-320. In: van der Heijden, M.G.A. and I.R. Sanders (eds.). Mycorrhizal ecology. Ecological studies 157. Springer, Berlin, Heidelberg. Doi: 10.1007/978-3-540-38364-2_12

Genre, A., M. Chabaud, T. Timmers, P. Bonfante, and D. Barkerb. 2005. Arbuscular mycorrhizal fungi elicit a novel intracellular apparatus in *Medicago truncatula* root epidermal cells before infection. Plant Cell 17, 3489-3499. Doi: 10.1105/tpc.105.035410

Genre, A., M. Chabaud, A. Faccio, D. Barker, and P. Bonfante. 2008. Prepenetration apparatus assembly precedes and predicts the colonization patterns of arbuscular mycorrhizal fungi within the root cortex of both *Medicago truncatula* and *Daucus carota*. Plant Cell 20, 1407-1420. Doi: 10.1105/tpc.108.059014

Gerdemann, J. and T. Nicholson. 1963. Spores of mycorrhizal *Endogone* species, extracted from soil by wet-sieving and decanting. Trans. Br. Mycol. Soc. 46, 235-244. Doi: 10.1016/S0007-1536(63)80079-0

Giovannetti, M. and B. Mosse. 1980. An evaluation of techniques for measuring vesicular arbuscular infection in roots. New Phytol. 84, 489-500. Doi: 10.1111/j.1469-8137.1980.tb04556.x

Goto, B., G.A. Silva, L. Maia, R.G. Souza, D. Coyne, A. Tchabi, L. Lawouin, F. Hountondji, and F. Oehl. 2011. *Racocetra tropicana*, a new species in the Glomeromycetes from tropical areas. Nova Hedwigia 92, 69-82. Doi: 10.1127/0029-5035/2011/0092-0069

Gove, J.H., G.P. Patil, B.F. Swinde, and C. Taille. 1994. Ecological diversity and forest management. pp. 409-462. In: Patil, G.P. and C.R. Rao (eds.). Handbook of statistics 12. Elsevier Science B.V., UK. Doi: 10.1016/S0169-7161(05)80014-8

Grime, P., L. Mackey, S.H. Hillier, and D.J. Read. 1987. Floristic diversity in a model system using experimental microcosms. Nature 328, 420-422. Doi: 10.1038/328420a0

Guadarrama, P. and F.J. Álvarez-Sánchez. 1999. Abundance of arbuscular mycorrhizal fungi spores in different environments in a tropical rain forest, Veracruz, Mexico. Mycorrhiza 8, 267-270. Doi: 10.1007/s005720050244

Guadarrama-Chávez, P., S.L. Camargo-Ricalde, L. Hernández-Cuevas, and S. Castillo. 2007. Los hongos micorrizógenos arbusculares de la región de Nizanda, Oaxaca, México. Bol. Soc. Bot. Mex. 81, 133-139. Doi: 10.17129/botsci.1770

Guadarrama, P., S. Castillo, J.A. Ramos-Zapata, L.V. Hernandez-Cuevas, and S.L. Camargo-Ricalde. 2014. Arbuscular mycorrhizal fungal communities in changing environments: the effects of seasonality and anthropogenic disturbance in a seasonal dry forest. Pedobiologia 57, 87-95. Doi: 10.1016/j.pedobi.2014.01.002

Hart, M. and J. Klironomos. 2002. Diversity of Arbuscular mycorrhizal fungi and ecosystem functioning. In: van der Heijden, M.G.A., and I.R. Sanders (eds). Mycorrhizal ecology. Springer, Berlin, Heidelberg. Doi: 10.1007/978-3-540-38364-2_9

Hassan, D.S., E. Boon, M. St-Arnaud, and M. Hijri. 2011. Molecular biodiversity of arbuscular mycorrhizal fungi in trace metal-polluted soils. Mol. Ecol. 20, 3469-3483. Doi: 10.1111/j.1365-294X.2011.05142.x

Helgason, T., T.J. Daniell, R. Husband, A.H. Fitter, and J.P.W. Young. 1998. Ploughing up the wood-wide web? Nature 394, 431. Doi: 10.1038/28764

Hijri, I., Z. Sykorova, F. Oehl, K. Ineichen, P. Mader, A. Wiemken, and D. Redecker. 2006. Communities of arbuscular mycorrhizal fungi in arable soils are not necessarily low in diversity. Mol. Ecol. 15, 227-2289. Doi: 10.1111/j.1365-294X.2006.02921.x

Huang, H., S. Zhang, X.Q. Shan, B.D. Chen, Y.G. Zhu, and J.N. Bell. 2007. Effect of arbuscular mycorrhizal fungus (*Glomus caledonium*) on the accumulation and metabolism of atrazine in maize (*Zea mays* L.) and atrazine dissipation in soil. Environ. Pollut. 146, 452-457. Doi: 10.1016/j.envpol.2006.07.001

Hurlbert, S.H. 1971. The nonconcept of species diversity: a critique and alternative parameters. Ecology 52, 577-586. Doi: 10.2307/1934145

Husband, R., E.A. Herre, S.L. Turner, R. Gallery, and J.P.W. Young. 2002a. Molecular diversity of arbuscular mycorrhizal fungi and patterns of host association over time and space in a tropical forest. Mol. Ecol. 11, 2669-2678. Doi: 10.1046/j.1365-294x.2002.01647.x

Husband, R., E.A. Herre, and J. Young. 2002b. Temporal variation in the arbuscular mycorrhizal communities colonizing seedlings in a tropical forest. FEMS Microbiol. Ecol. 42, 131-136. Doi: 10.1111/j.1574-6941.2002.tb01002.x

Jansa, J., A. Mozafar, G. Kuhn, T. Anken, R. Ruh, I.R. Sanders, and E. Frossard. 2003. Soil tillage affects the community structure of mycorrhizal fungi in maize roots. Ecol. Appl. 13, 1164-1176. Doi: 10.1890/1051-0761(2003)13[1164:STATCS]2.0.CO;2

Jansa, J., C. Thonar, and E. Frossard. 2009. Enhancement of symbiotic benefits through manipulation of the mycorrhizal community composition. Aspects Appl. Biol. 98, 9-15.

Jayachandran, K. and K. Shetty. 2003. Growth response and phosphorus uptake by arbuscular mycorrhizae of wet prairie sawgrass. Aquat. Bot. 76, 281-290. Doi: 10.1016/S0304-3770(03)00075-5

Jeffries, P., T. Spyropoulos, and E. Vardavarkis. 1988. Vesicular-arbuscular mycorrhizal status of various crops in different agricultural soils of northern Greece. Biol. Fertil. Soils. 5, 333-337. Doi: 10.1007/BF00262142

Johnson, N.C., D. Tilman, and D. Wedin. 1992. Plant and soil controls on mycorrhizal fungal communities. Ecology 73, 2034-2042. Doi: 10.2307/1941453

Kernaghan, G. 2005. Mycorrhizal diversity: cause and effect? Pedobiologia 49, 511-520. Doi: 10.1016/j.pedobi.2005.05.007

Koske, R.E. and B. Tessier. 1983. A convenient permanent slide mounting medium. Mycol. Soc. Am. Newsl. 34, 59.

Krishnamoorthy, R., K. Kim, C. Kim, and T. Sa. 2014. Changes of arbuscular mycorrhizal traits and community structure with respect to soil salinity in a coastal reclamation land. Soil Biol. Biochem. 72, 1-10. Doi: 10.1016/j.soilbio.2014.01.017

Kwaśna, H., G. Bateman, and E. Ward. 2008. Determining species diversity of microfungal communities in forest tree roots by pure-culture isolation and DNA sequencing. App. Soil Ecol. 40, 44-56. Doi: 10.1016/j.apsoil.2008.03.005

Landis, F.C., A. Gargas, and T.J. Givnish. 2004. Relationships among arbuscular mycorrhizal fungi, vascular plants and environmental conditions in oak savannas. New Phytol. 164, 493-504. Doi: 10.1111/j.1469-8137.2004.01202.x

Lekberg, Y., R.T. Koide, J.R. Rohr, L. Aldrich-Wolfe, and J.B. Morton. 2007. Role of niche restrictions and dispersal in the composition of arbuscular mycorrhizal fungal communities. J. Ecol. 95, 95-100. Doi: 10.1111/j.1365-2745.2006.01193.x

Li, Y., X. He, and L. Zhao. 2010. Tempo-spatial dynamics of arbuscular mycorrhizal fungi under clonal plant *Psammochloa villosa* Trin. Bor in Mu Us sandland. Eur. J. Soil Biol. 46, 295-301. Doi: 10.1016/j.ejsobi.2010.05.004

Lindahl, B., R. Nilsson, L. Tedersoo, K. Aberenkov, T. Carlsen, R. Kjoller, U. Kojalg, T. Pennanen, S. Rosendahl, J. Stenlid, and H. Kauserud. 2013. Fungal community analysis by high through put sequencing of amplified markers - a user's guide. New Phytol. 199, 288-299. Doi: 10.1111/nph.12243

Lodge, D.J. 1989. The influence of soil moisture and flooding on formation of VA-endo- and ectomycorrhizae in Populus and Salix. Plant Soil 117, 243-253. Doi: 10.1007/BF02220718

Lopes, P., J.O. Siqueira, and S.D. Stürmer. 2013. Switch of tropical Amazon forest to pasture affects taxonomic composition but not species abundance and diversity of arbuscular mycorrhizal fungal community. Appl. Soil Ecol. 71, 72-80. Doi: 10.1016/j.apsoil.2013.05.010

Mahdhi, M., T. Tounekti, T.A. Al-Turki, and H. Khemira. 2017. Composition of the root mycorrhizal community associated with *Coffea arabica* in Fifa Mountains (Jazan region, Saudi Arabia). J. Basic Microbiol. 57, 691-698. Doi: 10.1002/jobm.201700075

Mason, P.A., M.O. Musoko, and F.T. Last. 1992. Short-term changes in vesicular-arbuscular mycorrhizal spore populations in Terminalia plantations in Cameroon. pp. 261-267. In: Read, D.J., D.H. Lewis, A.H. Fitter, and L.J. Alexander (eds). Mycorrhizas in ecosystems. CAB International, UK.

Medina, M. 1991. El cultivo de la uchuva tipo exportación. Agricultura Tropical 28, 55-58.

Miller, P. 2000. Arbuscular mycorrhizal colonization of semi-aquatic grasses along a wide hydrologic gradient. New Phytol. 145,145-155. Doi: 10.1046/j.1469-8137.2000.00566.x

Miller, P. and R.R. Sharitz. 2000. Manipulation of flooding and arbuscular mycorrhizal formation influences growth and nutrition of two semi-aquatic species. Funct. Ecol. 14, 738-748. Doi: 10.1046/j.1365-2435.2000.00481.x

Ming, T. and C. Hui. 1999. Effects of arbuscular mycorrhizal fungi on alkaline phosphatase activities on *Hippophae rhamnoides* drought-resistance under water stress conditions. Trees 14, 113-115. Doi: 10.1007/PL00009757

Moebius-Clune, D.J., Z.U. Anderson, and T. Pawlowska. 2013. Arbuscular mycorrhizal fungi associated with a single agronomic plant host across the landscape: the structure of an assemblage. Soil Biol. Biochem. 64, 181-190. Doi: 10.1016/j.soilbio.2012.10.043

Moreira, M., D. Baretta, S.M. Tsai, and E.J. Cardoso. 2009. Arbuscular mycorrhizal fungal communities in native and in replanted *Araucaria* forest. Sci. Agric. 66, 677-684. Doi: 10.1590/S0103-90162009000500013

Morton, J. 1987. Cape Gooseberry. pp. 430-443. In: J. Morton (ed.). Fruits of warm climates. Florida Flair Books, Miami, USA.

Morton, J. and G. Benny. 1990. Revised classification of arbuscular mycorrhizal fungi (Zygomycetes): a new order Glomales, two new suborders Glomineae and Gigasporineae and two new families Acaulosporaceae and Gigasporaceae with an emendation of Glomaceae. Mycotaxon 37, 471-491.

Morton, J. and D. Redecker. 2001. Two new families of Glomales, Archaeosporaceae and Paraglomaceae, with two new genera *Archaeospora* and *Paraglomus*, based on concordant molecular and morphological characters. Mycologia 93, 181-195. Doi: 10.2307/3761615

Munyanziza, E., H.K. Kehri, and D.J. Bagyaraj. 1997. Agricultural intensification, soil biodiversity and agro-ecosystem function in the tropics: the role of mycorrhiza in crops and trees. Appl. Soil Ecol. 6, 77-85. Doi: 10.1016/S0929-1393(96)00152-7

Oehl, F., E. Sieverding, P. Ineichen, P. Mäder, T. Boller, and A. Wiemken. 2003. Impact of land use intensity on the species diversity of arbuscular mycorrhizal fungi in agroecosystems of Central Europe. Appl. Environ. Microb. 69, 2816-2824. Doi: 10.1128/AEM.69.5.2816-2824.2003

Oehl, F. and E. Sieverding. 2004. *Pacispora*, a new vesicular arbuscular mycorrhizal fungal genus in the Glomeromycetes. J. Appl. Bot. Food Qual. 78, 72-82.

Oehl, F., Z. Sýkorová, D. Redecker, A. Wiemken, and E. Sieverding. 2006. *Acaulospora alpina*, a new arbuscular mycorrhizal fungal species characteristic for high mountainous and alpine regions of the Swiss Alps. Mycologia 98, 286-294. Doi: 10.1080/15572536.2006.11832701

Oehl, F., F. de Souza, and E. Sieverding. 2008. Revision of *Scutellospora* and description of five new genera and three new families in the arbuscular mycorrhiza-forming Glomeromycetes. Mycotaxon 106, 311-360.

Oehl, F., E. Lackzo, A. Bogenrieder, K. Stahr, R. Bösch, M.G.A. van der Heijden, and E. Sieverding. 2010. Soil type and land use intensity determine the composition of arbuscular mycorrhizal

fungal communities. Soil Biol. Biochem. 42, 724-738. Doi: 10.1016/j.soilbio.2010.01.006

Oehl, F., J. Jansa, F. de Souza, and G. Silva. 2011a. *Cetraspora helvetica*, a new ornamented species in the Glomeromycetes from Swiss agricultural fields. Mycotaxon 114, 71-84. Doi: 10.5248/114.71

Oehl, F., D.K. Silva, L. Maia, N. Souza, H. Vieira, and G. Silva. 2011b. *Orbispora* ge. nov., ancestral in the Scutellosporaceae (Glomeromycetes). Mycotaxon 116, 161-169.

Oehl, F., Z. Sýkorová, J. Blaszkowski, I. Sànchez-Castro, D. Coyne, A. Tchabi, L. Lawouin, F. Hountondji, and G. Silva. 2011c. *Acaulospora sieverdingii*, an ecological diverse new fungus in the Glomeromycota, described from lowland temperate Europe and tropical West Africa. J. Appl. Bot. Food Qual. 87, 47-53.

Oehl, F., G. Silva, B. Goto, and E. Sieverding. 2011d. Glomeromycota: Three new genera and glomoid species reorganized. Mycotaxon 116, 78-120. Doi: 10.5248/116.75

Öpik, M., M. Moora, J. Liira, and M. Zobel. 2006. Composition of root-colonizing arbuscular mycorrhizal fungal communities in different ecosystems around the globe. J. Ecol. 94, 778-790. Doi: 10.1111/j.1365-2745.2006.01136.x

Öpik, M., M. Zobel, J.J. Cantero, J. Davison, J.M. Facelli, I. Hiiesalu, T. Jairus, J.S. Kalwij, K. Koorem, M.E. Leal, J. Liira, M. Metsis, V. Neshataeva, J. Paal, C. Phosri, S. Põlme, Ü. Reier, Ü. Saks, H. Schimann, O. Thiéry, M. Vasar, and M. Moora. 2013. Global sampling of plant roots expands the described molecular diversity of arbuscular mycorrhizal fungi. Mycorrhiza 23, 411-430. Doi: 10.1007/s00572-013-0482-2

Pagano, M.C., R.B. Zandavalli, and F.S. Araújo. 2013. Biodiversity of arbuscular mycorrhizas in three vegetational types from the semiarid of Ceará State, Brazil. Appl. Soil Ecol. 67, 37-46. Doi: 10.1016/j.apsoil.2013.02.007

Palenzuela, J., N. Ferrol, T. Boller, C. Azcón-Aguilar, and F. Oehl. 2008. *Otospora bareai*, a new fungal species in the Glomeromycetes from a dolomitic shrub land in Sierra de Baza National Park (Granada, Spain). Mycologia 100, 296-305. Doi: 10.1080/15572536.2008.11832484

Panwar, J. and J.C. Tarafdar. 2006. Distribution of three endangered medicinal plant species and their colonization with arbuscular mycorrhizal fungi. J. Arid Environ. 65, 337-350. Doi: 10.1016/j.jaridenv.2005.07.008

Peña-Venegas, C.P., G.L. Caronda, J.H. Arguelles, and A.L. Arcos. 2007. Micorrizas arbusculares del sur de la Amazonía Colombiana y su relación con algunos factores fisicoquímicos y biológicos del suelo. Acta Amaz. 37, 327-336. Doi: 10.1590/S0044-59672007000300003

Pérez, E. 1996. Plantas útiles de Colombia. Edición de Centenario, Cargraphics. Santander de Quilichao, Colombia.

Phillips, J.M. and D.S. Hayman. 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Trans. Br. Mycol. Soc. 55, 158-161. Doi: 10.1016/S0007-1536(70)80110-3

Rabatin, S.C. 1979. Seasonal and edaphic variation in vesicular-arbuscular mycorrhizal infection of grasses by *Glomus tenius*. New Phytol. 83, 95-102. Doi: 10.1111/j.1469-8137.1979.tb00730.x

Rabelo, P.C., D.K. Silva, A.C. Ferreira, B. Goto, and L. Maia. 2014. Diversity of arbuscular mycorrhizal fungi in Atlantic forest areas under different land uses. Agr. Ecosyst. Environ. 185, 245-252. Doi: 10.1016/j.agee.2014.01.005

Read, D.J. 1998. Plants on the web. Nature 396, 22-23. Doi: 10.1038/23822

Redecker, D., A. Schüler, H. Stockinger, S. Stümer, J. Morton, and C. Walker. 2013. An evidence-based consensus for the classification of arbuscular mycorrhizal fungi (*Glomeromycota*). Mycorrhiza 23, 515-531. Doi: 10.1007/s00572-013-0486-y

Rodríguez, N., C. Rivera-Calvo, and I. Paneque-Torres. 2005. Efecto de las tecnologías de extracción forestal sobre la diversidad de especies leñosas en ecosistemas de pinares naturales. Revista Chapingo 11, 125-131.

Roveda, G., A. Peñaranda, M. Ramírez, I. Baquero, and R. Galindo. 2012. Diagnóstico de la fertilidad química de los suelos de los municipios de Granada y Silvania para la producción de uchuva en Cundinamarca. Cienc. Tecnol. Agropec. 13, 179-188. Doi: 10.21930/rcta.vol13_num2_art:253

Schenck, N. and Y. Perez. 1988. Manual for the identification of VA mycorrhizal fungi. University of Florida, Gainesville, USA.

Schüßler, A., D. Scharzott, and C. Walker. 2001. A new fungal phylum, the *Glomeromycota*: phylogeny and evolution. Mycol. Res. 105, 1413-1421. Doi: 10.1017/S0953756201005196

Schnoor, T., I. Lekberg, S. Rosendahl, and P. Olsson. 2011. Mechanical soil disturbance as a determinant of arbuscular mycorrhizal fungal communities in semi-natural grassland. Mycorrhiza 21, 211-220. Doi: 10.1007/s00572-010-0325-3

Selvam, A. and A. Mahadevan. 2002. Distribution of mycorrhizas in an abandoned fly ash pond and mined sites of Neyveli Lignite Corporation, Tamil Nadu, India. Basic Appl. Ecol. 3, 277-284. Doi: 10.1078/1439-1791-00107

Sénes-Guerrero, C., G. Torres, S. Pfeiffer, M. Rojas, and A. Schüßler. 2014. Potato-associated arbuscular mycorrhizal fungal communities in the Peruvian Andes. Mycorrhiza 24, 405-417. Doi: 10.1007/s00572-013-0549-0

Sieverding, E. 1991. Vesicular-arbuscular mycorrhiza management in tropical agrosystems. Deutsche Gesellschaft fur Technische Zusammenarbeit. Eschborn, Germany.

Sieverding, E. and F. Oehl. 2006. Revision of *Entrophospora* and description of *Kuklospora* and *Intraspora*, two new genera in them arbuscular mycorrhizal Glomeromycetes. J. Appl. Bot. Food Qual. 80, 69-81.

Souza, R.G., L. Maia, M.F. Sales, and S.F.B. Trufem. 2003. Diversidade e potencial de infectividade de fungos micorrízicos arbusculares em área de caatinga, na Região de Xingó, Estado de Alagoas, Brasil. Rev. Bras. Bot. 26, 49-60. Doi: 10.1590/S0100-84042003000100006

Smith, S.D. and D.J. Read. 2008. Mycorrhizal symbiosis. Academic Press, Amsterdam.

Smith, S.E. and F.A. Smith. 2011. Roles of arbuscular mycorrhizas in plant nutrition and growth: new paradigms from cellular to ecosystem scales. Annu. Rev. Plant Biol. 62, 227-250. Doi: 10.1146/annurev-arplant-042110-103846

Stover, H.J., R.G. Thorn, J.M. Bowle, M.A. Bernards, and C.R. Jacobs. 2012. Arbuscular mycorrhizal fungi and vascular plant species abundance and community structure in tallgrass prairies with varying agricultural disturbance histories. Appl. Soil Ecol. 60, 61-70. Doi: 10.1016/j.apsoil.2012.02.016

Stürmer, S.L. and J.O. Siqueira. 2008. Diversidade de fungos micorrízicos arbusculares em ecossistemas Brasileiros. In: Moreira, F.M.S., J.O. Siqueira, and L. Brussaard (eds.). Biodiversidade do solo em ecossistemas Brasileiros. UFLA, Lavras, Brasilia.

Stürmer, S.L., R. Stürmer, and D. Pasqualini. 2013. Taxonomic diversity and community structure of arbuscular mycorrhizal fungi (Phylum Glomeromycota) in three maritime sand dunes in Santa Catarina state, south Brazil. Fungal Ecol. 6, 27-36. Doi: 10.1016/j.funeco.2012.10.001

Su, Y.Y. and L.D. Guo. 2007. Arbuscular mycorrhizal fungi in non-grazed, restored and over-grazed grassland in the Inner Mongolia steppe. Mycorrhiza 17, 689-693. Doi: 10.1007/s00572-007-0151-4

Tanja, R., K. Scheublin, P. Ridgway, J.P.W. Young, and M. van der Heijden. 2004. Nonlegumes, legumes, and root nodules harbor different arbuscular mycorrhizal fungal communities. Appl. Environ. Microbiol. 70, 6240-6246. Doi: 10.1128/AEM.70.10.6240-6246.2004

Trillos, O., J.M. Cotes, C.L. Medina, M. Lobo, and A. Navas. 2008. Caracterización morfológica de cuarenta y seis accesiones de uchuva (*Physalis peruviana* L.) en Antioquia (Colombia). Rev. Bras. Frutic. Jaboticabal. 30, 708-715. Doi: 10.1590/S0100-29452008000300025

Turrini, A., M. Agnolucci, M. Palla, E. Tomé, M. Tagliavini, F. Scandellari, and M. Giovannetti. 2017. Species diversity and community composition of native arbuscular mycorrhizal fungi in apple roots are affected by site and orchard management. Appl. Soil Ecol. 116, 42-54. Doi: 10.1016/j.apsoil.2017.03.016

Uhlmann, E., C. Görke, A. Petersen, and F. Oberwinkler. 2004. Arbuscular mycorrhizae from semiarid regions of Namibia. Can. J. Bot. 82, 645-653. Doi: 10.1139/b04-039

Vandenkoornhuyse, P., R. Husband, T. Daniell, I. Watson, J. Duck, A. Fitter, and J. Young. 2002. Arbuscular mycorrhizal community composition associated with two plant species in a grassland ecosystem. Mol. Ecol. 11, 1555-1564. Doi: 10.1046/j.1365-294X.2002.01538.x

van der Heijden, M.G.A., T. Boller, A. Wiemken, and I.R. Sanders. 1998a. Different arbuscular mycorrhizal fungal species are potential determinants of plant community structure. Ecology 79, 2082-2091. Doi: 10.1890/0012-9658(1998)079[2082:DAMFSA]2.0.CO;2

van der Heijden, M.G.A., J. Klironomos, M. Ursic, P. Moutoglis, R. Streitwolf-Engel, T. Boller, A. Wiemken, and I.R. Sanders. 1998b. Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. Nature 396, 69-72. Doi: 10.1038/23932

van der Heijden, M.G.A. and I.R. Sanders. 2002. Mycorrhizal ecology. Springer, Berlin, Heidelberg.

Violi, H.A., A. Barrientos-Priego, S.F. Wright, E. Escamilla-Prado, J.B. Morton, J.A. Menge, and C.J. Lovatt. 2008. Disturbance changes arbuscular mycorrhizal fungal phenology and soil glomalin concentrations but not fungal spore composition in montane rainforests in Veracruz and Chiapas, Mexico. For. Ecol. Manage. 254, 276-290. Doi: 10.1016/j.foreco.2007.08.016

Walker, C. and A. Schüßler. 2004. Nomenclatural clarifications and new taxa in the *Glomeromycota Pacispora*. Mycol. Res. 108, 979-982. Doi: 10.1017/S0953756204231173

Wang, Y.Y., M. Vestberg, C. Walker, T. Hurme, X. Zhang, and K. Lindstrom. 2008. Diversity and infectivity of arbuscular mycorrhizal fungi in agricultural soils of the Sichuan Province of mainland China. Mycorrhiza 18, 59-68. Doi: 10.1007/s00572-008-0161-x

Wilson, J., K. Ingleby, P.A. Mason, K. Ibrahim, and G.J. Lawson. 1992. Long-term changes in vesicular-arbuscular mycorrhizal spore populations in Terminalia plantations in Cote d'Ivoire. pp. 268-275. In: Read, D.J., D.H. Lewis, H.J. Fitter, and I.J. Alexander (eds.). Mycorrhizas in ecosystems. CAB International, London.

Zandavalli, R.B., S.L. Stürmer, and L.R. Dillenburg. 2008. Species richness of arbuscular mycorrhizal fungi in forests with *Araucaria* in Southern Brazil. Hoehnea 35, 63-68. Doi: 10.1590/S2236-89062008000100003

Zangaro, W. and M. Moreira. 2010. Micorrizas arbusculares nos biomas Floresta Atlântica e Floresta de Araucária. pp. 279-310. In: Siqueira, J.O., F.A. de Souza, E.J.B.N. Cardoso, and S.M. Tsai (eds.). Micorrizas 30 anos de pesquisa no Brasil. UFLA, Lavras, Brazil.

Zarei, M., S. Hempel, T. Wubet, T. Schäfer, G. Savaghebi, G.S. Jouzani, M.K. Nekouei, and F. Buscot. 2010. Molecular diversity of arbuscular mycorrhizal fungi in relation to soil chemical properties and heavy metal contamination. Environ. Pollut. 158, 2757-2765. Doi: 10.1016/j.envpol.2010.04.017

Zhang, Y., L.D. Guo, and R.J. Liu. 2004. Survey of arbuscular mycorrhizal fungi in deforested and natural forest land in the subtropical region of Dujiangyan, southwest China. Plant Soil 261, 257-263. Doi: 10.1023/B:PLSO.0000035572.15098.f6

Zhao, D. and Z. Zhao. 2007. Biodiversity of arbuscular mycorrhizal fugi in the hot-dry valley of the Jinsha River, southwest China. Appl. Soil Ecol. 37(2), 118-128. Doi: 10.1016/j.apsoil.2007.06.003

Rhizospheric rhizobia identification in maize (*Zea mays* L.) plants

Identificación de rizobios rizosféricos en plantas de maíz (*Zea mays* L.)

Reneé Pérez-Pérez[1]*, Maxime Oudot[2], Lizette Serrano[3], Ionel Hernández[1], María Nápoles[1], Daynet Sosa[3,4], and Simón Pérez-Martínez[4]

ABSTRACT

Rhizobia have been studied for the symbiosis that they establish with the roots of legumes. However, the colonization and promotion of growth in non-leguminous plants has also been demonstrated. The aim of this work was the biochemical and molecular identification of rhizosphere rhizobia present in the rhizosphere of two commercial maize cultivars. Cultivable isolates were obtained in yeast-mannitol-agar (YMA) medium from rhizospheric soil and the rhizoplane. The cultural (size, color, mucus, etc.), morphological, and staining (cell shape, response to staining and sporulation) characteristics were determined as well as isolate responses to eight biochemical tests (acid-base production, citrate, oxidase, catalase, H_2S production, urease, gelatinase and the oxidative-fermentative assay) that are valuable for rhizobia identification. The genus was determined by 16S rDNA gene sequencing. We obtained 81 total isolates of which 30.86% showed the cultural, morphological and staining characteristics expected for rhizobia and only 20% of these corresponded to the genus *Rhizobium*.

Key words: rhizobacteria, rhizosphere, molecular identification, *Rhizobium*.

RESUMEN

Los rizobios han sido estudiados por la simbiosis que establecen con las raíces de las leguminosas. Sin embargo, la colonización y promoción del crecimiento en plantas no leguminosas también ha sido demostrada. Este trabajo tuvo como objetivo la identificación bioquímica y molecular de rizobios rizosféricos presentes en la rizosfera de dos cultivares comerciales de maíz. Se obtuvieron aislamientos cultivables en medio Levadura-Manitol-Agar (YMA) a partir del suelo rizosférico y rizoplano de las plantas. Se determinaron las características culturales del cultivo (tamaño, color, mucosidad, etc.) y morfo-tintoriales (forma celular, respuesta a la tinción y esporulación), así como la respuesta de los aislados a ocho pruebas bioquímicas (producción ácido-base, citrato, oxidasa, catalasa, producción de H_2S, ureasa, gelatinasa y ensayo oxidativo-fermentativo) con valor predictivo para la identificación de rizobios. Se determinó el género mediante la secuenciación del gen ADNr-16S. Se obtuvieron 81 aislados totales de los cuales el 30.86% mostró las características culturales, morfológicas y tintoriales esperadas para los rizobios y solamente el 20% de estos correspondió al género *Rhizobium*.

Palabras clave: rizobacterias, rizosfera, identificación molecular, *Rhizobium*.

Introduction

From a nutritional point of view, maize (*Zea mays* L.) is one of the most important cereals in the world. Maize constitutes the nutritional basis for humans in many developing countries, and it is also the energy concentrate par excellence of intensive poultry and livestock feeding systems (Permuy, 2013). In Cuba, maize grain production has been affected by economic problems and by the use of inadequate soil practices and crop management that sometimes leads to significant environmental degradation.

Obtaining acceptable crop yields requires the application of large quantities of mostly nitrogen fertilizers. The use of chemical compounds as a means of fertilization in agriculture is a common practice among farmers. However, its indiscriminate use significantly alters the balance of the organic and living soil constituents (Vidal *et al*., 2015). In agricultural production systems based on crop rotation, maize is intercropped with some leguminous crops. This technique allows the maize to take advantage of the benefits provided by microbial communities that are associated with the leguminous rhizosphere. This nutrient

Received for publication: 11 June, 2019. Accepted for publication: 14 October, 2019 Doi: 10.15446/agron.colomb.v37n3.80189

[1] Departamento de Fisiología y Bioquímica Vegetal, Instituto Nacional de Ciencias Agrícolas INCA, San Jose de las Lajas, Mayabeque (Cuba).
[2] Département Génie Biologique, Institut Universitaire Technologie IUT, Villeurbanne Cedex, Lyon, (France).
[3] Centro de Investigaciones Biotecnológicas del Ecuador CIBE, Escuela Superior Politécnica del Litoral ESPOL, Guayaquil, Guayas (Ecuador).
[4] Universidad Estatal de Milagros UNEMI, Milagros, Guayas (Ecuador).
* Corresponding author: riny@inca.edu.cu

contribution is one of the main benefits helping to decrease the application of the mineral fertilizer load (Martín and Rivera, 2015).

Biofertilizers based on microorganisms that promote plant growth are considered a component of integrated plant nutritional management (Vessey, 2003) and allow substituting part of the inorganic fertilizers in many crops. The success in the use of these biopreparations lies (besides other aspects) in obtaining strains that are compatible with the crop of interest (Hernández *et al*., 2004) and that are efficient for obtaining the desired yields.

The rhizobia constitute a group of diazotrophic microorganisms that have been mainly studied for their symbiotic association with leguminous plant roots and on which differentiated organs called nodules are produced. In these structures, these bacteria perform biological nitrogen fixation (BNF), mediated by nitrogenase protein complex activity (Luyten and Vanderleyden, 2000). Currently, rhizobia are considered plant growth promoting rhizobacteria (PGPR) because they have the ability to promote non-leguminous plant growth at the rhizosphere level or as endophytes without nodule formation (Sessitsch *et al*., 2002). Several studies demonstrate these microorganisms' capacity to colonize non-leguminous plant rhizospheres such as rice, lettuce, tomato, pepper, wheat and maize (Bécquer *et al*., 2011; García-Fraile *et al*., 2012; Flores-Félix *et al*., 2013; Hernández, 2015). However, rhizobia used in these trials were isolated from legume nodules and not from the crop rhizosphere itself.

In Cuba, the presence of rhizobia associated with maize plants has not been reported; and, therefore, there are no biopreparations based on these microorganisms for crop biofertilization. Taking into account this background, the aim of this study was to identify maize rhizospheric rhizobia.

Materials and methods

Studies were carried out in the Department of Plant Physiology and Biochemistry at the Instituto Nacional de Ciencias Agrícolas (INCA) in Cuba and at the Centro de Investigaciones Biotecnologicas del Ecuador (CIBE), Escuela Superior Politecnica del Litoral (ESPOL).

Sampling

Two maize cultivars were sampled: Raúl and Canilla. They were cultivated in a Ferralitic Red Leached soil (Hernández *et al*., 2015) at "El Mulato" farm and at the Centro Nacional de Sanidad Agropecuaria (CENSA), respectively. Both centers are located at San Jose de las Lajas, Mayabeque province, Cuba. The bean (*Phaseolus vulgaris* L.) was the rotation crop. At both sites five random sampling points were established and two plants were taken from each of them for a total of 10 plants per cultivar. The extraction was performed taking a volume of soil of 20x20x20 cm. Samples were placed separately in polyethylene bags and stored in the laboratory at 4ºC until they were used.

Rhizobia isolation from Raúl and Canilla maize cultivar rhizospheres

Rhizobia isolation was conducted from rhizospheric soil and the maize rhizoplane using the methodology of Knief *et al*. (2012). For rhizospheric soil isolation, 1 g samples of soil were taken and serial dilutions were performed (10^{-1}-10^{-6}). One hundred µl suspensions were grown in YMA (Yeast Mannitol Agar) medium at pH 6.8 with congo red. Plates were incubated at 28°C for 10 d.

For rhizoplane isolation, roots were cut into 1 cm long portions and placed in Erlenmeyer flasks with 10 ml of sterile distilled water. Flasks were maintained while stirring for 1 h at 150 rpm. The same methodology was used later with dilutions from rhizospheric soils.

Roots from the previous methodology were placed on plates with LB (Luria Bertani agar) medium and incubated at 28ºC for 24 h. Subsequently, visible bacterial growth around the roots was collected and cultivated in YMA medium at pH 6.8 with Congo red. Plates were incubated at 28°C for 10 d. Strains obtained were stored at -20ºC in the INCA bacterial culture collection.

Rhizospheric rhizobia characterization of the maize cultivars Raúl and Canilla

Cultural and morphological characterization

The color, size (mm) and mucus of the colonies were determined as proposed for rhizobia (Wang and Martínez-Romero, 2001). Colonies with whitish coloration, semitranslucent, mucous or dry, and all those that absorbed YMA medium red pigment at its center were selected at different culture stages. Cultures that grew over a period of 1-3 d were considered as fast growth, and those up to 10 d as slow growth. Colonies with diameters between 1-2 mm and 2-4 mm were considered small or large, respectively. These were purified by successive passes and kept at 4°C in tubes with YMA medium. Cell morphology and the presence of spores were determined by gram staining.

Biochemical characterization

Eight biochemical tests were applied to each isolate: acid-base production, citrate utilization, oxidase, catalase, urease, hydrogen sulfide production (H_2S), gelatinase, and an oxidation-fermentation test.

To determine acid-base production isolates were cultured in YMA medium at pH 6.8 with bromothymol blue indicator (0.5% in sodium hydroxide (NaOH) 0.016 N). Subsequently, plates were incubated at 28°C for 10 d. Cultures that changed medium coloration from green to yellow were taken as positive results for acid production. Base production was found in the isolates that changed medium coloration from green to blue.

The use of citrate as carbon and energy sources was determined in tubes with Simmons citrate agar medium (Koser, 1923). Tubes were incubated at 28°C for 10 d. A change in medium coloration from green to yellow was interpreted as a positive result.

Determinations of oxidase and catalase activity were performed by the methods of Kovaks (1956) and Graham-Parker (1964), respectively.

To determine urease enzyme production, Christensen's urea agar medium (Christensen, 1946) was used. Tubes were incubated at 28°C for 10 d. A change in medium coloration from yellow to pink was considered positive urease and negative urease was considered when the medium remained yellow.

Hydrogen sulfide production (H_2S) was determined in TSI agar medium (Triple Sugar Iron) (MacFaddin, 2000). Tubes were incubated at 28°C for 10 d. The presence of a black precipitate at the bottom of the tube was interpreted as a positive result.

The gelatinase activity test was performed according to Leboffe and Pierce (2010) in nutrient gelatin medium (Difco Laboratories, 2009). Tubes were incubated at 28°C for 48 h. Those isolates that hydrolyzed the medium were taken as positive gelatinase and the others that maintained the medium in the solid state were considered as a negative gelatinase.

The type of energy metabolism (respiratory or fermentative) was performed following Hugh-Leifson's method (Hugh and Leifson, 1953) in O-F basal medium (Winn *et al*., 2006). Two tubes were used per isolate, one under anaerobic and the other one under aerobic conditions. Tubes were incubated at 28°C for 48 h. Isolates that totally changed medium coloration from green to yellow in both tubes were considered positive for anaerobic metabolism. Those that remained green in the anaerobic tube and then changed partially or totally to yellow in the other tube were considered positive for oxidative metabolism.

Identification by 16S rDNA gene sequencing

DNA extraction

Extraction of the genetic material was performed by alkaline lysis (von Post *et al*., 2003). A colony of the axenic culture was taken and placed in an Eppendorf tube with 40 µl of NaOH at 0.20 M. It was then heated in a microwave at 10% power or 700 W for 1 min.

Nucleic acid quantification by spectrophotometry was performed using the NanoDropTM 2000 at 260 nm; the ratios 260/280 and 260/230 were calculated in order to determine DNA concentration and quality.

PCR amplification

Universal primers forward 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and reverse 1492R (5'-GGTTACCTTGTTACGACTT-3') (Criollo *et al*., 2012) were used for 16S rDNA amplification. This procedure was carried out in a mini MS thermal cycler (Major Science, USA) under the following amplification parameters: initial denaturation 95°C for 10 min, additional denaturation for 1 min at 92°C and 35 cycles of 72°C each for 2 min. Amplification results were verified by electrophoresis in 1% agarose gel. PCR product sequencing was carried out by the Sanger method of Macrogen® (Republic of Korea).

Editing sequences and identification

Sequence quality analysis was performed through the FinchTV program (version 1.4.0, Geospiza Inc., Seattle, WA, USA). Consensus sequences were obtained with the MEGA-X software (version 10.0.4, Molecular Evolutionary Genetics Analysis) (Kumar *et al*., 2018) and compared with the NCBI (National Center for Biotechnological Information) database, using the BLASTn (Basic Local Alignment Search Tool - nucleotide, Maryland, USA) program (Benson *et al*., 2015). Alignments with an e-value lower than 1×10^{-5} and with a similarity and coverage greater than 80% of amplified region were considered as a hit.

Results and discussion

Rhizobia isolation from the maize cultivars Raúl and Canilla rhizosphere

A total of 81 isolates were obtained consisting of 46 strains of the Raúl cultivar and 35 strains of the Canilla cultivar. Forty-seven came from the rhizoplane equaling 58.3% and the rest came from rhizospheric soil. The rhizoplane is the rhizosphere zone most influenced by radical exudates and where their concentration is at a maximum (Atlas and Bartha, 2005). This is an attractive area for microbial colonization (Kapulnik, 2002). In addition, radical exudates from grasses provoke chemotactic responses in rhizobacteria such as rhizobia (van Rossum *et al*., 1995). Vanillic, p-coumaric, m-coumaric and cinnamic acids excreted by rice plants (and others) are important carbon sources for rhizobia and allow their saprophytic survival in this crop rhizosphere (Heidarzade *et al*., 2010).

Rhizospheric rhizobia characterization of the maize cultivars Raúl and Canilla

Cultural and morphological characterization

The 81 isolates complied with cultural descriptions proposed for rhizobia (Wang and Martínez-Romero, 2001). Taking into account the large number of isolates with similar morpho-cultural characteristics to those described for the rhizobia group, gram stain was used as a second discrimination criterion. Cellular morphology and staining responses allowed separating isolates into seven groups (Tab. 1).

Group number V was the most representative with a 30.9% of total bacteria detected. Coincidentally, morphological and staining isolate characteristics included in this group agree with those described by Frioni (1990) for rhizobia. In addition, these isolates showed a rapid growth rate (24 h) under used conditions. Rhizobia usually show fast growth rates with the exception of the genus *Bradyrhizobium*, which can take 7 to 10 d to grow (Berrada and Fikri-Benbrahim, 2014).

Biochemical characterization

Acid production in YMA medium with bromothymol blue was determined in 24 isolates, and only one showed base production (results not shown). Acid excretion is a characteristic shared by some rhizobia genera with fast growth rates, such as *Rhizobium*, *Allorhizobium* and *Sinorhizobium* (Berrada and Fikri-Benbrahim, 2014). One of the organic acids produced by rhizobia is indoleacetic acid (IAA), which promotes plant growth, since it allows cellular elongation, root initiation, and the formation of root hairs (van Loon, 2007).

Phosphate solubilization in soils constitutes another important function of these acids, which increase nutrient availability, an aspect that is advantageous for the plant (Zúñiga *et al*., 2013). Regarding base production, although less frequent, it has taxonomic value in slow-growing rhizobia identification, such as the genus *Bradyrhizobium*. In addition, this phenomenon intervenes in other mineral solubilization (Sugumaran and Janarthanam, 2007).

Eight isolates grew and produced a change of color in Simmons medium (Tab. 2), which indicates the capacity to metabolize sodium citrate (MacFaddin, 2000). Generally, rhizobia respond negatively to this assay (Sadowsky *et al*., 1983); this could be due to citrate molecule complexity in terms of its functional groups volume (three carboxyl groups), compared to other carbon sources used by these microorganisms that basically include sugars, alcohols and organic acids (Gaurav *et al*., 2016). The YMA medium includes mannitol, sucrose or glycerol as the sole carbon sources. These guarantee rhizobia multiplication with a suitable nitrogen source and under certain parameters of pH and temperature (Hernández and Nápoles, 2018).

TABLE 1. Bacterial groups established according to their morphological and staining characteristics.

Groups	Morphological and staining characteristics	Quantity	%
I	Gram-negative cocci, not sporulated	5	6.2
II	Gram-positive cocci, not sporulated	17	21.0
III	Gram-positive cocobacilli, not sporulated	4	4.9
IV	Coccobacilli and gram-negative long/short bacilli, sporulated	8	9.9
V	Coccobacilli and short Gram-negative bacilli, not sporulated	25	30.9
VI	Long bacilli, Gram-negative, not sporulated	20	24.7
VII*	White lumps with pink edges	2	2.5

*There was no response to gram staining. Probably not bacteria.

TABLE 2. Isolate rhizosphere biochemical characterization of the maize cultivars Raúl and Canilla.

Isolate	Provenance	Citrate	Oxidase	Catalase	H_2S production	Gelatinase	Oxidative/ Fermentative Metabolism
			***Zea mays* L. cv. Raúl**				
R1	RS	-	+	+	-	-	Oxidative
R2	RS	-	+	+	-	-	Fermentative
R3	RS	+	+	+	-	-	Oxidative
R4	RS	+	+	+	-	-	Oxidative
R5	RS	-	+	+	-	-	Oxidative
R6	RS	-	-	-	-	-	Oxidative
R7	RS	+	-	+	+	-	Oxidative
R8	RP	-	+	+	-	-	Oxidative
R9	RP	-	-	-	+	-	Oxidative
R10	RP	-	+	-	-	+	Oxidative
R11	RP	+	-	+	-	-	Oxidative
R12	RP	+	+	+	-	-	Fermentative
R13	RP	-	+	+	-	-	Oxidative
R14	RP	-	+	+	+	-	Oxidative
R15	RP	+	+	-	-	-	Oxidative
R16	RP	+	+	+	-	-	Fermentative
			***Zea mays* L. cv. Canilla**				
C1	RP	-	+	+	-	+	Oxidative
C4	RP	-	+	+	-	+	Oxidative
C6	RP	-	+	+	-	+	Oxidative
C8	RP	-	+	-	-	+	Fermentative
C16	RS	-	+	+	+	+	Oxidative
$C19_2$	RS	-	-	+	-	-	Oxidative
C21	RS	-	+	+	-	-	Oxidative
C22	RP	+	+	+	-	-	Oxidative
C24	RP	-	+	+	-	-	Oxidative

RP: rhizoplane, RS: rhizospheric soil.
Rhizobia are: citrate negative (-), oxidase positive (+), catalase positive (+), no H_2S production (-), gelatinase positive (+), urease positive (+), and have oxidative metabolism (usually).

Cytochrome oxidase enzyme activity was observed in 20 isolates and the same number for catalase activity (Tab. 2). Similar results were obtained by Sadowsky *et al.* (1983), which show that both slow-growing and fast-growing rhizobia share the positive results of these trials. However, urease enzyme production that is positive for rhizobia (Sadowsky *et al*., 1983), is negative in all isolates (results not shown).

Hydrogen sulfide precipitation was seen in four isolates. Rhizobia lack the capacity to reduce the sodium thiosulfate present in TSI medium, so there is no production of H_2S. Gelatinase activity was seen in six cases (Tab. 2), which could correspond to some types of rhizobia, since this is a biochemical characteristic of the group (Sadowsky *et al*., 1983).

The ability to metabolize glucose by fermentation was seen in four isolates that produced a change in medium coloration produced by organic acid excretion (Tab. 2). Oxidative metabolism was much superior to the previous variant with 21 isolates grown in aerobic conditions. Some gram-negative bacteria metabolize glucose through aerobic respiration; and, as a result, small amounts of weak acids are formed during Krebs cycle and the Entner Doudoroff pathway (Winn *et al*., 2006) that promote medium color changes, from green to yellow. In addition, this test allowed the determination of motility, since acid production is low, color changes are only observed in the tube's upper levels. In this case, total medium discoloration to yellow was observed in 100% isolates, which shows bacterial displacement throughout the tube.

Identification by 16S rDNA gene sequencing

In biochemical tests performed on rhizospheric isolates, null urease enzyme activity was determined that contradicts the biochemical pattern usually present in rhizobia. In this way, it could be concluded that none of the isolates belong to this group of microorganisms. However, molecular biology confirmed these results (Tab. 3).

Eight bacterial genera were identified from twenty-five isolates; five corresponded to the genus *Rhizobium* for 20% (Tab. 3.).

The most representative genera were *Pseudomonas* and *Stenotrophomonas* with 32 and 28%, respectively. The genera *Enterobacter, Starkeya, Achromobacter, Delftia* and *Flavobacterium* were identified, each one with 4% representation.

Most of these genera have been described as part of various crops' rhizospheric microbiota, including maize. These include mainly *Pseudomonas*, *Stenotrophomonas* and *Enterobacter* (Hernández *et al*., 2004; Morales-García *et al.,* 2011; Granada *et al*., 2015; Yang *et al.,* 2017; Gaviria-Giraldo *et al*., 2018).

The capacity of *Rhizobium* for rhizospheric and endophytic colonization of horticultural crops (García-Fraile *et al*., 2012; Flores-Félix *et al*., 2013) and grasses (Chabot *et al*., 1996; Gutiérrez-Zamora and Martínez-Romero, 2001; Sessitsch *et al*., 2002) has been studied. However, microorganisms that were used in these studies were isolated from legume nodules and not from the crop's rhizosphere.

Isolates obtained in this study come from Red Ferralitic soils, which have a low percentage of organic matter, and this translates into poor nitrogen content (Hernández *et al*., 2015). Maize requires high doses of nitrogen for growth and development, so it is common to rotate it with leguminous crops that enrich soil nitrogen concentration (Martín and Rivera, 2015). The presence of beans (*Phaseolus vulgaris* L.)

TABLE 3. Molecular identification of the isolates based on the 16S subunit rDNA sequencing.

Isolate	Genus	Possible identification	Identity%	Access Number
R1	*Stenotrophomonas*	*Stenotrophomonas rhizophila*	99%	NR _ 121739.1
R4		*Stenotrophomonas rhizophila*	100%	MH828345.1
R9		*Stenotrophomonas* sp.	99%	MH396743.1
R10		*Stenotrophomonas* sp.	95%	AM745261.1
R13		Stenotrophomonas rhizophila	99%	NR _ 121739.1
R16		Stenotrophomonas rhizophila	100%	MH828345.1
C16		Stenotrophomonas bentonitica	99%	NR _ 157765.1
C24		Stenotrophomonas pavanii	93%	NR _ 116793.1
R2	*Pseudomonas*	*Pseudomonas graminis*	99%	NR _ 026395.1
R3		*Pseudomonas hibiscicola*	97%	KX527638.1
R5		*Pseudomonas putida*	100%	MF952434.1
R6		*Pseudomonas* sp.	100%	MG833395.1
R8		*Pseudomonas* sp.	99%	KF542910.1
R14		*Pseudomonas graminis*	99%	NR _ 026395.1
R15		*Pseudomonas hibiscicola*	97%	KX527638.1
R7	*Rhizobium*	*Rhizobium* sp.	88%	MH899428.1
C1		*Rhizobium mesosinicum*	99%	NR _ 043548.1
C4		*Rhizobium aegyptiacum*	100%	NR _ 137399.1
C8		*Rhizobium mesosinicum*	99%	NR _ 043548.1
$C19_2$		*Rhizobium* sp.	99%	MK092996.1
R11	*Achromobacter*	*Achromobacter xylosoxidans*	99%	MK089550.1
R12	*Enterobacter*	*Enterobacter bugandensis*	99%	NR _ 148649.1
C6	*Starkeya*	*Starkeya novella*	99%	NR _ 074219.1
C21	*Flavobacterium*	*Flavobacterium anhuiense*	99%	NR _ 044388.1
C22	*Delftia*	*Delftia lacustris*	91%	NR _ 116495.1

as a predecessor crop to maize from which the isolation was performed and the type of soil characteristics constitute two important factors that explain the rhizobia population presence associated with this plant rhizosphere. Bean establishes a greater symbiotic interaction with Rhizobiaceae family representatives, fundamentally with the genus *Rhizobium* (Ramírez-Bahena *et al*., 2008; Dall'Agnol *et al*., 2013), an aspect that could explain the presence of this genus in the crop's rhizosphere. In this way, *Rhizobium* can be demonstrated as a common component of maize rhizospheric microbiota.

Conclusions

In the maize rhizosphere, planted in Red Ferralitic soil in Mayabeque province, Cuba, there are rhizobia populations of the genus *Rhizobium* spp. Biochemical characterizations are important elements for microorganism identification. However, with the development of molecular techniques, a more accurate and reliable taxonomic diagnosis can be made.

Acknowledgments

The authors would like to thank all CIBE analysts and researchers who supported this research.

Literature cited

Atlas, R. and R. Bartha. 2005. Ecología microbiana y microbiología ambiental. Addison Wesley, Madrid.

Bécquer, C.J., B. Salas, D.J. Archambault, J.J. Slaski, and A. Anyia. 2008. Selección de rizobios adaptados a ecosistemas ganaderos de Sancti Spíritus, Cuba, inoculados en maíz (*Zea mays* L.). Fase I: Invernadero. Pastos y Forrajes 31(3), 229-246.

Bécquer, C.J., U. Ávila, L. Palmero, A. Nápoles, L. Ulloa, Y. Suárez, and O. Colina. 2011. Selección de cepas aisladas de rizobios, inoculadas en maíz (*Zea mays* L.), en condiciones de campo en ecosistemas ganaderos de Sancti Spíritus, Cuba. Rev. Cub. Cienc. Agric. 45(4), 445-449.

Benson, D.A., K. Clark, I. Karsch-Mizrachi, D.J. Lipman, J. Ostell, and E.W. Sayers. 2015. GenBank. Nuc. Acids Res. 43(Database issue), D30-5. Doi: 10.1093/nar/gku1216

Berrada, H. and K. Fikri-Benbrahim. 2014. Taxonomy of the rhizobia: current perspectives. Br. Microbiol. Res. J. 4(6), 616-639. Doi: 10.9734/BMRJ/2014/5635

Chabot, R., H. Antoun, and M.P. Cescas. 1996. Growth promotion of maize and lettuce by phosphate-solubilizing *Rhizobium leguminosarum* bv. *phaseoli*. Plant Soil 184(2), 311-321. Doi: 10.1007/BF00010460

Christensen, W.B. 1946. Urea decomposition as a means of differentiating *Proteus* and Paracolon cultures from each other and from *Salmonella* and *Shigella* types. J. Bacteriol. 52(4), 461-466.

Criollo, P.J., M. Obando, L. Sánchez, and R. Bonilla. 2012. Efecto de bacterias promotoras de crecimiento vegetal (PGPR) asociadas a *Pennisetum clandestinum* en el altiplano cundiboyacense. Cienc. Tecnol. Agropec. 13(2), 189-195. Doi: 10.21930/rcta.vol13_num2_art:254

Dall'Agnol, R.F., R.A. Ribeiro, E. Ormeno-Orrillo, M.A. Rogel, J.R.M. Delamuta, D.S. Andrade, and M. Hungria. 2013. *Rhizobium freirei* sp. nov., a symbiont of *Phaseolus vulgaris* that is very effective at fixing nitrogen. Int. J. Syst. Evol. Microbiol. 63(11), 4167-4173. Doi: 10.1099/ijs.0.052928-0

Difco Laboratories. 2009. Difco & BBL manual: manual of microbiological culture media. Becton Dickinson and Company, Sparks, USA.

Flores-Félix, J.D., E. Menéndez, L.P. Rivera, M. Marcos-García, P. Martínez-Hidalgo, P.F. Mateos, and E. Martínez-Molina. 2013. Use of *Rhizobium leguminosarum* as a potential biofertilizer for *Lactuca sativa* and *Daucus carota* crops. J. Plant Nutr. Soil. Sci. 176(6), 876-882. Doi: 10.1002/jpln.201300116

Frioni, L. 1990. Comunidad del Sur-Edinor. Ecología microbiana del suelo. Colección Reencuentro, Universidad de la República, Montevideo.

García-Fraile, P., L. Carro, M. Robledo, M.H. Ramírez-Bahena, J.D. Flores-Félix, M.T. Fernández, and P.F. Mateos. 2012. *Rhizobium* promotes non-legumes growth and quality in several production steps: towards a biofertilization of edible raw vegetables healthy for humans. PLOS ONE 7(5), e38122. Doi: 10.1371/journal.pone.0038122

Gaurav, K., A.K. Singh, and G. Singh. 2016. Spent wash as an alternative medium for growth of Rhizobium. J. Acad. Indust. Res. 5(6), 81-84.

Gaviria-Giraldo, J., G. Restrepo-Franco, N. Galeano-Vanegas, and A. Hernández-Rodríguez. 2018. Bacterias diazotróficas con actividad promotora del crecimiento vegetal en *Daucus carota* L. Cienc. Agricult. 15(1), 19-27. Doi: 10.19053/01228420.v15.n1.2018.7753

Graham, P.H. and C.A. Parker. 1964. Diagnostic features in the characterization of the root-nodule bacteria of legumes. Plant Soil 20, 383-396. Doi: 10.1007/BF01373828

Granada-Mora, K.I., R. González, Y. Alvarado, A.R. Robles, and R. Torres. 2015. Caracterización de rizobacterias y estimulación de parámetros morfológicos y biomasa en maíz (*Zea mays* L.). Centro de Biotecnología 4(1), 14-22.

Heidarzade, A., H. Pirdashti, and M. Esmaeili. 2010. Quantification of allelopathic substances and inhibitory potential in root exudates of rice (*Oryza sativa*) varieties on Barnyardgrass (*Echinochloa crus-galli* L.). Plant Omics 3(6), 204-209.

Hernández, A., J.M. Pérez, and D. Bosch. 2015. Clasificación de los suelos de Cuba. Ediciones INCA, Instituto Nacional de Ciencias Agrícolas, Cuba.

Hernández, A., N. Rives, A. Caballero, A.N. Hernández, and M. Heydrich. 2004. Caracterización de rizobacterias asociadas al cultivo del maíz en la producción de compuestos indólicos, sideróforos y ácido salicílico. Rev. Colomb. Biotecnol. 6(1), 6-13.

Hernández, I. 2015. Rizobios asociados a plantas de arroz (*Oryza sativa* L.) cultivar INCA LP-5. Efecto en el crecimiento del cultivo. Msc thesis, Universidad de La Habana, La Habana.

Hernández, I. and M.C. Nápoles. 2018. Efecto de diferentes fuentes de carbono sobre el crecimiento de un aislado de rizobio. Cult. Trop. 39(3), 87-90.

Hugh, R. and E. Leifson. 1953. The taxonomic significance of fermentative versus oxidative metabolism of carbohydrates by various gram-negative rods. J. Bacteriol. 66(1), 24-26.

Kapulnik, Y. 2002. Plant roots the hidden half. Plant growth promotion by rhizosphere bacteria. Marcel Dekker, New York, USA.

Knief, C., N. Delmotte, S. Chaffron, M. Stark, G. Innerebner, R. Wassmann, and C. von Mering. 2012. Metaproteogenomic analysis of microbial communities in the phyllosphere and rhizosphere of rice. Isme J. 6(7), 1378. Doi: 10.1038/ismej.2011.192

Koser, S.A. 1923. Utilization of the salts of organic acids by the colon-aerogenes group. J. Bacteriol. 8(5), 493-520.

Kovaks, N. 1956. Identification of *Pseudomonas pyocyanea* by the oxidase reaction. Nature 178, 703. Doi: 10.1038/178703a0

Kumar, S., G. Stecher, M. Li, C. Knyaz, and K. Tamura. 2018. MEGA X: Molecular evolutionary genetics analysis across computing platforms. Mol. Biol. Evol. 35(6), 1547-1549. Doi: 10.1093/molbev/msy096

Leboffe, M.J. and B.E. Pierce. 2010. Microbiology laboratory theory and application. Morton Publishing Company, Englewood, USA.

Luyten, E. and J. Vanderleyden. 2000. Survey of genes identified in *Sinorhizobium meliloti* spp., necessary for the development of an efficient symbiosis. Eur. J. Soil Biol. 36(1), 1-26. Doi: 10.1016/S1164-5563(00)00134-5

MacFaddin, J.F. 2000. Biochemical tests for identification of medical bacteria. Lippincott, Williams, and Wilkins. Philadelphia, USA.

Martín, G.M. and R. Rivera. 2015. Influencia de la inoculación micorrízica en los abonos verdes. Efecto sobre el cultivo principal. Estudio de caso: el maíz. Cult. Trop. 36, 34-50.

Permuy, A.N. 2013. El cultivo de maíz. Conferencia sobre el cultivo de maíz. Estación territorial de investigaciones agropecuaria de Holguín (ETIAH), Holguín, Cuba.

Ramírez-Bahena, M.H., P. García-Fraile, A. Peix, A. Valverde, R. Rivas, J.M. Igual, P.F. Mateos, E. Martínez-Molina, and E. Velázquez. 2008. Revision of the taxonomic status of the species *Rhizobium leguminosarum* (Frank 1879) Frank 1889AL, *Rhizobium phaseoli* Dangeard 1926AL and *Rhizobium trifolii* Dangeard 1926AL. *R. trifolii* is a later synonym of *R. leguminosarum*. Reclassification of the strain *R. leguminosarum* DSM 30132 (=NCIMB 11478) as *Rhizobium pisi* sp. nov. Int. J. Syst. Evol. Microbiol. 58(11), 2484-2490. Doi: 10.1099/ijs.0.65621-0

Sadowsky, M.J., H.H. Keyser, and B.B. Bohlool. 1983. Biochemical characterization of fast- and slow-growing rhizobia that nodulate soybeans. Int. J. Syst. Bacteriol. 33(4), 716-722. Doi: 10.1099/00207713-33-4-716

Sessitsch, A., J.G. Howieson, X. Perret, H. Antoun, and E. Martinez-Romero. 2002. Advances in Rhizobium research. Critical Rev. Plant Sci. 21, 323-378. Doi: 10.1080/0735-260291044278

Sugumaran, P. and B. Janarthanam. 2007. Solubilization of potassium containing minerals by bacteria and their effect on plant growth. World J. Agricul. Sci. 3(3), 350-355.

van Loon, L.C. 2007. Plant responses to plant growth-promoting bacteria. Eur. J. Plant. Pathol. 119, 243-254. Doi: 10.1007/s10658-007-9165-1

van Rossum, D., F.B. Schuuramns, M. Gilli, A. Muyotcha, H.W. van Versveld, A.H. Stouthamer, and F.C. Boogerd. 1995. Genetic and phenetic analyses of Bradyrhizobium strains nodulating peanut (*Arachis hypogaea* L.) roots. Appl. Environ. Microbiol. 61(4), 1599-1609.

Vessey, J.K. 2003. Plant growth promoting rhizobacteria as biofertilizers. Plant Soil 255(2), 571-586. Doi: 10.1023/A:1026037216893

Vidal, M., R. Ruiz, A. Antúnez, and C. Araya. 2015. Antecedentes nutricionales del cultivo de maíz en Chile. pp. 83-93. In: Antúnez, A., M. Vidal, S. Felmer, and M. González (eds.). Riego por pulsos de maíz grano. Boletín INIA no. 312. Instituto de Investigaciones Agropecuarias INIA, Rengo, Chile.

von Post, R., C. Dayteg, M. Nilsson, B. Forster, S. Tuvesson, and L. von Post. 2003. A high-throughput DNA extraction method for barley seed. Euphytica 130, 255-260. Doi: 10.1023/A:1022863006134

Wang, E.T. and J.C. Martínez-Romero. 2001. Taxonomía del Rhizobium. Centro de investigaciones sobre fijación de Nitrógeno. Microbios, Universidad Nacional Autónoma de México, Mexico.

Winn, W., S. Allen, W. Janda, E. Koneman, G. Procop, P. Schreckenberger, and G. Woods. 2006. Color atlas and textbook of diagnostic microbiology. Lippincott Williams and Wilkins, Philadelphia, USA.

Yang, Y., N. Wang, X. Guo, Y. Zhang, and Y. Boping. 2017. Comparative analysis of bacterial community structure in the rhizosphere of maize by high-throughput pyrosequencing. PLOS ONE 12(5), e0178425. Doi: 10.1371/journal.pone.0178425

Zúñiga, A., M.J. Poupin, R. Donoso, T. Ledger, N. Guiliani, R.A. Gutiérrez, and B. González. 2013. Quorum sensing and indole-3-acetic acid degradation play a role in colonization and plant growth promotion of *Arabidopsis thaliana* by *Burkholderia phytofirmans* PsJN. Mol. Plant Microbe. Interact. 26(5), 546-553. Doi: 10.1094/MPMI-10-12-0241-R

Impact of soil use on aggregate stability and its relationship with soil organic carbon at two different altitudes in the Colombian Andes

Impacto del uso del suelo sobre la estabilidad de agregados y su relación con el carbono orgánico en dos pisos altitudinales en Los Andes de Colombia

Efraín Francisco Visconti-Moreno[1] and Ibonne Geaneth Valenzuela-Balcázar[1]*

ABSTRACT

The stability of soil aggregates depends on the organic matter, and the soil use and management can affect the soil organic matter (SOM) content. Therefore, it is necessary to know the relationship between aggregate stability and the content of SOM in different types of soil use at two different altitudes of the Colombian Andes. This study examined the conditions of soil aggregate stability expressed as a distribution of the size classes of stable aggregates (SA) and of the mean weighted diameter of the stable aggregates (MWD). To correlate these characteristics with the soil organic carbon (OC), we measured the particulate organic matter pool (POC), the OC associated with the mineral organic matter pool (HOC), the total organic carbon content (TOC), and the humification rate (HR). Soils were sampled at two altitudes: 1) Humic Dystrudepts in a cold tropical climate (CC) with three plots: tropical mountain rainforest, pastures, and crops; 2) Fluvaquentic Dystrudepts in a warm tropical climate (WC) with three plots: tropical rainforest, an association of oil palm and pastures, and irrigated rice. Soils were sampled at three depths: 0-5, 5-10 and 10-20 cm. The physical properties, mineral particle size distribution, and bulk density were measured. The content of SA with size>2.36 mm was higher in the CC soil (51.48%) than in the WC soil (9.23%). The SA with size 1.18-2.36 mm was also higher in the CC soil (7.78%) than in the WC soil (0.62%). The SA with size 0.60-1.18 mm resulted indifferent. The SA with size between 0.30 and 0.60 mm were higher in the WC soil (13.95%) than in the CC soil (4.67%). The SA<0.30 mm was higher in the WC soil (72.56%) than in the CC soil (32.15%). It was observed that MWD and the SA>2.36 mm increased linearly with a higher POC, but decreased linearly with a higher HR. For the SA<0.30 mm, a linear decrease was observed at a higher POC, while it increased at a higher HR.

Key words: soil degradation, soil structure, organic matter, agriculture.

RESUMEN

La estabilidad de agregados del suelo depende de la materia orgánica y el uso y manejo del suelo puede afectar el contenido de materia orgánica (MOS) del mismo. Por lo tanto, es necesario conocer la relación entre la estabilidad de agregados y el contenido de MOS en diferentes tipos de uso del suelo a diferentes altitudes en Los Andes de Colombia. El presente estudio examinó las condiciones de estabilidad de agregados del suelo expresados como una distribución por tamaño de las clases de agregados estables (AE) y el diámetro medio ponderado (DMP) de agregados estables. Para relacionar estas características con el contenido de carbono orgánico del suelo (CO), se midieron la materia orgánica particulada (COP), el CO asociado con la materia orgánica mineral (COM), el contenido total de carbono orgánico (COT) y el índice de humificación (IH). Se realizaron muestreos de dos suelos de pisos altitudinales diferentes: 1) Un Humic Dystrudepts en clima frío (CF) con tres lotes: bosque natural, pastura y cultivos; 2) Un Fluvaquentic Dystrudepts en clima cálido (CC) con tres lotes: bosque natural, palma de aceite asociada con pastura y arroz con riego. Se muestreo el suelo a tres profundidades: 0 a 5, 5 a 10, y 10 a 20 cm. Se midieron las propiedades físicas, distribución por tamaño de la partícula mineral y densidad aparente. El contenido de AE con tamaño >2.36 mm fue mayor en el suelo de clima frío (51.48%) que en el de clima cálido (9.23%). Los AE de tamaño 1.18 a 2.36 mm fueron también mayores en clima frío (7.78%) que en clima cálido (0.62%). Los AE de tamaño 0.60 a 1.18 mm resultaron indiferentes. Los AE de tamaño entre 0.30 y 0.60 mm presentaron un contenido más alto en el suelo de clima cálido (13.95%) en comparación al de clima frío (4.67%). Los AE<0.30 mm fueron mayores en clima cálido (72.56%) con respecto al clima frío (32.15%). Se observó que el DMP y los AE>2.36 mm aumentaron linealmente con el contenido de COP más alto, pero disminuyeron linealmente con un IH más alto. Para los AE<0.30 mm se observa una disminución lineal a mayor COP, mientras que este aumenta a un IH más alto.

Palabras clave: degradación del suelo, estructura del suelo, materia orgánica, agricultura.

Received for publication: 30 January, 2019. Accepted for publication: 5 December, 2019 Doi: 10.15446/agron.colomb.v37n3.77601

[1] Grupo de Investigación Ambiente y Vida, Facultad de Ciencias Agrarias y del Ambiente, Universidad Francisco de Paula Santander UFPS, Cucuta (Colombia).

* Corresponding author: ibonnegeanethvb@ufps.edu.co

Introduction

The Norte de Santander province is located in the northeastern area of Colombia, on the eastern ramification of the Andes range. This province is characterized by strong altitudinal variations (approximately from 50 to 4000 m a.s.l.). The variations in landscape configuration and changes in the vegetation modify its tropical weather (IGAC, 2006a).

Food production is one of the most important socioeconomic activities in this region. For this reason, it is essential to acknowledge that the soil use is adapted to each particular biophysical condition in terms of the altitude. The zones of the region with cold and warm climate represent the areas with the most intense agricultural land use. This fact has generated soil degradation problems, such as compaction or erosion (IGAC, 2006a), which may be related to the soil's physical properties, in particular, to its structure and its aggregates stability (Pla, 2010).

Soil aggregates are the product of a complex physical-chemical and biological aggregation process, in which various factors such as climate, soil use, plants, and soil properties interact (Bronick and Lal, 2005). Among the soil properties that determine directly the aggregation and its stability, the texture, total organic carbon, microbiological activity, soil fauna and inorganic cementing agents (exchangeable Ca and oxyhydroxides) stand out (Six *et al*., 2004).

According to Oades (1984), macroaggregation is controlled by management in soils where organic matter is the major binding agent. In general, the numbers of macroaggregates (>0.250 mm) can increase by the addition of decomposable organic materials. The best distribution of organic materials and mix with inorganic colloids occur through the root systems, particularly the fine, bushy and extensive root systems of grasses. However, in intensively disturbed soil or with lack of root growth, the opposite effect may be observed.

Changes in soil use, from natural forest to agricultural use, frequently generate modifications in the soil organic carbon content. This affects the aggregates and soil stability directly, which leads to soil productivity loss (Sena *et al*., 2017). According to Lozano *et al.* (1997), the soil structure and stability of aggregates are the most deteriorated physical properties in agricultural tropical soils that are exposed to intense conventional tillage. This generates limitations, such as surface seal or compaction, that alter the normal soil hydrological functioning and generate erosion.

The problems of aggregate stability in soils have been associated with changes in the organic carbon of soil, caused by changes in the soil use (Nascente *et al*., 2015). Aggregation is a hierarchical process, in which the union of mineral components of the soil with organic components occurs at a microscopic scale. In this process, humified organic carbon participates to form micro aggregates, and then, at a larger scale, the particulate organic carbon or light organic carbon takes part in the formation of macro aggregates and mega aggregates. Therefore, the increment in organic carbon increases the dynamic of aggregation and in turn, the greater aggregation favors the preservation of soil organic carbon (Six *et al.,* 2004; Bronick and Lal, 2005).

Consequently, there has been a growing interest in identifying the soil use systems that best suit the stability of soil aggregates from the preservation of soil organic carbon. In this sense, this study aimed to examine the effect of three soil use systems at two altitudes of the Andes mountain range, located in the Norte de Santander province of Colombia.

Materials and methods

The analyzed soils were located in two municipalities of the province of Norte de Santander in the northeast of Colombia. The first one was a Humic Dystrudepts soil in the high mountain area with a cold climate, located in the Vereda Monteadentro, at the municipality of Pamplona. Three adjacent lots on this soil were selected, each one with different soil use and cover: natural forest, kikuyo pastures, and intensive horticultural crops. All lots were located within the premontane wet forest zone, with an average annual temperature of 13.5°C, an average annual rainfall of 900 mm, at an approximate altitude of 2558 m a.s.l. and with geographic reference coordinates 7°20'47.59" N and 72°39'50.62" W.

The other soil was a Fluvaquentic Dystrudepts in the low area of alluvial valley with a warm climate, located in the Vereda Astilleros, at the municipality of El Zulia. Three adjacent lots were selected by the following soil uses and covers: natural forest, oil palm with pasture and intensive rice cultivation with irrigation. These lots were located within the tropical humid forest zone, with an annual average temperature of 27°C, an average rainfall of 2200 mm, at an approximate altitude of 76 m a.s.l. and with geographic reference coordinates 8°12'13.5" N and 72°32'52.1" W.

The study was conducted with an experimental arrangement of a 2x2x3 factorial design with three replicates, with the climate, soil use and soil depth as factors. In each selected plot, undisturbed soil samples were collected in metallic cylinders and disturbed composite samples were also taken. Soil samples were collected at three depths: 0 to 5 cm, 5 to 10 cm and 10 to 20 cm. The study was focused on the arable layer or soil tillage depth (0 to 20 cm), which is separated into three layers: the first 5 cm is considered the place where the largest amount of particulate organic matter would accumulate; the layer from 10 to 20 cm is the depth where the largest amount of stable or humified organic matter would be present, while the medium layer could be considered as a transition layer.

A systematic sampling strategy was performed on a diagonal transect line of 100 m with three equidistant sampling points (10 m from the edge and 40 m between them). In each sampling point, five soil subsamples were collected for each depth to integrate the composite sample of that point. Those sub-samples were collected on a cross traced over each sampling point, where the distance between the crosses was 1 meter. The undisturbed samples were extracted in metallic cylinders of 5 cm of diameter and 5 cm of height, taken in threefold at each depth in each sampling point.

The soil aggregate stability was determined by the modified Yoder's wet sieving method proposed by Pla (1983). Stability tests were performed on aggregates with diameter from 2.36 to 4.10 mm, obtained by dry sieving of disturbed soil samples. The wet sieving was carried out for 10 min with an array of sieves corresponding to 2.36, 1.18, 0.60, and 0.30 mm of mesh opening, after a pre-wetting of 10 min on the aggregates.

Sands retained in each sieve were determined by dispersion with a solution of 10% sodium hexametaphosphate and further mechanical agitation. This allowed making the proper correction when calculating the size distribution of stable aggregates retained on each sieve. Also, the aggregates smaller than 0.30 mm were estimated by difference, resulting in five classes of aggregates size.

The mean weighted diameter (MWD) was calculated as an important stability index, from the percentage of the total weight of the aggregate fraction retained in each sieve (Wi) and the average diameter of the fraction for each sieve (Xi). To calculate the MWD, expressed in mm, Equation 1 was used:

$$MWD\ (mm) = \sum Xi * Wi / 100 \quad (1)$$

The disturbed soil samples also allowed the measurement of mineral particles contents (sand, clay, and silt) by the modified Bouyoucos method (IGAC, 2006b), and, to know the organization of soil constituents, the structural arrangement description was performed according to the guidelines for soil description (FAO, 2006). The oxidizable total organic carbon (TOC) was determined by the digestion and wet acid oxidation method of Walkley and Black with colorimetric measurement by spectrophotometry (IGAC, 2006b). Measurement of organic carbon in the particulate fraction of soil organic matter (POC) and the organic carbon of the humified fraction of soil organic matter (HOC) was performed by physical fractioning of the soil organic matter (SOM), by the method of suspension and agitation in water with sieving, using sieves with openings of 2.36 mm and 0.053 mm. After the separation with sieves of the particulate fraction and the humified fraction of the SOM, the organic carbon content was determined in each one by dry combustion in a muffle at 580°C for 12 h (IGAC, 2006b).

The contents of organic carbon in the different pools indicated as TOC, POC, and HOC, were expressed in weight percentage. They were transformed to a weight expression in megagrams (Mg) of organic carbon (OC) ha^{-1}, for which the soil bulk density (Bd) and the thickness of the soil layer were considered; the first expressed in Mg m^{-3} and the second expressed in cm, through Equation 2:

$$OC\ (Mg\ ha^{-1}) = OC * Bd * Thickness \quad (2)$$

The humification index (HI) was calculated as the quotient of the HOC divided by the TOC and it was expressed in percentage terms (%) (IGAC, 2006b).

The soil bulk density (Bd) was determined by the modified method of the metallic cylinder of Uhland (Pla, 2010).

The carbon of the soil microbial biomass (MBOC) was measured indirectly using the method of substrate-induced respiration (glucose) in disturbed soil samples that were kept refrigerated (4°C) from their sampling until their analysis (Lozano *et al*., 2005), and it was expressed as mg of MBOC kg^{-1} of soil.

The results were analyzed concerning the compliance of the normality statistic assumptions and the variance homogeneity, by the Shapiro-Wilk and Kolmorogov tests. When assumptions were not accomplished, the Kruskal-Wallis not parametric variance test was used to determine the statistical differences with a reliability degree of 95% and to know the effect of factors such as climate, soil use, and depth.

The Pearson correlation coefficients were determined and the dispersion graphics between the related variables with the structural stability and the SOC content were made to interpret their behavior and the ratio of the stability of aggregates with the SOC in their different pools.

Results and discussion

Data of the mineral particle content in the Humic Dystrudepts soil evaluated in the lots at cold climate presented clay in a proportion of 153.8 to 253.8 g kg^{-1} and sand in proportions of 476.9 to 676.9 g kg^{-1}. Sand predominates at all depths from 0 to 20 cm and in all the soil use systems turns in coarse and medium coarse class textures.

Considering the Bd of the evaluated soil in the cold climate (Tab. 1), an average Bd of 1.33 was observed. This fact is normal in soils of medium texture class, and low in soils of coarse texture class. This represents a favorable condition, which means the existence of a good relationship between the volume occupied by the solids and the pores in this soil (Pla, 2010).

The Fluvaquentic Dystrudepts soil that was evaluated in the warm climate expressed contrasting data of sand, silt and clay content compared to the other studied soil. Clay is in proportions from 253.8 to 597.8 g Kg^{-1}, while sand is from 70.2 to 459.5 g Kg^{-1}, defining a texture class from fine to medium.

TABLE 1. Principal statistics for the data of the examined variables in the soils under cold and warm climates (three soil depths, three soil uses, and three replicates).

Variable	Mean	Median	Std. Dev.	Asymmetry	Kurtosis	Shapiro-Wilks		Kolmogorov	
						W	*P*-value	D	*P*-value
Cold tropical climate (n = 27)									
Sand	585.72	623.50	78.71	-0.09	-1.47	0.860	0.000	1.000	0.000
Clay	204.20	200.50	43.34	0.01	-0.67	0.910	0.090	1.000	0.000
Silt	210.07	236.00	58.13	-0.62	-0.76	0.890	0.020	1.000	0.000
Bd	1.33	1.40	0.22	-0.36	-1.39	0.840	0.000	0.840	0.000
SA>2.36	51.48	49.50	15.92	0.87	-0.68	0.780	0.000	1.000	0.000
SA 1.18 to 2.36	7.78	6.00	4.29	1.23	-0.02	0.760	0.000	1.000	0.000
SA 0.6 to 1.18	3.93	3.00	1.99	0.4	-1.41	0.820	0.000	0.940	0.000
SA 0.3 to 0.6	4.67	3.50	2.36	0.7	-1.14	0.800	0.000	0.980	0.000
SA<0.3	32.15	34.50	14.05	-0.38	-1.28	0.850	0.000	1.000	0.000
MWD	2.02	1.92	0.54	0.81	-0.72	0.810	0.000	0.910	0.000
TOC	36.01	30.13	14.60	1.22	0.84	0.860	0.000	1.000	0.000
POC	32.19	25.27	19.00	1.3	0.48	0.830	0.000	1.000	0.000
HOC	67.27	72.42	37.00	0.43	-0.13	0.950	0.440	1.000	0.000
MBOC	39.16	32.51	21.73	0.61	-0.96	0.860	0.000	1.000	0.000
HI	64.07	74.13	20.52	-0.78	-0.8	0.850	0.000	1.000	0.000
Warm tropical climate (n = 27)									
Sand	278.78	363.52	173.05	-0.54	-1.47	0.770	0.000	1.000	0.000
Clay	378.26	292.48	153.19	0.71	-1.47	0.690	0.000	1.000	0.000
Silt	342.96	348.00	58.67	0.21	-0.33	0.940	0.400	1.000	0.000
Bd	1.44	1.46	0.18	-0.68	-0.87	0.840	0.000	0.860	0.000
SA>2.36	9.23	7.00	8.70	0.84	-0.62	0.840	0.000	0.770	0.000
SA 1.18 to 2.36	0.62	0.33	0.69	2.09	2.56	0.610	0.000	0.560	0.000
SA 0.6 to 1.18	3.67	1.67	3.87	1.43	0.96	0.800	0.000	0.660	0.000
SA 0.3 to 0.6	13.95	11.00	15.89	3.04	10.08	0.710	0.000	0.840	0.000
SA<0.3	72.56	72.00	18.55	-2.30	6.79	0.820	0.000	0.960	0.000
MWD	0.46	0.33	0.34	0.82	-0.40	0.890	0.020	0.520	0.000
TOC	20.76	16.48	11.15	1.92	3.63	0.830	0.000	1.000	0.000
POC	7.79	6.61	4.11	0.85	-0.39	0.870	0.000	1.000	0.000
HOC	49.60	45.68	20.86	1.22	0.67	0.850	0.000	1.000	0.000
MBOC	8.47	7.99	2.77	1.57	3.54	0.900	0.040	1.000	0.000
HI	85.53	85.18	7.02	-0.31	-0.82	0.950	0.460	1.000	0.000

Sand; Clay; Silt= g kg^{-1}; Bd= Mg m^{-3}; SA= %; MWD= mm; TOC= Mg ha^{-1}.

MBOC= mg OC Biomass kg^{-1} soil.

The Bd of the soil in the warm climate (Tab. 1) had an average of 1.44 Mg m^{-3}. This represents a high value in soils with fine and medium-class textures, indicating an unfavorable condition that could result in structural problems by compaction in the soil (Pla, 2010).

The contrasts in the contents of sand and clay in both soils are important because the SOC amount can be influenced by the distribution of these particles (Mujuru *et al*., 2013). In addition, the SOC has an important effect on the soil particle aggregation, with a correlation between the content and kind of SOC and the size and aggregates stability (Martinez *et al*., 2008). Therefore, it is necessary to be aware of the particle distribution in the studied soils.

The results of aggregates stability in the cold climate soil (Tab. 1) show the stable aggregates (SA)>2.36 mm are the highest in proportion (51.48%) followed by the SA<0.30 mm with a proportion of 32.15% and in the third place the SA from 1.18 to 2.36 mm with a proportion of 7.78%. The SA from 0.60 to 1.18 and the SA from 0.30 to 0.60 mm represent, together, the remaining 8.60%. These data reveal a favorable condition of structural stability, considering that the 59.26% of the SA are larger than 1.18 mm, which favors the proper physical conditions in the surface soil (Pinto *et al*., 2016).

In the case of the aggregate stability in the warm climate soil (Tab. 1), the results show the SA<0.30 mm is 72.56%. The SA from 0.30 to 0.60 mm represents 13.95%, and the remaining 13.52% are distributed in the bigger sizes classes. This high proportion of micro aggregates is clear evidence of the unfavorable physical conditions in this soil.

Table 2 shows that the structural stability measured by the SA proportions of size classes presents a response with significant statistical effect due to the climate in 4 of the 5 size classes of SA. The class without significant effect in SA is between 0.60 to 1.18 mm. It is deduced that this effect matches the condition of higher SOC content in soils of cold climate.

It can be observed that there was a significant effect only over the size class of SA from 0.30 to 0.50 mm. The other four classes remained without significant effect. This corresponds with the statement that micro-aggregation is not so sensitive to management. Therefore, it is more difficult to improve micro-aggregation through normal farming practices (Oades, 1984). In the case of depth, there is no significant effect in any of the size classes of SA.

The lack of effect over the SA due to the depth of the soil is understood by the fact that the three depths belong to the horizon A of each soil. Therefore, there are no relevant differences in factors involved in the stability of aggregates, such as texture, mineralogy and structural arrangement (type, grade, and class of aggregates) between the three layers of each soil (Tabs. 3 and 4).

The lack of a significant statistical effect of the soil use system in most of the size classes of SA may be interpreted as a situation generated by the extremes on TOC, POC and MBOC observed in the evaluated soils. The soil in cold climate has very positive conditions of structure and aggregate stability, where the SA>2.36 mm prevails. Hence, none of the three evaluated use systems affects significantly this good condition. In contrast, it was found that the soil in a warm climate has very negative conditions of structure and aggregate stability, where the SA<0.30 mm prevails, and, therefore, none of the three evaluated uses affects significantly this negative condition.

TABLE 2. Results of the Kruskal-Wallis non-parametric test for the stable aggregates according to the effect of each factor involved.

	SA>2.36	SA 1.18 to 2.36	SA 0.6 to 1.18	SA 0.3 to 0.6	SA<0.3
			Climate by altitudinal level		
Parameter H	39.76	39.76	3.56	4.53	33.99
***P*-value**	0.0001*	0.0001*	0.0573	0.0331*	0.0001*
			Soil use		
Parameter H	2.77	2.18	4.69	8.3	0.38
***P*-value**	0.2496	0.321	0.0942	0.0156*	0.8251
			Soil depth		
Parameter H	2.59	0.56	1.97	0.45	1.42
***P*-value**	0.2734	0.749	0.3716	0.7961	0.4908

SA = %; *significant statistical difference ($P<0.05$).

TABLE 3. Description of the structural arrangement of the cold tropical climate soil.

Use	Depth	Type	Class	%	Grade	Consistency		
						Dry	Humid	Wet
Mountain rainforest	0 - 5	Granular and Crumb Structures	Medium	90	Weak	Loose	Very friable	Non Sticky
			Coarse	10				
	5 - 10	Granular and Crumb Structures	Fine	80	Weak	Loose	Very friable	Non Sticky
			Medium	20				
	10 - 20	Granular and Crumb Structures	Fine	80	Moderate	Loose	Very friable	Non Sticky
			Medium	20				
Pastures	0 - 5	Granular and Crumb Structures	Medium	60	Moderate	Slightly hard	Very friable	Slightly Sticky
			Fine	40				
	5 - 10	Granular and Crumb Structures	Very Coarse	70	Moderate	Slightly hard	Very friable	Slightly Sticky
			Medium	30				
	10 - 20	Subangular Blocky Structures	Very Coarse	80	Strong	Slightly hard	Very friable	Slightly Sticky
			Fine	20				
Crops	0 - 5	Granular and Crumb Structures	Medium	50	Weak	Slightly hard	Very firm	Very Sticky
			Fine	50				
	5 - 10	Granular and Crumb Structures	Medium	60	Moderate	Slightly hard	Very firm	Very Sticky
			Fine	40				
	10 - 20	Subangular Blocky Structures	Medium	70	Moderate	Slightly hard	Extra firm	Very Sticky
			Fine	30				

TABLE 4. Description of the structural arrangement of the warm tropical climate soil.

Use	Depth	Type	Class	%	Grade	Consistency		
						Dry	Humid	Wet
Rainforest	0 - 5	Subangular Blocky Structures	Coarse	95	Strong	Very hard	Very firm	Very Sticky
			Fine	5				
	5 - 10	Subangular Blocky Structures	Coarse	90	Strong	Very hard	Very firm	Very Sticky
			Fine	10				
	10 - 20	Subangular Blocky Structures	Coarse	80	Strong	Very hard	Extremely firm	Very Sticky
			Fine	20				
Oil Palm	0 - 5	Granular and Crumb Structures	Medium	85	Moderate	Slightly hard	Firm	Slightly Sticky
			Fine	15				
	5 - 10	Granular and Crumb Structures	Very Coarse	80	Moderately strong	Slightly hard	Firm	Slightly Sticky
			Medium	20				
	10 - 20	Granular and Crumb Structures	Very Coarse	60	Moderately strong	Slightly hard	Firm	Slightly Sticky
			Fine	40				
Irrigated Rice	0 - 5	Subangular Blocky Structures	Medium	70	Strong	Very hard	Very firm	Very Sticky
			Fine	30				
	5 - 10	Subangular Blocky Structures	Medium	60	Strong	Very hard	Very firm	Very Sticky
			Fine	40				
	10 - 20	Subangular Blocky Structures	Medium	70	Strong	Very hard	Extremely firm	Very Sticky
			Fine	30				

For the MWD, the soil in cold climate has the biggest structural stability (Fig. 1), being superior in the two first soil layers (0 to 5 and 5 to 10 cm) of the rainforest, with values of 2.93 and 2.91 mm, respectively. The crop presents MWD values in the layers of 0 to 5 and 5 to 10 cm, of 1.96 and 1.73 mm, respectively, which overcome the MWD of the pasture in the same layers. For the deepest layer (10 to 20 cm), the MWD of the crop (2.13mm) is superior to the values of the rainforest and the pasture, which confirms the general behavior of the aggregate stability in the three soil use systems in cold climate (Fig. 2).

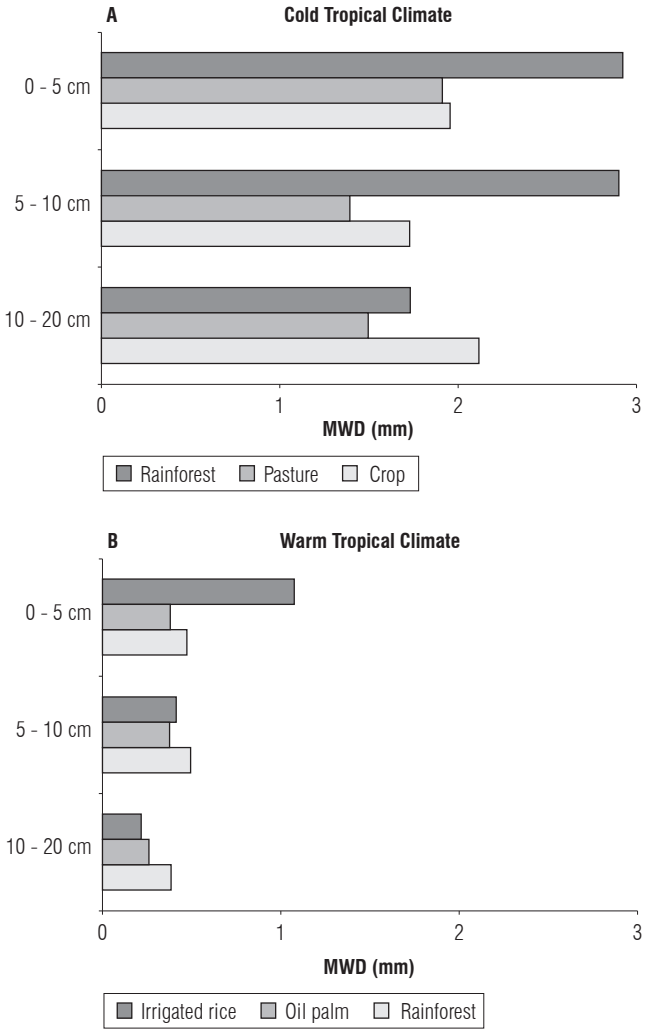


FIGURE 1. Mean weighted diameter (mm) according to the depth of the soil in each soil use system evaluated for each altitudinal level studied. A) cold tropical climate and B) warm tropical climate.

The stratification observed in the MWD coincides with previous studies (Loss, *et al*., 2017), in which important changes of the MWD were observed, with the depth in soils with different soil use systems and management. This situation can be related to differences of higher organic matter and biological activity, and a higher number of roots in some soil layers, which favor a better size of the stable aggregates.

For the soil in a warm climate, the MWD can help to confirm the low structural stability of the soil. When such soils are irrigated for rice cultivation, the surface layer (0 to 5 cm) presents the largest MWD of this soil with a value of 1.08 mm. All the remaining values are equal or under 0.50 mm, which is very negative and confirms the general worse behavior of the aggregate stability in the three soils use systems in a warm climate (Fig. 3).

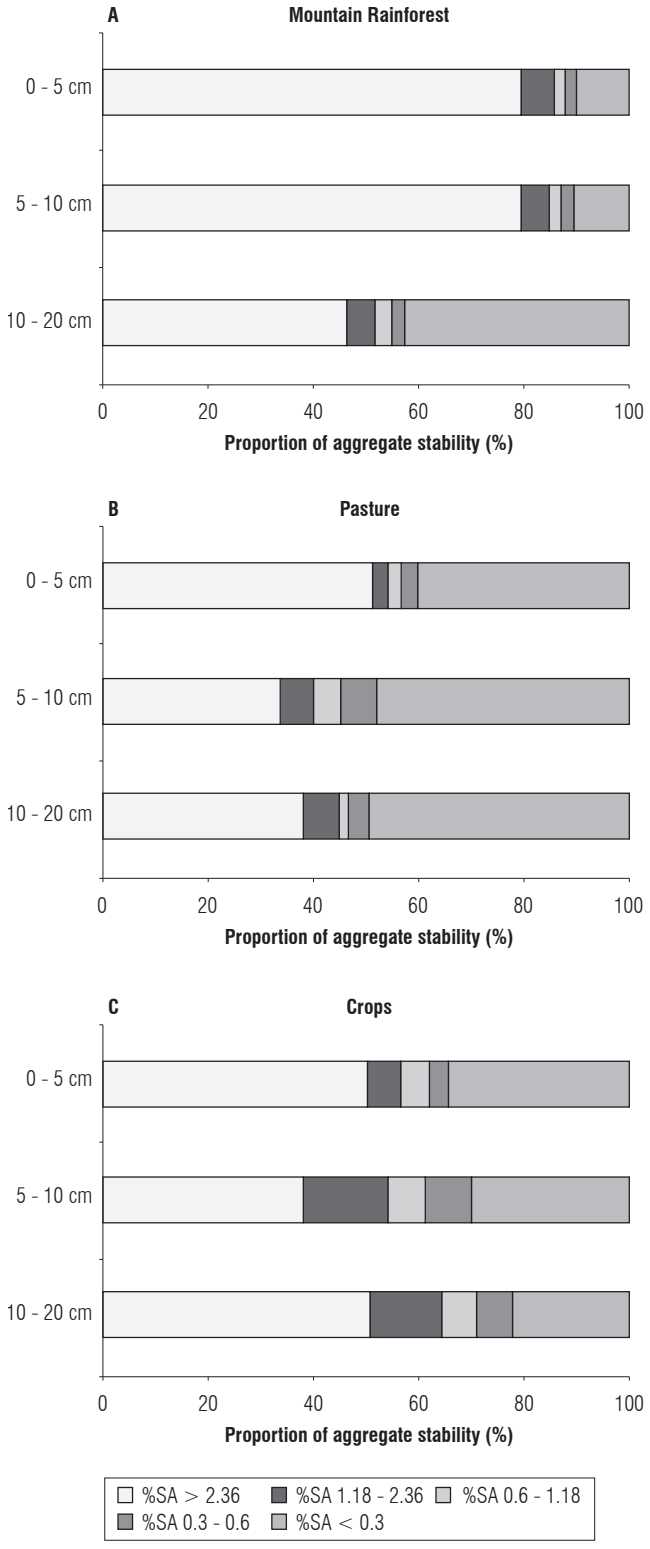


FIGURE 2. Proportion of aggregate stability (%) distributed by size classes according to the soil depth in each use system evaluated in the altitudinal level of cold climate. A) Mountain rainforest, B) pasture and C) crops.

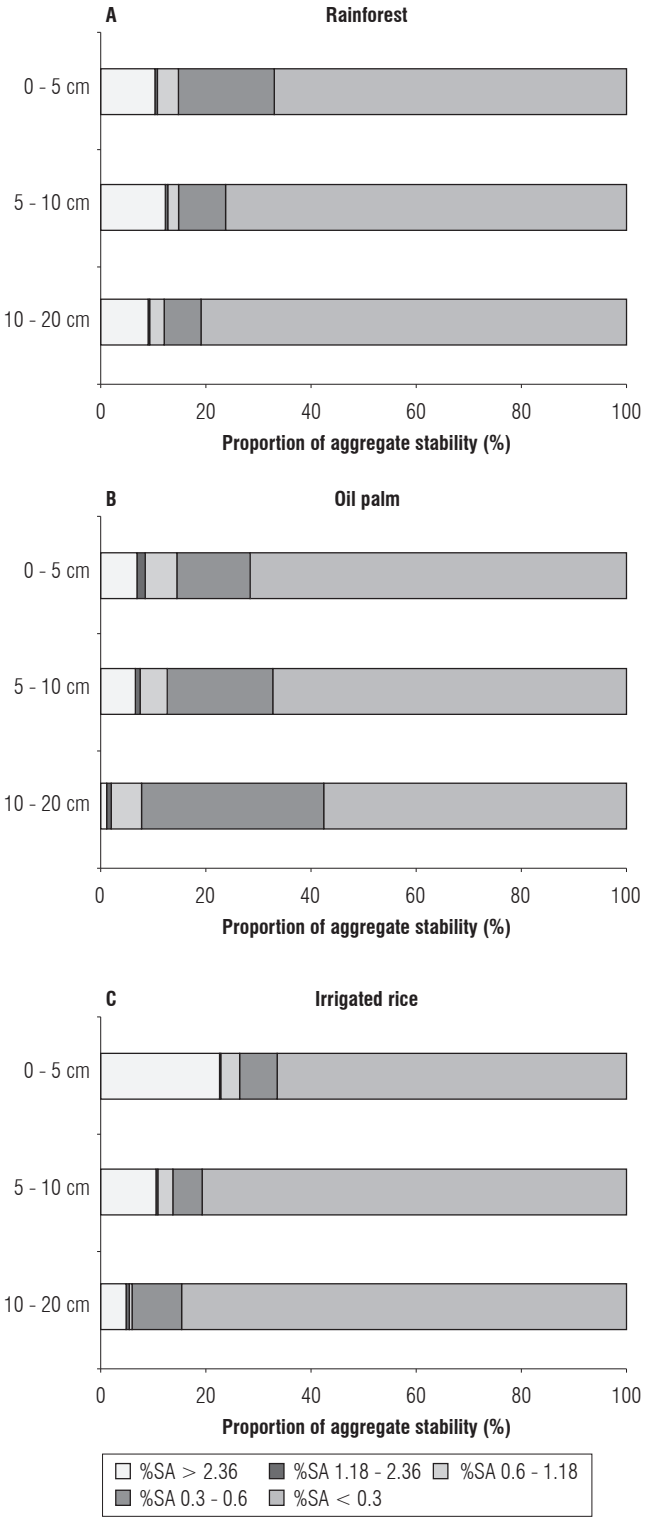


FIGURE 3. Proportion of stable aggregates (%) distributed by size classes according to the soil depth in each use system evaluated in the altitudinal level of warm climate. A) Rainforest, B) oil palm and C) irrigated rice.

The analysis of soil TOC content (Tab. 5) showed a statistically significant effect of climate and soil use system, indicating that cold climate had the highest TOC content. The use system of pasture had the highest content and the crop along with the rainforest had the lowest TOC content.

When relating the behavior of the TOC content and the structural stability of the soil, it could be observed that the soil with the highest TOC also showed the best structural stability. This coincides with the studies that have shown that soils with high TOC content present stable aggregates of larger sizes (Lozano *et al*., 1997; An *et al*., 2010; Oliveira, *et al*., 2015).

For the results of POC content (Tab. 5), a statistically significant effect of the climate was found, being the highest content of POC in the cold climate.

Figueiredo *et al.* (2010) pointed out that POC increases with a higher amount of organic waste returned to the soil and that the use systems of pastures and forests frequently enhanced this pool of the SOC. Besides, Briedis *et al.* (2012) found that the SA with a larger size were more abundant when the POC was higher. Therefore, the dominant presence of SA>2.36 mm in the soil of a cold climate has a direct relation with the higher POC content.

Results of the HOC revealed significant statistical differences by the effects of the climate and depth, without differences found for the soil use system. Since HOC is higher in a cold climate according to the depth it is higher in the deepest layer (10 to 20 cm) and lower in the surface (0 to 5 and 5 to 10 cm).

The statistical analysis of the MBOC showed that there was a statistically significant effect due to the climate, the use, and the depth. A cold climate presents the highest amount of MBOC. The first two layers present a higher MBOC and the effect of the soil is shown in the higher contents in pasture and oil palm, while a lower content is found in crops and forests. These results are similar to the ones reported by Moraes-Sa *et al.* (2009), who reported a positive linear correlation between the MBOC and the POC.

A high positive correlation between the SA>2.36 mm and the sand contents and POC was found. On the other hand, a negative correlation was found between the SA>2.36 mm with the clay content, the silt content, the Bd and the HI (Tab. 6). For the SA<0.30 mm, a high positive correlation is observed with the clay content, the silt content and the HI. In contrast, the correlation is negative between the SA<0.30 mm with the sand content and the POC.

TABLE 5. Mean values of organic carbon in different pools of the soil organic matter and humification index, for each climate altitudinal level and according to the use system in the three depths studied.

Use	Depth	TOC (Mg ha^{-1})	POC (Mg ha^{-1})	HOC (Mg ha^{-1})	MBOC (mg CO kg^{-1})	HI (%)
Cold Tropical Climate						
Mountain rainforest	0 - 5 cm	43.00 b	48.05 c	26.28 e	20.43 i	35.07
	5 - 10 cm	30.82 b	25.67 c	28.02 e	19.49 i	46.79
	10 - 20 cm	45.29 b	25.06 c	70.36 f	12.35 i	72.90
Pasture	0 - 5 cm	13.47 a	60.85 c	74.05 e	70.41 i	55.46
	5 - 10 cm	46.71 a	26.11 c	45.09 e	76.56 i	53.21
	10 - 20 cm	79.15 a	42.16 c	88.21 f	49.43 i	67.68
Crops	0 - 5 cm	31.43 b	16.01 c	63.85 e	32.28 j	79.74
	5 - 10 cm	30.15 b	14.95 c	81.30 e	30.31 j	84.71
	10 - 20 cm	59.95 b	30.85 c	128.30 f	41.19 j	81.09
Warm Tropical Climate						
Rainforest	0 - 5 cm	13.00 b	10.14 d	45.25 g	7.62 k	81.77
	5 - 10 cm	11.95 b	3.45 d	44.10 g	9.22 k	92.75
	10 - 20 cm	27.68 b	5.10 d	96.89 h	6.96 k	94.96
Oil palm	0 - 5 cm	20.66 a	11.64 d	39.97 g	8.55 k	78.94
	5 - 10 cm	20.23 a	8.58 d	39.84 g	7.45 k	84.14
	10 - 20 cm	40.22 a	7.52 d	58.26 h	7.88 k	88.72
Irrigated rice	0 - 5 cm	15.06 b	7.71 d	29.79 g	7.13 l	79.50
	5 - 10 cm	13.92 b	6.34 d	29.06 g	8.79 l	82.14
	10 - 20 cm	24.11 b	9.63 d	63.27 h	12.63 l	86.82

TOC: Total organic carbon content, POC: Particulate organic matter pool, HOC: Mineral organic matter pool, MBOC: Carbon of the soil microbial biomass, HI: Humification index.
Same letters represent statistically homogeneous groups.

TABLE 6. Pearson correlation coefficients for the stable aggregates and the weighted average diameter with the physical variables and the organic carbon content in different pools of soil.

	SA>2.36	SA 1.18 to 2.36	SA 0.6 to 1.18	SA 0.3 to 0.6	SA<0.3	MWD
Sand	0.63*	0.51	0.12	-0.10	-0.66*	0.63*
Clay	-0.54*	-0.44	-0.11	0.07	0.57*	-0.54*
Silt	-0.59*	-0.47	-0.09	0.11	0.60*	-0.60*
Bd	-0.55*	0.00	0.08	0.29	0.38	-0.54*
TOC	0.36	0.31	-0.14	-0.17	-0.30	0.35
POC	0.65*	0.33	-0.08	-0.25	-0.55*	0.64*
HOC	0.05	0.46	0.08	-0.09	-0.10	0.09
MBOC	0.45	0.47	0.08	-0.24	-0.41	0.46
HI	-0.70*	-0.20	0.09	0.20	0.59*	-0.68*

SA: Stable agregates, MWD: Mean weighted diameter of the stable aggregates, Bd: Soil bulk density, TOC: Total organic carbon content, POC: Particulate organic matter pool, HOC: Mineral organic matter pool, MBOC: Carbon of the soil microbial biomass, HI: humification index.

*Represents a high correlation.

With regard to the MWD, a high positive correlation with the sand content and the POC was observed. However, a negative correlation with the Bd, the humification and the clay and silt contents was observed. This agrees with the study by Pulido *et al*. (2009) who observed that the predominance of silts may cause disintegration and affect structural stability. Additionally, when there is a lower contribution of the organic compounds that stabilize the structure (higher humification), the structural stability decreases.

From the comparison functions of variable pairs (Fig. 4), the most relevant correlations are evident, and this helps to understand the relationship between structural stability

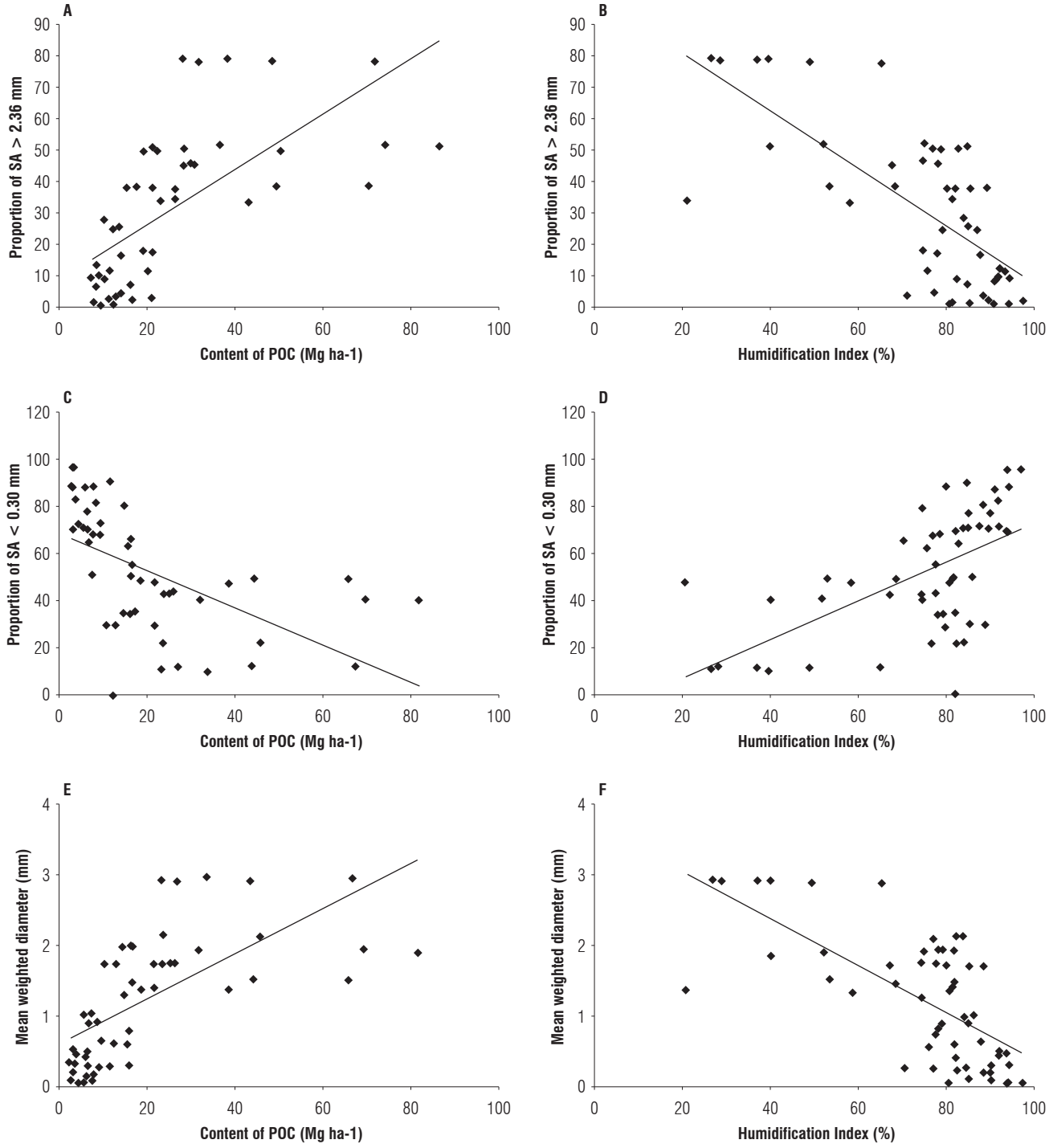


FIGURE 4. Relevant correlations between variables of structural stability and variables of soil organic carbon.

and the SOC. The MWD and the SA>2.36 mm increase linearly when there is a higher POC, but they decrease when the humification index increases. For the SA<0.30 mm, a linear decrease can be observed when there is a higher content of POC, but an increase can be observed when there is a higher humification index. Similar results were obtained by Tivet *et al.* (2013) who found a positive relationship between the particulate OC with an increase of the SA of larger sizes.

Conclusions

From the two climates considered in the study, the soil of a cold climate showed better aggregate stability, which

confirms the correlation of macroaggregates with high TOC and POC in soils. This also indicates that the poor structural stability in the soil of warm climate is related to low TOC and POC.

None of the soil use systems has significantly affected the good structural stability of soil in a cold climate. However, a decrease in the structural stability of the soil was observed with crop use. This confirms the effect of intensive agricultural management on aggregation because of the reduction of organic carbon in the soil.

It is necessary to promote the increase of the TOC and POC in the soil of a warm climate to improve aggregate stability. This can be achieved by increasing the incorporation of organic residues in the soil. In soils of cold climate, all the soil use systems must preserve the TOC and the maintenance of a high POC content.

Literature cited

An, S., A. Mentler, H. Mayer, and W. Blum. 2010. Soil aggregation, aggregate stability, organic carbon and nitrogen in different soil aggregate fractions under forest and shrub vegetation on the Loess Plateau, China. Catena 81, 226-233. Doi: 10.1016/j.catena.2010.04.002

Briedis, C., J. Moraes-Sá, E. Caires, J. Navarro, T. Massao-Inagaki, A. Boer, C. Quadros-Neto, A. Oliveira-Ferreira, L. Canalli, and J. Burkner-Dos Santos. 2012. Soil organic matter pools and carbon-protection mechanisms in aggregate classes influenced by surface liming in a no-till system. Geoderma 170, 80-88. Doi: 10.1016/j.geoderma.2011.10.011

Bronick, C. and R. Lal. 2005. Soil structure and management: a review. Geoderma 124, 3-22. Doi: 10.1016/j.geoderma.2004.03.005

FAO, Food and Agriculture Organization of the United Nations. 2006. Guidelines for soil description. FAO, Rome.

Figueiredo, C, D. Siqueira-Resck, and M. Carbone-Carneiro. 2010 Labile and stable fractions of soil organic matter under management systems and native Cerrado. Rev. Bras. Cienc. Solo 34, 907-916. Doi: 10.1590/S0100-06832010000300032

IGAC, Instituto Geográfico Agustín Codazzi. 2006a. Estudio general de suelos y zonificación de tierras del departamento Norte de Santander. Instituto Geográfico Agustín Codazzi, Bogota.

IGAC, Instituto Geográfico Agustín Codazzi. 2006b. Métodos analíticos de laboratorio de suelos. Instituto Geográfico Agustín Codazzi, Bogota.

Loss, A., E. Dos Santos, D. Schmitz, M. Da Veiga, C. Kurtz, and J. Comin. 2017. Atributos físicos do solo em cultivo de cebola sob sistemas de plantio direto e preparo convencional. Rev. Colomb. Cienc. Hort. 11(1), 105-113. Doi: 10.17584/rcch.2017v11i1.6144

Lozano, Z., S. Cabrera, J. Peña, and M. Adams. 1997. Efecto de los sistemas de labranza sobre dos inceptisoles de los llanos occidentales de Venezuela. II. Propiedades físicas de los suelos. Revista Venesuelos, 5, 25-33.

Lozano, Z., R. Hernández, and A. Ojeda. 2005. Manual de métodos para la evaluación de la calidad física, química y biológica de los suelos. Universidad Central de Venezuela, Maracay, Venezuela.

Martínez, E., J. Fuentes, and E. Acevedo. 2008. Carbono orgánico y propiedades del suelo. J. Soil Sci. Plant Nutr. 8(1), 68-96.

Moraes-Sa, J. and R. Lal. 2009. Stratification ratio of soil organic matter pools as an indicator of carbon sequestration in a tillage chronosequence on a Brazilian Oxisol. Soil Tillage Res. 133, 65-74. Doi: 10.1016/j.still.2008.09.003

Mujuru, L., A. Mureva, E. Velthorst, and M. Hoosbeek. 2013. Land use and management effects on soil organic matter fractions in Rhodic Ferralsols and Haplic Arenosols in Bindura and Shamva districts of Zimbabwe. Geoderma 209-210, 262-272. Doi: 10.1016/j.geoderma.2013.06.025

Nascente, A., Y. Li, and C. Costa-Crusciol. 2015. Soil aggregation, organic carbon concentration, and soil bulk density as affected by cover crop species in a no-tillage system. Rev. Bras. Ciênc. Solo 39, 871-879. Doi: 10.1590/01000683rbcs20140388

Oades, J. 1984. Soil organic matter and structural stability: mechanisms and implications for management. Plant Soil 76, 319-337. Doi: 10.1007/BF02205590

Oliveira-Marques, J., F. Luizão, W. Teixeira, M. Sarrazin, S. Filgueira-Ferreira, T. Beldini, and E. Araújo-Marques. 2015 Distribution of organic carbon in different soil fractions in ecosystems of central Amazonia. Rev. Bras. Cienc. Solo 39, 232-242. Doi: 10.1590/01000683rbcs20150142

Pinto, Y., J. Álvarez, and F. Forero. 2016. Efecto de la labranza en la estabilidad estructural y resistencia a la penetración en un inceptisol sembrado con arracacha (*Arracacia xanthorrhiza* Bancroft) en Boyacá. Rev. Col. Cienc. Hort. 10(1), 99-112. Doi: 10.17584/rcch.2016v10i1.5049

Pla, I. 1983. Metodología para la caracterización física con fines de diagnóstico de problemas de manejo y conservación de suelos en condiciones tropicales. Rev. Fac. Agron. Alcance 32, 5-91.

Pla, I. 2010. Medición y evaluación de propiedades físicas de los suelos: dificultades y errores más frecuentes. I - Propiedades mecánicas. Suelos Ecuatoriales 40(2), 75-93.

Pulido, M., D. Lobo, and Z. Lozano. 2009. Asociación entre indicadores de estabilidad estructural y la materia orgánica en suelos agrícolas de Venezuela. Agrociencia 43, 221-230.

Sena, K., K. Maltoni, G. Amorim-Faria, and A. Rodrigues-Cassiolato. 2017. Organic carbon and physical properties in sandy soil after conversion from degraded pasture to *Eucalyptus* in the Brazilian Cerrado. Rev. Bras. Cienc. Solo 41, e0150505. Doi: 10.1590/18069657rbcs20150505

Six, J., H. Bossuyt, S. Degryze, and K. Denef. 2004. A history of research on the link between (micro)aggregates, soil biota, and soil organic matter dynamics. Soil Till. Res. 79, 7-31 Doi: 10.1016/j.still.2004.03.008

Tivet, F., J. Moraes-Sa, R. Lal, C. Briedis, P. Borszowskei, J. Burkner-Dos Santos, A. Farias, G. Eurich, D. Hartman, M. Nadolny, S. Bouzinac, and L. Seguy. 2013. Aggregate C depletion by plowing and its restoration by diverse biomass-C inputs under no-till in sub-tropical and tropical regions of Brazil. Soil Till. Res. 126, 203-218 Doi: 10.1016/j.still.2012.09.004

Water potential in cape gooseberry (*Physalis peruviana* L.) plants subjected to different irrigation treatments and doses of calcium

Potencial hídrico en plantas de uchuva (*Physalis peruviana* L.) sometidas a diferentes regímenes de riego y dosis de calcio

Javier Álvarez-Herrera[1]*, Hernán González[2], and Gerhard Fischer[3]

ABSTRACT

To determine whether the management of irrigation and nutrition in cape gooseberry crops with calcium to reduce the cracking of fruits affects the water potential of the plants, the present study was carried out using a randomized block design with 12 treatments in a 4×3 factorial arrangement. The blocks were the irrigation frequencies (4, 9 and 14 days apart). The first factor was the irrigation coefficient (0.7, 0.9, 1.1 and 1.3 of the evaporation tank of class A), and the second factor was the calcium dose (0, 50 and 100 kg ha^{-1}), representing 36 experimental units. Seed propagated cape gooseberries were transplanted in 20 L pots using peat moss as substrate. The water potential in the leaves (ψ_{leaf}) and stems (ψ_{stem}) was measured as well as the water consumption and irrigation water-use efficiency (WUEi) of the plants. The ψ_{leaf} and ψ_{stem} of the cape gooseberry plants presented a sinusoidal trend throughout the day. The water frequency of 4 days with an irrigation coefficient of 1.1 showed the highest values of ψ_{leaf} and ψ_{stem}. The ψ_{stem} and ψ_{leaf} reached the highest values at predawn (4 am) as a result of the low vapor pressure deficit (VPD) levels that occurred at that time and reached their lowest point in the midday hours. The irrigation coefficient of 1.1 had the second largest WUEi and, thus, represented the water level most suitable for growing cape gooseberry since it generated the largest number of big fruits and the smallest percentage of cracked fruits.

Key words: Solanaceae, fertilization, water level, WUEi, consumptive use.

RESUMEN

Con el objetivo de establecer si el manejo del riego y de la nutrición con calcio que se le da al cultivo de uchuva para disminuir el rajado de los frutos afecta el potencial hídrico de la planta, se llevó a cabo el presente trabajo, en donde se empleó un diseño en bloques al azar con 12 tratamientos en arreglo factorial de 4×3. Los bloques fueron las frecuencias de riego (4, 9 y 14 días distanciadas). El primer factor fue la lámina de riego (0.7; 0.9; 1.1 y 1.3 de la evaporación del tanque clase A) y el segundo la dosis de calcio (0, 50 y 100 kg ha^{-1}), lo que representó 36 unidades experimentales. Las uchuvas propagadas por semilla se trasplantaron en materas de 20 L usando turba rubia como sustrato. Se determinó el potencial hídrico en hojas (ψ_{hoja}) y tallos (ψ_{tallo}), así como el consumo de agua y la eficiencia en el uso del agua de riego (EUAr) por parte de las plantas de uchuva. El ψ_{hoja} y el ψ_{tallo} en las plantas presentó una tendencia sinusoidal a lo largo del día. La frecuencia de riego de 4 días con una lámina de riego de 1.1 mostró los valores más altos de ψ_{hoja} y ψ_{tallo}. Los ψ_{hoja} y ψ_{tallo} alcanzaron los valores más altos al alba (4 a.m.) producto de los bajos niveles en el déficit de presión de vapor (DPV) existentes a esa hora, y llegaron a su punto más bajo en las horas del mediodía. La lámina de riego de 1.1 presentó la segunda mayor EUAr, y es la lámina de riego más adecuada para el cultivo de uchuva pues generó la mayor cantidad de frutos de tamaño grande y menores porcentajes de rajado de frutos.

Palabras clave: solanácea, fertilización, lámina de riego, EUAr, uso consuntivo.

Introduction

Although the area planted with cape gooseberry in Colombia increased in the period between 2010 and 2016, from 745 to 1,023 ha, the yield was reduced from 13.8 to 12.5 t ha^{-1} (Agronet, 2019), which constitutes a decrease of 9.4%. This can be probably caused by an increase in cracked fruits (Gordillo *et al*., 2004) and by other aspects, such as inadequate fertilization and disease management plans; the lowest yield was in 2013 with 9.8 t ha^{-1} (Agronet, 2019). In addition, cape gooseberry crops have been greatly impacted by adverse weather conditions, including the irregular supply of water by rainfall (Villareal-Navarrete *et al*., 2017; Aparecido *et al*., 2019).

Received for publication: 28 May, 2019. Accepted for publication: 5 December, 2019 Doi: 10.15446/agron.colomb.v37n3.79935

[1] Group of Agricultural Research - GIA, Faculty of Agricultural Sciences, Universidad Pedagógica y Tecnológica de Colombia (UPTC), Tunja (Colombia).
[2] Marketing Field Specialist. Bayer, Villavicencio (Colombia).
[3] Scientific consultant, Emeritus researcher of Colciencias, Bogota (Colombia).
* Corresponding author: javier.alvarez@uptc.edu.co

One of the restrictions presented by the export of cape gooseberry fruits is the cracking problem, which causes the product to reach its destination with the presence of fungi and results in rejection of the fruits and consequent economic losses. The cracking of fruits results from mainly two causes: the first may be a poor and irregular supply of water to the crop with excessive enlargement of the fruits (e.g. through high water and nutrient supply) in the last developmental stage beyond what the tissue can support (Fischer, 2005), and the second occurs with deficiencies of calcium, which is probably associated with morphogenetic reasons that cause cape gooseberry fruits to not absorb the amounts of calcium needed to provide resistance in the cell walls and, thus, avoid cracking (Alvarez-Herrera *et al*., 2012).

At the field level, irrigation coefficients of 1.2 (120% of evaporation) increased the yield of fruits per plant in the plot in which Ca was added, compared with the application of a smaller amount of water (Gordillo *et al*., 2004). However, in a greenhouse with an application of a net irrigation coefficient of 1.3 and 100 kg ha^{-1} of Ca, the percentage of cracked fruits decreased, but the fruit production was lower (Álvarez-Herrera *et al*., 2012).

The water potential reveals the moment of maximum water requirements by a plant in a day or a crop cycle and determines the water potential of the soil when measurements are taken at predawn. It also facilitates irrigation scheduling, which results in better control of water stress at certain times of the day or at times when the temperature increases considerably (Cole and Pagay, 2015). In this regard, Taiz and Zeiger (2010) stated that, among the resources required by a plant for its growth, development, and physiology, water is the largest in terms of amount, and so is the aggravating factor that can be the most limiting, which accentuates the importance of irrigation and its key role in the production of crops. It is also known that the water status of a plant, combined with climatic conditions, regulates the rate of transpiration, which plays an important role in the absorption and mobility of calcium (Marschner, 2012).

Because irrigation and nutrition management with calcium greatly influence the obtention of good quality fruits (Álvarez-Herrera *et al*., 2014) and recent research recommends different irrigation doses, depending on whether quality (not cracked fruits) or quantity (large fruits) is desired (Álvarez-Herrera *et al*., 2015), it is necessary to know the water status of a plant under different irrigation regimes, for which, the determination of the water potential acquires great importance. Therefore, this research aimed to determine the water potential of cape gooseberry plants subjected to different irrigation regimes and calcium doses.

Materials and methods

Location of the experiment

The experiment was carried out in a greenhouse with a plastic cover at the Faculty of Agricultural Sciences, Universidad Nacional de Colombia, Bogota campus, at an altitude of 2,556 m a.s.l. and with coordinates 4º38'7" N and 74º5'20" W. The average greenhouse temperature was 18°C and the relative humidity was 60%.

Experimental design

A randomized complete block design was used with 12 treatments. The blocking criterion was the irrigation frequencies (4, 9 and 14 d). The treatments were in a 4×3 factorial arrangement, in which the first factor corresponded to the applied irrigation coefficients (0.7, 1.0, 1.3 and 1.6 of evaporation of the evaporimeter tank), and the second factor was the calcium doses (0, 50 and 100 kg ha^{-1}), which generated 36 experimental units (EU). Each EU was composed of two cape gooseberry plants, for a total of 72 plants, which were planted in 20 L pots, 80 cm in diameter and 50 cm deep. Peat moss was used as a substrate.

Setup of the experiment

The plant material used was *Physalis peruviana* L., ecotype Colombia, because it is the most desired on the international markets due to its high content of sugars and vitamins (Fischer, 2000). The cape gooseberry seeds were planted in plastic trays with 72 cells, which germinated after 45 d and remained in the trays for 4 months from the time of sowing until they reached an average height of 25 cm.

The plants were planted at a distance of 2 m between plants and rows, and traditional cultural activities (phytosanitary management, pruning, trellis, and harvesting) of commercial crops in the producing areas were carried out. The high V trellis system was used. Edaphic and foliar fertilization were carried out based on the requirements of the crop and considering the fact that peat provides nutrients for a short period of time. The doses used in kg ha^{-1} were N: 150; P_2O_5: 220; K_2O: 150; MgO: 60; S: 40 B: 1; Zn: 3; Cu: 2; and Mn: 0.5 fractionated from the time of planting, first in two quarterly applications and then three bi-monthly applications, and monthly foliar applications.

For the application of the irrigation levels, a drip irrigation system was used (two drippers per plant with a flow rate of 4 L h^{-1}, each). Once the plants were planted, the different doses of calcium were applied in the substrate around the plants, distributed monthly with a class A evaporimeter tank built on a scale of 1:1 and installed inside the greenhouse in order to establish the amount of water applied according to the equation for calculating the consumptive use, which takes into account the potential evapotranspiration. The data measured in the evaporimeter inside the greenhouse were correlated with the data from the type A evaporation tank at the climatological station of the IDEAM (regional main category 6 and code 2403513) to validate the latter and extrapolate the date for open field conditions (Eq. 1).

$$Irrigation\ level = \frac{Etp \times C \times A}{\eta_r} \quad (1)$$

where,

Etp = evapotranspiration in mm measured in the evaporimeter tank.
C = multiplication coefficient according to treatments.
A = area of the pot (254.4 cm^2).
η_r = efficiency of drip irrigation (0.9).

The irrigation water-use efficiency (*WUEi,* %) was calculated from the dry biomass of the leaves divided by the total water applied in each treatment.

The leaf and stem water potentials were determined in the plants during the reproductive phase, beginning at 30 d in the vegetative phase, following the treatments with determinations every 4 h (8 am, 12 pm, 4 pm, 8 pm, 12 am, and 4 am (at predawn) using a Scholander pump (Model 615, PMS Instrument Company, Albany, OR, USA). For the determination of the leaf water potential (ψ_{leaf}), a leaf from the tertiary branch of each plant was taken, which was bagged in a plastic bag covered with aluminum foil for 15 min, taken out and placed in a pressure chamber to determine the potential. For the determination of the stem water potential (ψ_{stem}), a leaf of the tertiary branch was pocketed for 3 h and inserted into a chamber; the measurement was taken according to the methodology followed by Nortes *et al*. (2005).

The instantaneous measurement of the temperature (T) and relative humidity (RH) of the air were recorded with an Extech RHT20 Datalogger (Extech Instruments, Waltham, MA, USA). With these data, the calculation of the vapor pressure deficit (VDP), given in kPa, was performed using the equation used by Murray (1967):

$$VDP = \left(1 - \frac{RH}{100}\right) \times \left[6.1078^{\left\{\frac{17.27 \times T_{air}}{T_{air} + 273.15 - 35.86}\right\}}\right] \quad (2)$$

Data analysis

An analysis of variance (ANOVA) was performed for a completely randomized block design in order to determine if there were significant differences between the irrigation frequencies (blocks) and between the treatments (water level by calcium doses) for each of the measured response variables. In addition, a Tukey comparison test of averages was used at 5% to classify each of the levels of the evaluated factors. The VDP measurements calculated for the hours of measurement of the water potential were correlated with the stem and leaf water potential. The software SAS (version 9.2) was used for the data analysis.

Results and discussion

Leaf water potential (ψ_{leaf})

When analyzing the behavior of the ψ_{leaf} in the cape gooseberry plants, circadian rhythm was observed (Fig. 1A), similar to what was found in persimmon (*Diospyros kaki* L. f.) by Griñan *et al*. (2019). There were only significant differences between the evaluated irrigation levels at 8 pm. Likewise, it was noted that, when the amount of water applied increased, the ψ_{leaf} was higher than 0.1 MPa approx. on average throughout the different measurements throughout the day. This result coincides with that reported by Singh and Singh (2006), who found that increases in water stress caused a reduction in ψ_{leaf} at predawn in *Dalbergia sissoo* Roxb., which varied from 0.4 to 1.16 MPa. The same authors pointed out that the higher the photosynthetic rate, the greater the water potential (ψ), which implies that well-watered plants will have normal development and an adequate ψ_{leaf}.

The ψ_{leaf} showed significant differences between the different irrigation frequencies applied only at certain hours of the day (morning, 8 am, noon, and in the afternoon, 4 pm, Fig. 1B), while in the hours without sunlight (predawn, 4 am, night, 8 pm, and 12 pm), the water status of the plant was homogeneous regardless of the frequency of irrigation. This indicates that the effect of the treatments on the ψ_{leaf} increased when the plants received sunlight, which is in agreement with what is reported by Girona *et al.* (2006), who found that, in hazelnut, the daily variation of the leaf water potential is more related to the climate components, especially to solar radiation, than to the water potential of the soil. Likewise, Sousa *et al.* (2006) mentioned that the

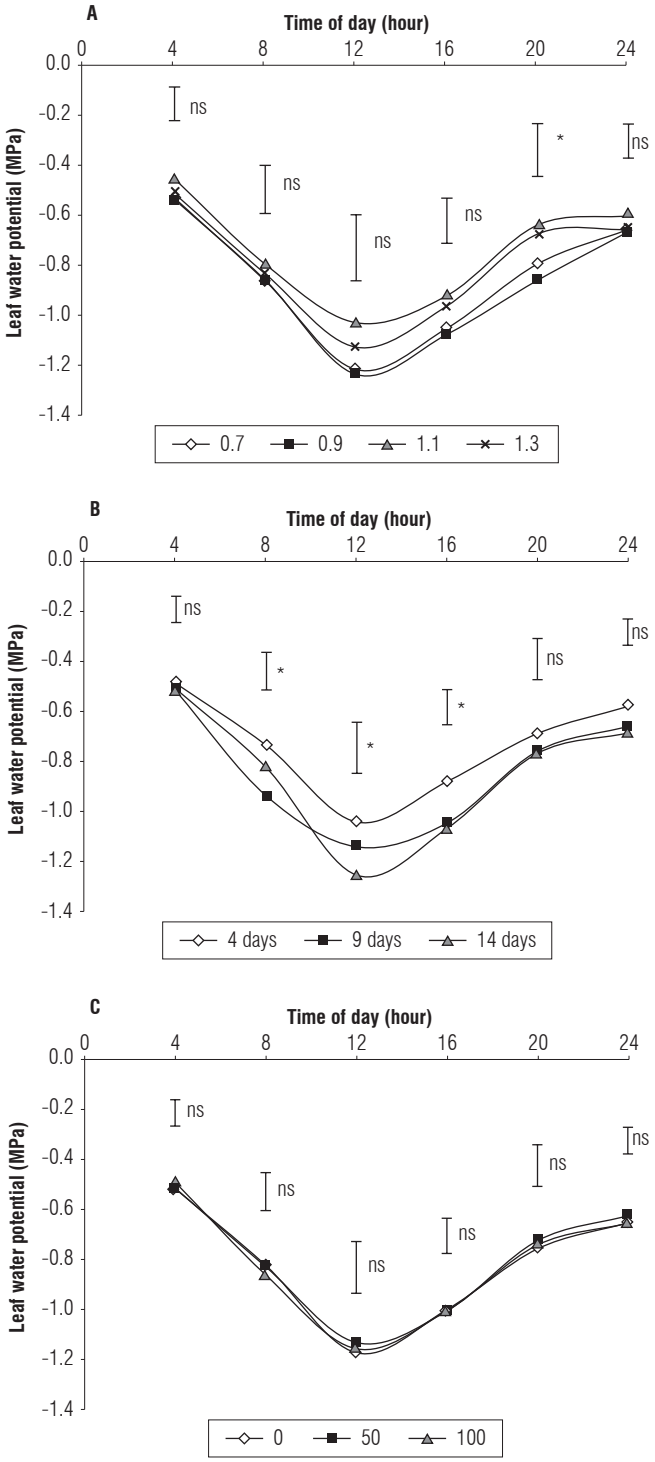


FIGURE 1. Leaf water potential in cape gooseberry plants planted in peat moss in greenhouses and subjected to A) different irrigation levels; B) irrigation frequencies and C) calcium doses in kg ha^{-1}. The bars indicate minimal significant difference according to the Tukey test ($P \leq 0.05$). ns: not significant. *: indicates significant effect with $P \leq 0.05$.

ψ_{leaf} of grape plants at night has a high correlation with the water potential in the soil. The ψ_{leaf} in the cape gooseberry plants showed values of -0.51; -0.84; -1.15; -1.01; -0.74 and -0.65 MPa for the different hours (4 am, 8 am, noon, 4 pm, 8 pm and midnight, respectively), with the maximum differences between treatments at noon.

The irrigation frequency of 4 d presented the highest values of ψ_{leaf}, followed by irrigation every 9 and 14 d, probably because, as the water stress is greater, the ψ_{leaf} decreases in cape gooseberry plants. Similarly, Zhu *et al.* (2004) determined that the ψ_{leaf} in apple trees decreased as the water stress increased and mentioned that the concentration of cytokinins at the leaf level also decreased as a result of the lower activity of this hormone in the root zone, which implies delays in processes such as cell division. Also, Kitsaki and Drossopoulos (2005) stated that, as the ψ_{leaf} becomes more negative, the higher the ABA content is. Therefore, it can be inferred that the ψ_{leaf} is a reliable measure of the level of stress to which a plant is subjected (Girona *et al.*, 2006). In addition, in grapes, the growth rate of the shoots and the net assimilation of CO_2 had greater correlation with the ψ_{leaf} at noon than with the ψ_{leaf} at predawn (Baeza *et al.*, 2007).

Jaimez *et al.* (1999) stated that, in areas with high evaporative demand, plants have a loss of turgor that is related to low values of water potential. In addition, if the frequency of irrigation affects the water potential, the plant likely has difficulties with osmotic adjustment under stress conditions and, below values of -1.6 to -1.8 MPa, the stomatal conductance decreases and, consequently, the photosynthetic rates decrease. Similarly, Marsal and Girona (1997) evaluated the leaf water potential in peach trees and mentioned that values below -3 MPa severely affect plants. They also stated that, in full fruit growth, the sink effect is likely to generate greater water mobilization towards the fruit and decrease the amount of water in the leaves.

Regarding the effect of the different doses of calcium applied to the cape gooseberry plants, they did not affect the ψ_{leaf}, as shown in figure 1C. Although increasing the application of Ca^{2+} increased [Ca^{2+}] in the leaves (unpublished data), there was no effect on the ψ_{leaf}, probably because changes in the ψ_{leaf} are strongly influenced by environmental conditions, especially by a vapor pressure deficit (VPD) (Singh and Singh, 2006).

Stem water potential (ψ_{stem})

This potential had a behavior very similar to that of the ψ_{leaf}, with the ψ_{stem} greater at predawn (4 am) and at night (Fig. 2). However, between 8 am and 4 pm, lower values were reached, and, at midday, the minimum averaged -0.97 MPa. These values match those found for citrus at the same time, from -0.6 to -1.3 MPa (García-Tejero *et al.*, 2010), and

those defined by De Swaef *et al*. (2009) for most fruit trees, between -0.5 and -1.5 MPa.

The ψ_{stem} showed significant differences between the irrigation levels only in the measurements taken at noon (Fig. 2A). The irrigation level that presented the highest ψ_{stem} values had a coefficient of 1.1, both at midday and at other times of the day, while the levels of 0.7, 0.9 and 1.3 throughout the day presented the lowest ψ_{stem} values. It is likely that the amount with a coefficient of 1.3 caused a temporary root hypoxia effect that affected the potential value. The ψ_{stem} values at predawn oscillated on average around -0.50 MPa, which are similar to the oscillations found by Nortes *et al.* (2005) in almond trees with average values of -0.4 MPa. These results are also in agreement with that found by Navarro *et al.* (2007), who stated that, in plants subjected to stress, the ψ_{stem} at predawn and noon was less than in the control plants, which reflected the hydraulic signals that the roots send to the shoots. These authors also confirmed that, under stress conditions, maintaining the turgor pressure at the cellular level implies an osmotic adjustment and probable changes in the elasticity of the cell wall in such a way that a decrease in the ψ_{stem} results. Likewise, measuring the ψ_{stem} directly reflects the water status of the plant since it maintains a strong relationship with the flow in the vascular bundles, which highlights the importance of determining the ψ_{stem} (García-Tejero *et al*., 2010).

The irrigation frequency generated significant differences in the ψ_{stem} at predawn, but not at midday (Fig. 2B). This can be explained because ψ_{stem} is a measurement that correlates very well with ψ_{soil}, according to Sousa *et al.* (2006). Therefore, measuring ψ_{stem} at predawn would indicate the moisture content of the substrate, while the ψ_{stem} at midday has a strong correlation with the environmental conditions that are indicative of the evaporative demand (De Swaef *et al*., 2009). However, in wheat, Sato *et al.* (2006) found no correlation between the ψ_{stem} at predawn and the ψ_{soil}, which could have originated from the moisture retention capacity of the soils, the ability of the plants to absorb water, the species, the growth status, and the environmental conditions.

In this regard, the values of both ψ_{stem} and ψ_{leaf} were lower at noon because, as the transpiration increases and the pressure decreases in the xylem, the cells begin to dehydrate, so it is common to find pressures of between -1 to -2 MPa (Boyer, 1995). This decrease was observed in the ψ_{stem} of the cape gooseberry plants because stress generates problems in the development and growth of plants since it affects processes such as photosynthesis. Under stress conditions,

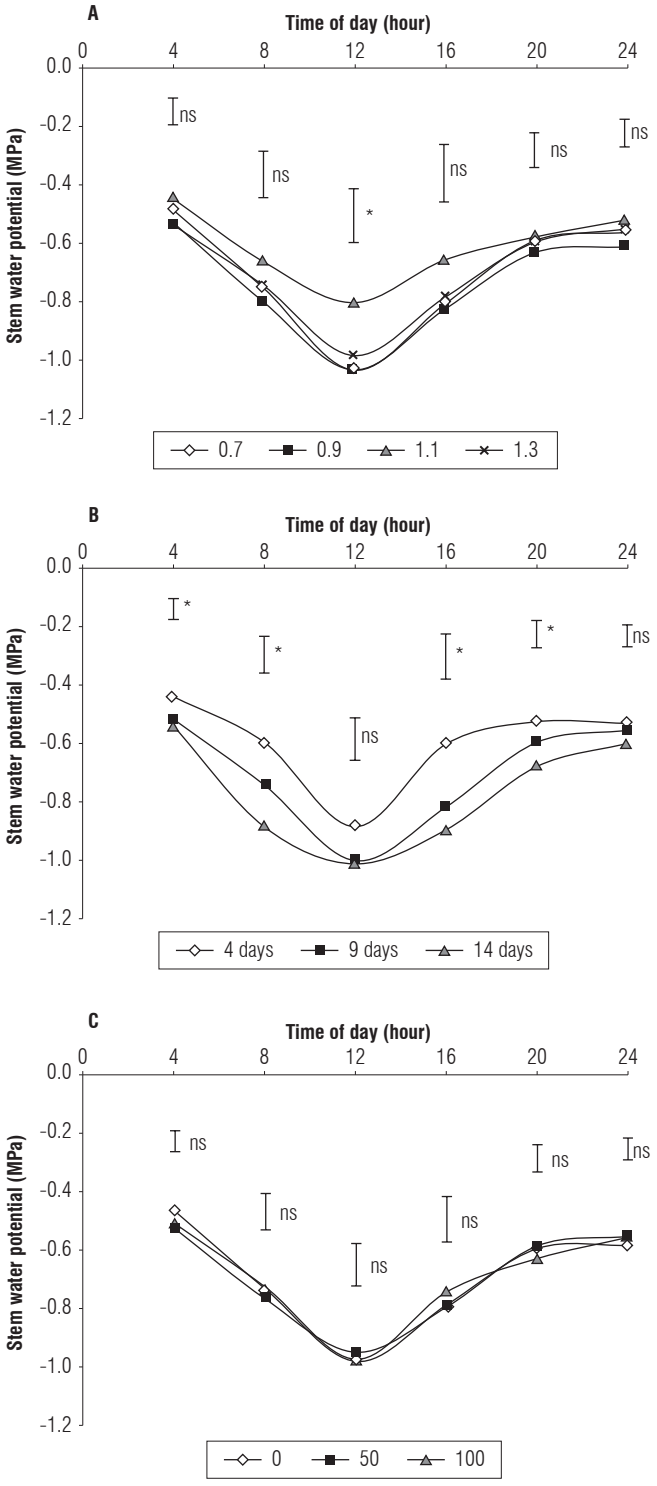


FIGURE 2. Stem water potential in cape gooseberry plants planted in peat moss in greenhouses and subjected to A) different irrigation levels; B) irrigation frequencies and C) calcium doses in kg ha^{-1}. The bars indicate minimal significant difference according to the Tukey test ($P \leq 0.05$). ns: not significant. *: indicates significant effect with $P \leq 0.05$.

the first defense mechanism in response to a water deficit is the partial closure of the stomata, which restricts the intake of CO_2 (De Pauw *et al*., 2008), affecting the growth of fruits

as well as other quality parameters (Álvarez-Herrera *et al*., 2015). In addition, Moriana *et al*. (2012) found that, in olive trees under stress conditions, the ψ_{stem} at noon reached -2.0 MPa and that, when the plants had an isohydric response to drought conditions, stomatal control prevented dehydration and potential values were not affected. However, when the response was anisohydric, the plant water potential was more sensitive to water stress.

On the other hand, when analyzing the effect of the doses of calcium applied to the cape gooseberry plants, they did not show significant differences in the behavior of the ψ_{stem} for any of the hours of the day (Fig. 2C), as happened with the ψ_{leaf}, and showed a pattern of variation that described the ψ_{stem} of the plants throughout the day. As with the ψ_{leaf}, it is likely that this measurement, being strongly influenced by climate, was not altered by the calcium fertilization. In contrast, Li *et al*. (2003) found that, when external Ca^{2+} applications were performed directly on the foliage, there was an increase in tolerance to drought and a decreased water potential because this element influenced the mitigation of oxidative stress by decreasing the activity of catalase, superoxide dismutase, and polyphenol oxidase.

Comparison between leaf and stem water potential and relationship with VPD

Figure 3 shows the average behavior throughout the day of the ψ_{leaf} and ψ_{stem} of the cape gooseberry plants. The ψ_{stem} was lower than the ψ_{leaf} with significant differences during all hours of the day except for predawn (4 am), where the values were -0.50 and -0.51 MPa for ψ_{stem} and ψ_{leaf}, respectively. This is consistent with the findings of Vélez *et al*. (2007), who mentioned that, in citrus, the measurement of ψ_{stem} and ψ_{leaf} at predawn, in treatments with an irrigation deficit and with an adequate water supply, had similar values. This suggests that, to have better control of the imposed water deficit, it is necessary to measure the daily variation of the stem. The equilibrium behavior between the observed potentials implies that at predawn, the water status was uniform throughout the whole plant, and the environmental conditions had a minimal influence, which in turn reflected the water potential of the substrate used according to the findings of Nortes *et al*. (2005). In addition, it could be suggested that this measurement would have a greater correlation in substrates than in clayey soils because of strong moisture retention that would limit the effect of deficit irrigation treatments, as expressed by Sato *et al*. (2006).

During the rest of the day, the differences between the measured potentials, probably, occurred as a result of the longer bagging time (3 h) for the measurement of the ψ_{stem}. This process limits the influence of environmental conditions (temperature and radiation) and decreases the photosynthesis of the bagged leaf, creating a continuum between the stem and the leaf (Boyer, 1995). As a result, values that are lower than those recorded in the leaf are obtained. The bagging time (15 min) only decreased the temperature and the effects of solar radiation but did not generate a continuous equilibrium in the vascular system, as reflected in the differences of the potentials at different times of day with the exception of the measurement at predawn. Likewise, the greatest difference between the potentials was measured at noon, with which it can be inferred that this is the time when the environmental conditions affected the potential the most. For this reason, for cape gooseberry plants, the measurement of this parameter at midday would be a good indicator of maximum water stress because of the high sensitivity that it presents, similar to that found by Nortes *et al*. (2005).

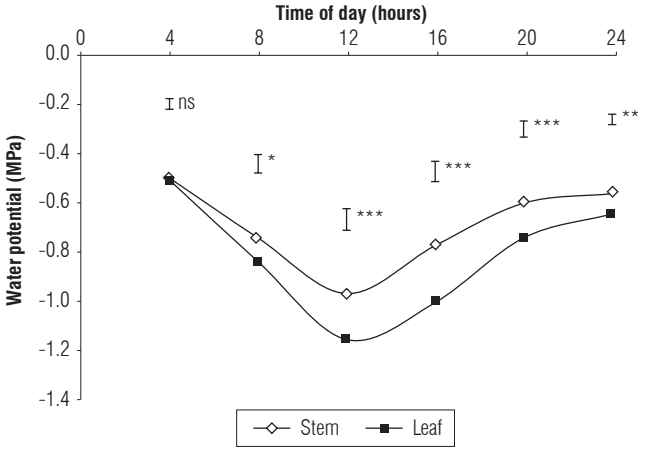


FIGURE 3. Leaf and stem water potential in cape gooseberry plants planted in peat moss in greenhouses. Bars indicate minimal significant difference according to the Tukey test ($P \leq 0.05$). ns: not significant. *: indicates significant effect with $P \leq 0.05$.

Because of the fact that the ψ_{stem} and ψ_{leaf} have been related to the environmental conditions, a relationship between these parameters of the water status of plants with the VPD was expressed, which has shown to have a strong correlation with the ψ in plum trees (Intrigliolo and Castel, 2006) and almonds (Nortes *et al*., 2005). Figure 4 shows that, as the VPD increased, both the ψ_{stem} and ψ_{leaf} decreased towards more negative values, and the VPD explained this decrease with a correlation of 95.3% and 83.2%, respectively. This behavior is similar to that reported by different researchers for plum (Intrigliolo and Castel, 2006), rose (Urban and Langelez, 2003) and *Dalbergia sissoo* Roxb. (Singh and Singh, 2006), who found that the highest values of ψ_{leaf} were due to the lower values of VPD. Likewise, the

relationship between the measured potentials and the VPD presented a polynomial trend. In this regard, Hogg and Hurdle (1997) stated that the relationship between the water potential and the VPD is not linear because stomatal closure is gradual as the VPD increases and stabilizes as a necessary response to keep the water potential high enough and avoid damage to the plant. However, Intrigliolo and Castel (2006) observed linear relationships in plums, and Sato *et al*. (2006) found a linear relationship between measurements at predawn of the water potential and the VPD, explained by night perspiration. Likewise, De la Rosa *et al*. (2013) stated that, in peaches, the ψ_{stem} at noon had a better correlation with the average temperature and with the VPD than with other climatic variables. However, they suggested that, to automate the measurements of the water status of plants, measurements of the maximum daily trunk contraction at noon would be a reliable alternative, similar to measuring the ψ_{stem} (Intrigliolo and Castel, 2005; Vélez *et al*., 2007) or stomatal conductance, which has an inversely proportional relationship with to ψ_{stem} in forest species (Hogg *et al*., 2000).

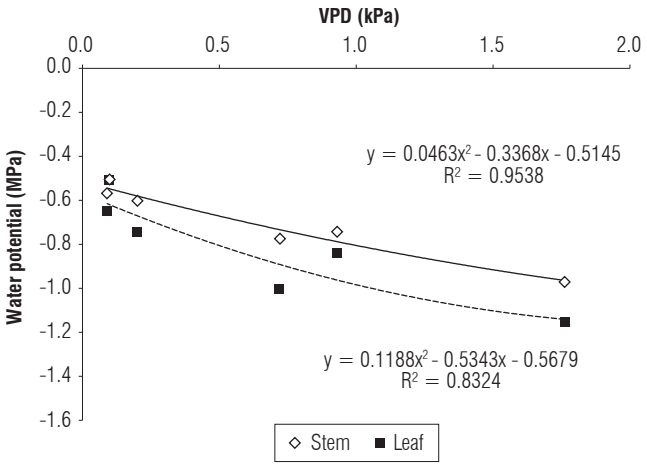


FIGURE 4. Relationship between the water potential of leaves and stems in cape gooseberry plants with a vapor pressure deficit (VPD).

Water consumption and irrigation water-use efficiency (WUEi)

Table 1 shows the amount of water applied during the treatment phase to the cape gooseberry plants. The plants that received the most water corresponded to the treatment of 1.3 with an irrigation frequency of 9 d, while the lower application of water corresponded to the irrigation frequency of 4 d with a coefficient of 0.7. The daily water consumption varied from 1.4 to 2.8 L/plant-d (Tab. 2) for the treatments with an irrigation frequency of 4 d with a coefficient of 0.7 and an irrigation frequency of 9 d with a coefficient of 1.3, respectively, which reflects the fact that the variation in the irrigation regimes used reached 100%.

TABLE 1. Total application of water (L) in cape gooseberry plants throughout the production period (6 months).

Irrigation coefficient	Irrigation frequency (d)		
	4	9	14
0.7	257	271	262
0.9	331	348	337
1.1	404	425	412
1.3	478	503	487

TABLE 2. Daily water consumption (L/plant-d) in cape gooseberry plants.

Irrigation coefficient	Irrigation frequency (d)		
	4	9	14
0.7	1.4	1.5	1.5
0.9	1.8	1.9	1.9
1.1	2.2	2.4	2.3
1.3	2.7	2.8	2.7

The WUEi did not show significant differences for the irrigation level interaction with the calcium dose (P=0.8946), nor for the irrigation frequency (P=0.6402). However, there were differences when analyzing the effect separately from the irrigation level factor (P=0.0357*). The coefficient that showed the highest WUEi was 0.7, which was 30%, 39%, and 50% higher than in the irrigation levels of 1.1; 0.9 and 1.3, respectively. Thus, it can be stated that the less water applied to cape gooseberry plants, the greater the efficiency in the production of biomass and the lower the water potential (Tab. 3), as reported in cucumber (Rahil and Qanadillo, 2015) and tomato (Zotarelli *et al*., 2009; Savic *et al*., 2011). These authors mentioned that the treatments with higher WUEi had more daily applications of water with more frequent irrigations and that a higher number of applications saw less water loss by infiltration. In addition, Intrigliolo and Castel (2010) confirmed that, in plums in the first season, the irrigation deficit generated a higher WUEi. However, for the second and third seasons, the treatments began to decrease the WUEi, as a product of the wastage of the plant subjected to permanent stress. In parallel, Fischer and Lüdders (1999) found that the WUEi in cape gooseberry plants is affected by the temperature of the root zone and that, when the soil temperature approaches 30°C, the WUEi is higher than when the plants are planted in root zones of 22 and 14°C.

Because the irrigation level of 1.1 had the second largest WUEi and generated the largest number of large fruits and the highest production (Alvarez-Herrera *et al*., 2015), it can be stated that this is the suitable irrigation coefficient for the production of cape gooseberry since an irrigation level of 0.7 had a higher WUEi, but lower production with smaller fruits, as found in olive trees by Miranda *et al*. (2018).

Likewise, in spite of the water deficit, the cape gooseberry fruits had a higher amount of total soluble solids and lower total titratable acids, similar to those found in plums (Intrigliolo and Castel, 2010) and tomatoes (Savic *et al*., 2011).

TABLE 3. Effect of the irrigation coefficient, the calcium dose and the frequency of irrigation on the irrigation water-use efficiency (WUEi) in cape gooseberry plants.

Factor	Factor level	WUEi (g L^{-1})
Irrigation coefficient	0.7	3.15 a
	0.9	2.27 ab
	1.1	2.43 ab
	1.3	2.10 b
Calcium doses (kg ha^{-1})	0	2.33 a
	50	2.59 a
	100	2.55 a
Irrigation frequency (d)	4	2.66 a
	9	2.42 a
	14	2.39 a

Average values with different letters in the same column and classified by factor indicate significant statistical differences according to the Tukey test ($P \leq 0.05$).

Conclusions

The ψ_{leaf} and ψ_{stem} in the cape gooseberry plants presented a behavior similar to a circadian rhythm. The irrigation frequency of 4 d with an irrigation level of 1.1 showed the highest values of ψ_{leaf} and ψ_{stem}. The ψ_{leaf} and ψ_{stem} reached the highest values at predawn (4 am). The application of irrigation levels lower than the ETc produced water stress in the plants and led to crop wilting. The irrigation coefficient of 0.7 presented the highest WUEi; however, the plants in this treatment showed strong symptoms of water stress and lower values of ψ_{leaf} and ψ_{stem}.

Literature cited

Agronet. 2019. Sistema de estadísticas agropecuarias. Producción nacional por producto. Uchuva. URL: www.agronet.gov.co (accessed March 2019).

Álvarez-Herrera, J., G. Fischer, and J.E. Vélez-Sánchez. 2015. Producción de frutos de uchuva (*Physalis peruviana* L.) bajo diferentes láminas de riego, frecuencias de riego y dosis de calcio. Rev. Colomb. Cienc. Hortíc. 9(2), 222-233. Doi: 10.17584/rcch.2015v9i2.4177

Álvarez-Herrera, J., G. Fischer, L.P. Restrepo, and M. Quicazán. 2014. Contenidos de carotenoides totales y ácido ascórbico en frutos sanos y rajados de uchuva (*Physalis peruviana* L.). Acta Hortic. 1016, 77-82. Doi: 10.17660/ActaHortic.2014.1016.8

Álvarez-Herrera, J., H. Balaguera-López, and G. Fischer. 2012. Effect of irrigation and nutrition with calcium on fruit cracking of the cape gooseberry (*Physalis peruviana* L.) in the three strata of the plant. Acta Hortic. 928, 163-170. Doi: 10.17660/ActaHortic.2012.928.19

Aparecido, L.E.O., R.M. Batista, J.R.S.C. Moraes, C.T.S. Costa, and A.F. Moraes-Oliveira. 2019. Agricultural zoning of climate risk for *Physalis peruviana* cultivation in Southeastern Brazil. Pesq. Agropec. Bras. 54, e00057. Doi: 10.1590/S1678-3921.pab2019.v54.00057

Baeza, P., P. Sánchez-de-Miguel, A. Centeno, P. Junquera, R. Linares, and J.R. Lisarrague. 2007. Water relations between leaf water potential, photosynthesis and agronomic vine response as a tool for establishing thresholds in irrigation scheduling. Sci. Hortic. 114, 151-158. Doi: 10.1016/j.scienta.2007.06.012

Boyer, J. 1995. Measuring the water status of plants and soils. Academic Press, San Diego, USA.

Cole, J. and V. Pagay. 2015. Usefulness of early morning stem water potential as a sensitive indicator of water status of deficit-irrigated grapevines (*Vitis vinifera* L.). Sci. Hortic. 191, 10-14. Doi: 10.1016/j.scienta.2015.04.034

De la Rosa, J.M., M.R. Conesa, R. Domingo, R. Torres, and A. Pérez-Pastor. 2013. Feasibility of using trunk diameter fluctuation and stem water potential reference lines for irrigation scheduling of early nectarine trees. Agr. Water Manage. 126, 133-141. Doi: 10.1016/j.agwat.2013.05.009

De Pauw, D.J.W., K. Steppe, and B. De Baets. 2008. Identifiability analysis and improvement of a tree water flow and storage model. Math. Biosci. 211, 314-332. Doi: 10.1016/j.mbs.2007.08.007

De Swaef, T., K. Steppe, and R. Lemeur. 2009. Determining reference values for stem water potential and maximum daily trunk shrinkage in young apple trees based on plant responses to water deficit. Agr. Water Manage. 96, 541-550. Doi: 10.1016/j.agwat.2008.09.013

Fischer, G. 2000. Crecimiento y desarrollo de la uchuva. pp. 9-26. In: Flórez, V., G. Fischer, and A. Soria (eds.). Producción, poscosecha y exportación de la uchuva. Unibiblos, Universidad Nacional de Colombia, Bogota.

Fischer, G. 2005. El problema del rajado del fruto de uchuva y su posible control. pp. 55-82. 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. Unibiblos, Universidad Nacional de Colombia, Bogota.

Fischer, G. and P. Lüdders. 1999. Efecto de la temperatura del substrato sobre el consumo de agua y la transpiración en la uchuva (*Physalis peruviana* L.). Suelos Ecuat. 29(1), 45-49.

García-Tejero, I., R. Romero-Vicente, J.A. Jiménez-Bocanegra, G. Martínez-García, V.H. Durán-Zuazo, and J.L. Muriel-Fernández. 2010. Response of citrus trees to deficit irrigation during different phenological periods in relation to yield, fruit quality, and water productivity. Agr. Water Manage. 97, 689-699. Doi: 10.1016/j.agwat.2009.12.012

Girona, J., M. Mata, J. del Campo, A. Arbone, E. Bartra, and J. Marsal. 2006. The use of midday leaf water potential for scheduling deficit irrigation in vineyards. Irrig. Sci. 24, 115-127. Doi: 10.1007/s00271-005-0015-7

Gordillo, O., G. Fischer, and R. Guerrero. 2004. Efecto del riego y de la fertilización sobre la incidencia del rajado en frutos de

uchuva (*Physalis peruviana* L.) en la zona de Silvania (Cundinamarca). Agron. Colomb. 22(1), 53-62.

Griñan, I., P. Rodriguez, Z.N. Cruz, H. Nouri, E. Borsato, A.J. Molina, A. Moriana, A. Centeno, M.J. Martin-Palomo, D. Perez-Lopez, A. Torrecillas, and A. Galindo. 2019. Leaf water relations in *Diospyros kaki* during a mild water deficit exposure. Agr. Water Manage. 217, 391-398. Doi: 10.1016/j.agwat.2019.03.008

Hogg, E.H. and P.A. Hurdle. 1997. Sap flow in trembling aspen: implications for stomatal responses to vapor pressure deficit. Tree Physiol. 17, 501-509. Doi: 10.1093/treephys/17.8-9.501

Hogg, E.H., B. Saugier, J.Y. Pontailler, T.A. Black, W. Chen, P.A. Hurdle, and A. Wu. 2000. Responses of trembling aspen and hazelnut to vapor pressure deficit in a boreal deciduous forest. Tree Physiol. 20, 725-734. Doi: 10.1093/treephys/20.11.725

Intrigliolo, D.S. and J.R. Castel. 2005. Usefulness of diurnal trunk shrinkage as a water stress indicator in plum trees. Tree Physiol. 26, 303-311. Doi: 10.1093/treephys/26.3.303

Intrigliolo, D.S. and J.R. Castel. 2006. Performance of various water stress indicators for prediction of fruit size response to deficit irrigation in plum. Agr. Water Manage. 83, 173-180. Doi: 10.1016/j.agwat.2005.12.005

Intrigliolo, D.S. and J.R. Castel. 2010. Response of plum trees to deficit irrigation under two crop levels: tree growth, yield and fruit quality. Irrig. Sci. 28, 525-534. Doi: 10.1007/s00271-010-0212-x

Jaimez, R.E., F. Rada, and C. García-Nuñez. 1999. The effect of irrigation frequency on water and carbon relations in three cultivars of sweet pepper (*Capsicum chinense* Jacq), in a tropical semiarid region. Sci. Hortic. 81, 301-388. Doi: 10.1016/S0304-4238(99)00017-5

Kitsaki, C.K. and J.B. Drossopoulos. 2005. Environmental effect on ABA concentration and water potential in olive leaves (*Olea europaea* L. cv. "Koroneiki") under non-irrigated field conditions. Environ. Exp. Botany 54, 77-89. Doi: 10.1016/j.envexpbot.2004.06.002

Li, M., G. Xuan-Wang, and J. Lin. 2003. Application of external calcium in improving the PEG-induced water stress tolerance in licorice cells. Bot. Bull. Acad. Sin. 44, 275-284. Doi: 10.7016/BBAS.200310.0275

Marsal, J. and J. Girona. 1997. Relationship between leaf water potential and gas exchange activity at different phenological stages and fruit loads in peach trees. J. Am. Soc. Hort. Sci. 122, 297-464. Doi: 10.21273/JASHS.122.3.415

Marschner, P. (ed). 2012. Marschner's mineral nutrition of higher plants. Elsevier, Amsterdam.

Miranda, R.D., M.V. Cuevas, A. Diaz-Espejo, and V. Hernandez-Santana. 2018. Effects of water stress on fruit growth and water relations between fruits and leaves in a hedgerow olive orchard. Agr. Water Manage. 210, 32-40. Doi: 10.1016/j.agwat.2018.07.028

Moriana, A., D. Pérez-López, M.H. Prieto, M. Ramírez-Santa-Pau, and J.M. Pérez Rodríguez. 2012. Midday stem water potential as a useful tool for estimating irrigation requirements in olive trees. Agr. Water Manage. 112, 43-54. Doi: 10.1016/j.agwat.2012.06.003

Murray, F.W. 1967. On the computation of saturation vapor pressure. J. Appl. Meteorol. 6, 203-204. Doi: 10.1175/1520-0450(1967)006<0203:OTCOSV>2.0.CO;2

Navarro, A., S. Bañon, E. Olmos, and M.J. Sánchez-Blanco. 2007. Effects of sodium chloride on water potential components, hydraulic conductivity, gas exchange and leaf ultrastructure of *Arbutus unedo* plants. Plant Sci. 172, 473-480. Doi: 10.1016/j.plantsci.2006.10.006

Nortes, P.A., A. Pérez-Pastor, G. Egea, W. Conejero, and R. Domingo. 2005. Comparison of changes in stem diameter and water potential in young almond trees. Agric. Water Manage. 77, 296-307. Doi: 10.1016/j.agwat.2004.09.034

Rahil, M.H. and A. Qanadillo. 2015. Effects of different irrigation regimes on yield and water use efficiency of cucumber crop. Agr. Water Manage. 148, 10-15. Doi: 10.1016/j.agwat.2014.09.005

Sato, T., O. Abdalla, T. Oweis, and T. Sakuratani. 2006. The validity of predawn leaf water potential as an irrigation-timing indicator for field-grown wheat in northern Syria. Agr. Water Manage. 82, 223-236. Doi: 10.1016/j.agwat.2005.07.023

Savic, S., R. Stikic, V. Zaric, B. Vucelic-Radovic, Z. Jovanovic, S. Djordjevic, and D. Petkovic. 2011. Deficit irrigation technique for reducing water use of tomato under polytunnel conditions. J. Cent. Eur. Agr. 12(4), 597-607. Doi: 10.5513/JCEA01/12.4.960

Singh, B. and G. Singh. 2006. Effects of controlled irrigation on water potential, nitrogen uptake and biomass production in *Dalbergia sissoo* seedlings. Environ. Exp. Botany 55, 209-219. Doi: 10.1016/j.envexpbot.2004.11.001

Sousa, T., M. Oliveira, and M. Pereira. 2006. Physiological indicators of plant water status of irrigated and non-irrigated grapevines grown in a low rainfall area of Portugal. Plant Soil 282, 127-134. Doi: 10.1007/s11104-005-5374-6

Taiz, L. and E. Zeiger. 2010. Plant physiology. Sinauer Associates, Sunderland, USA.

Urban, L. and I. Langelez. 2003. Effect of high-pressure mist on leaf water potential, leaf diffusive conductance, CO_2 fixation and production of cultivar 'Sonia' rose plants grown on rockwool. Sci. Hortic. 50, 229-244. Doi: 10.1016/0304-4238(92)90176-D

Vélez, J.E., D.S. Intrigliolo, and J.R. Castel. 2007. Scheduling deficit irrigation of citrus trees with maximum daily trunk shrinkage. Agr. Water Manage. 90, 197-204. Doi: 10.1016/j.agwat.2007.03.007

Villarreal-Navarrete, A., G. Fischer, L.M. Melgarejo, G. Correa, and L. Hoyos-Carvajal. 2017. Growth response of the cape gooseberry (*Physalis peruviana* L.) to waterlogging stress and *Fusarium oxysporum* infection. Acta Hortic. 1178, 161-168. Doi: 10.17660/ActaHortic.2017.1178.28

Zhu, Li-Hua., A. van de Peppel, X.Y. Li, and M. Welander. 2004. Changes of leaf water potential and endogenous cytokinins in young apple trees treated with or without paclobutrazol under drought conditions. Sci. Hortic. 99, 133-141. Doi: 10.1016/S0304-4238(03)00089-X

Zotarelli, L., J. Scholberg, M. Dukes, R. Muñoz-Carpena, and J. Icerman. 2009. Tomato yield, biomass accumulation, root distribution and irrigation water use efficiency on a sandy soil, as affected by nitrogen rate and irrigation scheduling. Agr. Water Manage. 96, 23-34. Doi: 10.1016/j.agwat.2008.06.007

Heritage and Patrimony of the Peasantry: an analytical framework to address rural development

Herencia y Patrimonios de Campesinado: un marco analítico para abordar el desarrollo rural

Fabio Alberto Pachón-Ariza[1]*, Wolfgang Bokelmann[2], and César Ramírez-Miranda[3]

ABSTRACT

The term "rural development" is exceptionally multifaceted, which makes it difficult to define. This and other features make it a 'wicked problem', which means the consequences of rural developmental problems can create other complications. To date, the important discussion of rural development has dealt with productivity and economic concerns. This discussion has many crucial aspects such as the environment, infrastructure, and respect for fundamental rights. This paper describes the 'Heritage and Patrimony of the Peasantry' as an alternative analytical framework for addressing rural development. This analytical framework takes important topics from other rural development perspectives (primarily focused on food sovereignty principles). The heritage and patrimony of the peasantry framework moves away from the market point of view, which converts everything into an asset that can be marketed, and utilizes other sources of heritage. The peasantry has seven kinds of 'heritages' or 'patrimonies': natural, cultural, economic, physical, social, institutional, and human. These heritages or patrimonies are the bases of construction for a decent standard of living which will accomplish full rights for all rural inhabitants, i.e. rural development.

Key words: peasants, interdisciplinary research, quality of life, rural communities, rural development strategies.

RESUMEN

El término desarrollo rural es excepcionalmente multifacético, lo que dificulta su definición. Esta y otras características lo convierten en un "problema complejo", lo que significa que las consecuencias de los problemas de desarrollo rural pueden crear otros problemas. Hasta la fecha, la importante discusión sobre el desarrollo rural ha sido sobre productividad y asuntos económicos. Sin embargo, esta discusión tiene muchos aspectos cruciales como el medio ambiente, la infraestructura y el respeto de los derechos fundamentales. Este estudio describe los Patrimonios del Campesinado, un marco analítico alternativo para abordar el desarrollo rural. Este marco analítico toma temas importantes de otras perspectivas de desarrollo rural, pero está enfocado principalmente en los principios de la soberanía alimentaria. Patrimonios del campesinado se aleja del punto de vista del mercado, que convierte todo en un activo que se puede comercializar, y se enfoca en otras facetas del patrimonio. El campesinado tiene siete tipos de patrimonios: naturales, culturales, económicos, físicos, sociales, institucionales y humanos. Estos patrimonios son la base de la construcción de un nivel de vida que, a su vez, permitirá alcanzar plenos derechos para todos los habitantes rurales, es decir, el desarrollo rural.

Palabras clave: campesinos, investigación interdisciplinaria, calidad de vida, comunidades rurales, estrategias de desarrollo rural.

Introduction

Rural development and the alleviation of poverty have been a primary concern for many governments in developing countries over the last few decades. Though we have seen impactful advances in many communities, the strategies and solutions proposed have not ensured changes to an acceptable quality of rural life nor have they been able to guarantee respect for all rural inhabitants' rights (Scoones, 2015).

This paper is designed to suggest an alternative analytical framework for addressing rural development in a straightforward way. By analyzing and factoring in heritage and patrimony of the peasantry, this paper takes into consideration different points of view, based on a literature review and taking into account the idea of heritages and patrimonies, suggests a way in which all heritages can cooperate and, thereby, achieve a better life for all rural inhabitants.

Received for publication: 10 December, 2018. Accepted for publication: 03 December 2019 Doi: 10.15446/agron.colomb.v37n3.76757

[1] Department of Agri-food and Rural Development, Universidad Nacional de Colombia, Bogota (Colombia).
[2] Department of Agricultural Economics, Economics of Horticultural Production, Humboldt Universität zu Berlin, Berlin (Germany).
[3] Universidad Autónoma de México, Mexico City (Mexico).
* Corresponding author: fapachona@unal.edu.co

Rural development, a 'wicked problem'

Rittel and Webber (1973) defined a 'wicked problem' as a malignant, tricky or aggressive condition enclosed in a vicious circle. A 'wicked problem' is difficult to explain and solve for several reasons. The first challenge steams from an incomplete understanding of a situation or contradicting information (Roberts, 2012). In other words, it is hard to define and fix something clearly and completely if there is a lack of comprehension (Kuhmonen, 2018). Second, with many people there are many opinions that make it difficult to decide how to tackle a problem (Norris *et al*., 2016). Third, there are often great financial burdens and barriers associated with wicked problems (Gharehgozli *et al*., 2017). Finally, it is difficult to make accurate assessments and thorough changes since there are so many intertwined problems (Dutta, 2018). On top of that, it is difficult to know if taking action could create unwanted/unforeseen complications (Probst and Bassi, 2014; Innes and Booher, 2016).

Rittel and Webber (1973) defined ten characteristics of wicked problems that could be applied in the scope of understanding the complexities of addressing and applying rural development issues and strategies. First, wicked problems have no conclusive formulation (Zijp *et al*., 2016). Concerning rural development, several approaches from the technocratic point of view to a new political approach represented by food sovereignty have tried to address many issues. Each approach offers a set of steps and solutions for rural development problems. However, so far these solutions have not been comprehensive enough to have a definitive understanding of the entire problem(s) and how to fix it (Pachón *et al*., 2016).

Second, it is difficult to quantify or declare success with wicked problems, primarily because they create many other problems (opposed to the limits of conventional problems that can be explained or interpreted) (Elia and Margherita, 2018). There is often a disagreement about the causes of problems of rural development. Sometimes politicians and technicians blame the idiosyncrasy of rural people (Castro-Arce and Vanclay, 2019). Others blame the policies, especially in developing countries. The fact is that rural inhabitants in many places remain trapped in poverty, illiteracy, and illness. In other words, rural development has exceeded the capacity and/or willingness of their governments' ability to deal with these very problems (Head and Alford, 2015).

Third, the solutions to wicked problems are dichotomous. There is no suggestion that some of these answers are perfect or better than any other answer. It is important that these approaches are tractable methods for the condition we are trying to enhance (Farrell and Hooker, 2013). Rural development approaches, especially from the technocratic perspectives, have proposed alternatives for solving the problems of rural communities. Unfortunately, these attempts have often led to unforeseen outcomes that can occasionally be extremely deleterious for community dynamics, economics, and the environment (Kay, 2009). New solutions create extra dimensions that must be integrated into an analysis before steps towards change are made that ensure that unintentional consequences do not arise (Luckey and Schultz, 2001).

Fourth, there is no pattern to follow when confronting a wicked problem, despite the guidance the past can offer. People working with wicked problems must build new ways and ideas as they go along (Dentoni and Bitzer, 2015). First and foremost, the widespread approaches have offered partial solutions for rural development challenges. Their focuses have mainly been on economic activities rather than on the people themselves. Their solutions have aimed to increase incomes as a way to isolate rural people. Every rural community has its needs and wishes, and the solutions to these needs must be constructed taking into consideration the opinion of rural people themselves. These processes, constructed from the bottom-up, require flexibility to accommodate dissimilar situations and, therefore, to maintain the legitimacy of the inclusion of people in the decision-making processes (Chambers, 1983).

Fifth, there are several explanations for a wicked problem, and the pertinence of the explanations depends on the particular perception of the designer. As described previously, the main approaches to rural development for explaining the consequences of rural problems is to propose a course of action to solve them (Gold *et al*., 2018). The perspectives of the technocratic approach have focused their proposals on an economic point of view. From the green revolution to neoliberalism to the import substitution industrialization (ISI) to neostructuralism, the modernization of agricultural production has been deemed the answer to rural development problems. In contrast, a sociological approach has focused on the rural inhabitants' personal and communal needs. In the center we find the socio-technocratic approach, which analyses productive problems in a social context and proposes competitiveness as the way to solve them (Kay, 2009). Another example is the political approach

that has used food sovereignty to focus on the rights of rural inhabitants and consumers as its response to rural development problems (Pachón *et al*., 2016).

Sixth, every negative consequence of a wicked problem is a symptom of another problem. Equally, the causes of problems are, at the same time, the consequences of others. Rural development problems are narrowly interconnected with the causes and consequences of many other problems (Andersson and Törnberg, 2018). For instance, illiteracy and a low level of education in rural areas are some of the reasons for other phenomena such as poverty, lack of participation, and low agricultural production. Likewise, when people do not know how to read and write, their integration into society is harder for them than it is for those who do know how to read and write (Leverenz, 2014). Rural poverty is narrowly related to low agricultural production, although a high agricultural production does not guarantee freedom from poverty. Clearly, identifying the main causes of rural development problems is a complicated task. That is why a multidisciplinary approach is necessary when addressing these problems (Pacanowsky, 1995; Norris *et al*., 2016).

Seventh, a lack of an alleviation policy for a wicked problem has a decisive scientific test because society and scientists understand problems differently. The scientific approaches to addressing rural development are incomplete (Tietjen and Jørgensen, 2016). A multidisciplinary approach that takes the interactions and connections into consideration and then places the emphasis on the peoples' rights over economic concerns might be better for tackling a wicked problem, such as rural development. Rural development policy actions have partially failed in the last decades because of the lack of a "people first" mindset. For instance, the distribution of power among rural stakeholders remains concentrated in those that hold land, money, and political influence (Roberts, 2000).

Eighth, finding a "solution" to a wicked problem usually focuses on a design effort, opposed to a rigid strategy which reduces the likelihood of trial and error (Came and Griffith, 2018). Rural development seems to go beyond the capacity of the governments and public policies, which creates dissatisfaction among rural and, sometimes, urban inhabitants (Brugue *et al*., 2015). Traditionally, public policies have addressed rural development problems based on a disciplinary policy, almost entirely avoiding integrating other concerns (Pachón *et al*., 2016).

Ninth, every wicked problem is exceptional (Kolko, 2011; Andersson and Törnberg, 2018; Elia and Margherita, 2018). Even though rural development challenges are similar in many places, the solutions vary drastically. The problems are similar because public policies, especially in developing countries, have followed the same pattern based on the green revolution and neoliberalism (Kay, 2009; Pachón *et al*., 2016). Hence, the consequences of such policies trigger analogous problems and difficulties. However, the solutions to these problems are different everywhere (Bitsch, 2009), because they must be formulated based on the peculiarities of the rural areas and the idiosyncrasy of their people. Obviously, the rural inhabitants themselves should construct such solutions, furthering solution variances.

Tenth, the designers trying to tackle a wicked problem must be held responsible and accountable for their actions. Governments must acknowledge that they are responsible for the consequences of the application of rural policies that have tried to solve rural development problems (Xiang, 2013). However, in many places the rural inhabitants themselves have been suffering from the effects of such policies, due to a lack of accountability. Rural inhabitants are often isolated from society where their importance is not often recognized (Probst and Bassi, 2014).

Rural development is a complex and interdependent situation that is difficult to explain and comprehend (Anderson, 2003). It has been improperly understood, which means that the different approaches to address it have been incomplete. Some strategies have successfully helped to manage and solve problems. However, many problems related to rural development such as poverty, illiteracy, income inequality, lack of access to health care and education, degradation of the environment, and lack of access to credit and technical assistance still remain. Especially in developing countries, the persistence of issues such as poor infrastructure, isolation, and absence of social recognition only fuel the difficulties of solving problems of rural development (Chambers and Conway, 1992; Ellis and Biggs, 2001; Brass, 2002; Molina, 2010). Two significant points emerge from the above debate. What have the central themes for successful approaches to rural development been? And, what are the most important characteristics to take into consideration to approach and solve a wicked problem such as rural development?

How to address a wicked problem

The most efficient way to tackle a wicked problem, such as rural development, is through an interdisciplinary and transdisciplinary framework. The integration of different disciplines, points of view, and an innovative analytical

framework based on such amalgamation allows us to address the complexity of real life (Norris *et al*., 2016; Elia and Margherita, 2018).

The characteristics of social problems regarding rural development are complex, ambiguous, and uncertain (König *et al*., 2013). However, the disciplines and traditional approaches to planning try to simplify their approaches, splitting them up for the purpose of analyzing every component separately (Espina, 2007). Such separation reduces the scope of analysis of the methods, minimizing the attributes that emerge from the interaction of all the factors. Indeed, reality requires comprehensive analytical frameworks that overcome the boundaries of disciplines. Comprehensive analytical frameworks enable us to address complex problems successfully and efficiently throughout the process (McKee *et al*., 2015; Henriksen, 2016).

A holistic analytical framework allows the identification of a complete and wide-ranging image of the problems. Such methodology attempts to tackle the complexity of problems and allows a better understanding of all their synergies and connections (Delgado and Rist, 2011). Equally, a comprehensive analytical framework realizes the emerging capacity of the problems in rural territories that are ever-changing. Usually, new situations, attributes, and problems appear according to the interaction of every component.

Besides the holistic analytical framework, adequate organization is necessary to address wicked problems. Members of an organization who usually come from diverse disciplines must share similar objectives, cooperate, and, most importantly, be able to manage heterogeneity and the complexity of the disciplines (König *et al*., 2013). The organization must be able to manage conflicts stemming from various points of view. Finally, and maybe most importantly, the organization must take into consideration previous research and proposals that have addressed problems to avoid wasting significant time and energy trying to do something that somebody else has already done.

Interdisciplinary and transdisciplinary frameworks

The academic community (Dewey, 1938; Miguélez, 2009; Olivé, 2011; Raasch *et al*., 2013) commonly defines an interdisciplinary framework as the integration, combination, or mixture of scientists of two or more disciplines, fields, bodies of knowledge, or modes of thinking. An interdisciplinary framework brings skills, techniques, concepts, and expertise to create meaning, explanations, solutions, understanding, and alternatives for tackling complex problems that have been incompletely understood or are socially complicated (Norris *et al*., 2016).

Scientists working under an interdisciplinary framework must demonstrate willingness, temperament, and commitment to cross the boundaries of disciplines because their results depend on the relationships, judgement, and dialogue with the scientists of other areas (Dentoni and Bitzer, 2015; Gharehgozli *et al*., 2017). An interdisciplinary framework is necessary for innovation and, in fact, it has been stimulated by international funding (Millar, 2013). It operates primarily at a university level, because there is greater access to know-how, tools, and funds. In addition, universities offer transversal enrichment, prestige and the acquisition of reputation, learning of techniques, efficiency enhancement, and recruitment of scholars (van Rijnsoever and Hessels, 2011). However, its implementation and outcomes at the institutional level are still doubted by the scientific community (Elia and Margherita, 2018).

A transdisciplinary framework aims to understand and address complex problems through the interaction of diverse disciplines (Dentoni and Bitzer, 2015). Besides scientists of specific fields, this interaction includes other stakeholders who come from any discipline, for instance, peasants who can make relevant contributions (Olivé, 2011). The main goal of a transdisciplinary framework, besides tackling complexity, is to create novel concepts, methods, and approaches that improve on disciplines. Hence, in a transdisciplinary framework, there is a dialogue between the scientific and empirical knowledge, and as a result, interesting epistemological bridges are created (Miguélez, 2009) that strengthen both science and practice.

A transdisciplinary framework is greater than a mere sum of the disciplines. It is a collaboration among them, a method to merge knowledge where the boundaries of the disciplines are blurry (Espina, 2007). These methodologies are characterized by an emergent attribute that bridges the gap between disciplines and implies a novel transcultural, transnational, and transpolitical approach.

Zemelman (2001) argues that a transdisciplinary framework must take into consideration all the inputs and outputs as a unity of all the sides to explain and solve problems. He suggests avoiding methodologies focused on factorial logic. Instead, he proposes the implementation of a methodology focused on a matrix of complex relationships with reciprocal effects. In this matrix, the problem is analyzed as a network, emphasizing all the dimensions and connections

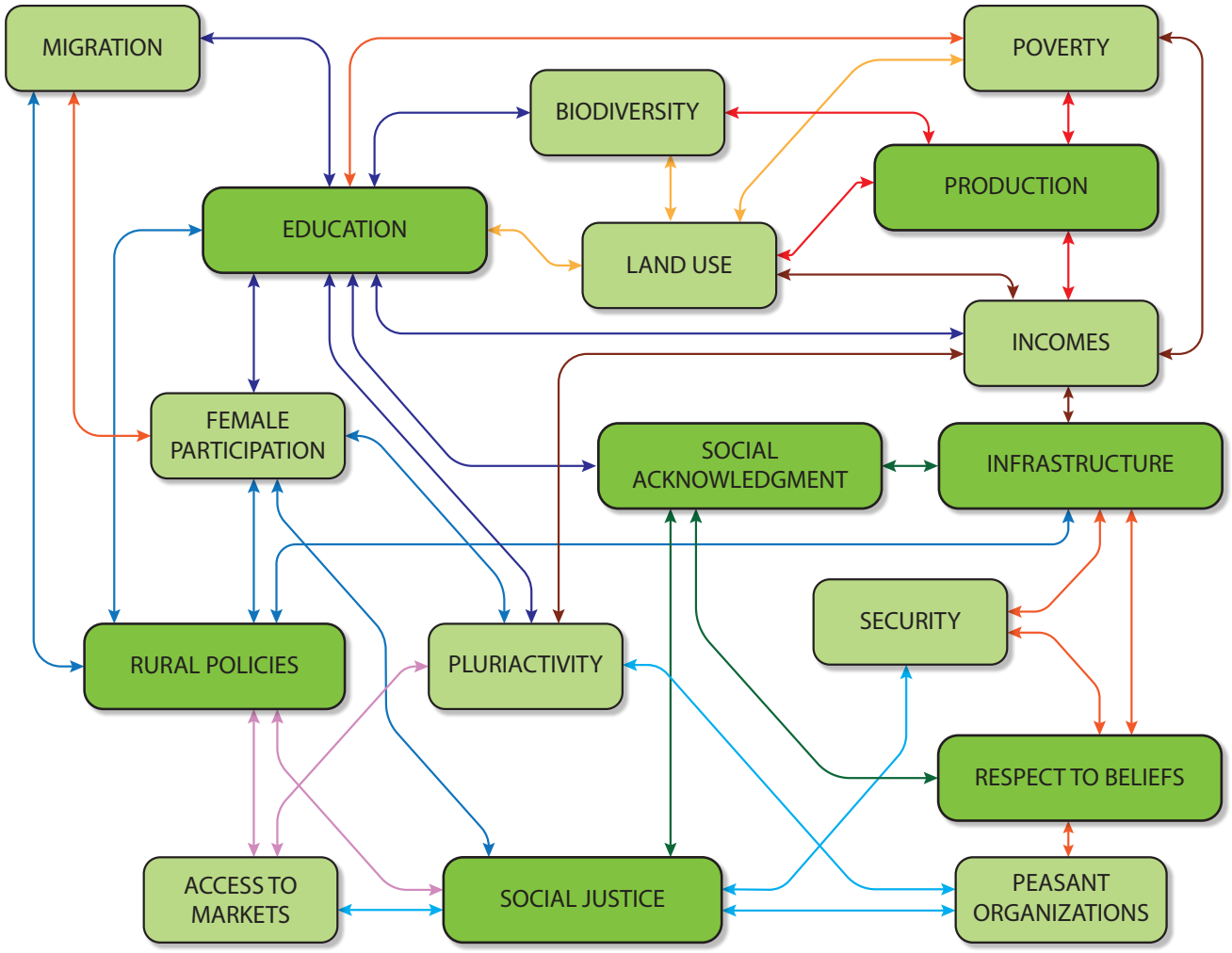


FIGURE 1. Rural development connections.

that are reliant on each other (Dutta, 2018). In the scope of rural development, challenges must be addressed and measured individually and communally to better understand output causes. In other words, the problems of rural development addressed in a transdisciplinary framework identify all the connections among the problems and the consequences of these relations (Fig. 1).

Figure 1 displays some of the problems of rural territories and some of their consequences. It also establishes the relationships among them, whether as cause or consequence. For example, education is one of the most important topics that determines the quality of life and exerts a strong influence on other subjects such as migration, land use, and poverty (Brown and Park, 2002). Education affects migration because in some rural areas young people who hold a medium or high educational level usually migrate to urban areas looking for jobs related to their backgrounds. However, when educated people remain in rural areas, positive changes in land use, conservation of biodiversity, and female participation in decision making are evident (Gustafsson and Li, 2004). A similar description could be established with the other problems. For example, social justice, one of the main demands of the peasantry around the world, is directly connected to rural policies, social acknowledgement, and access to markets. Since rural developmental problems are narrowly associated with one another, none of them should be addressed separately. An interdisciplinary and transdisciplinary framework is decisive for solving most of the main problems and their consequences integrally. In this scenario, 'Heritage and Patrimony of the Peasantry' is the proposal of an analytical framework to address rural development that integrates many of the concerns of rural populations and incorporates the main characteristics of the most important rural developmental approaches, especially

food sovereignty (Desmarais, 2002; Holt-Giménez and Altieri, 2013).

Heritage and patrimony of the peasantry, an alternative analytical framework

Initially, it is important to define rural development and heritages that the peasantry offer us as an alternative viewpoint. This first stage aims to provide all rural residents with a basic standard of living, which can only be accomplished through the protection of the human rights of rural residents (Rosset, 2003; Borras Jr., 2009). Heritage and patrimony of the peasantry aims to organize, as much as possible the topics involved in problems of rural development by addressing them in an interdisciplinary and transdisciplinary framework. Heritage and patrimony of the peasantry framework is based on four milestones: rural territory, heritage and patrimony, quality of rural life, and respect for human rights. Figure 2 shows the interaction of these milestones.

Rural territory

It is important to understand, in general, what rural territory means. A territory is defined as a space that holds feelings of identity and collectively constructed ideas of development whose transformation is a result of the mobilization and appropriation of the inhabitants (Schejtman and Berdegué, 2003; Jouini *et al*., 2019). Besides the differences between the rural and urban concepts based on population totals, three main approaches have analyzed this concept: as a historical process; its functionality; and its environmental viewpoint. Rural territory as a historical

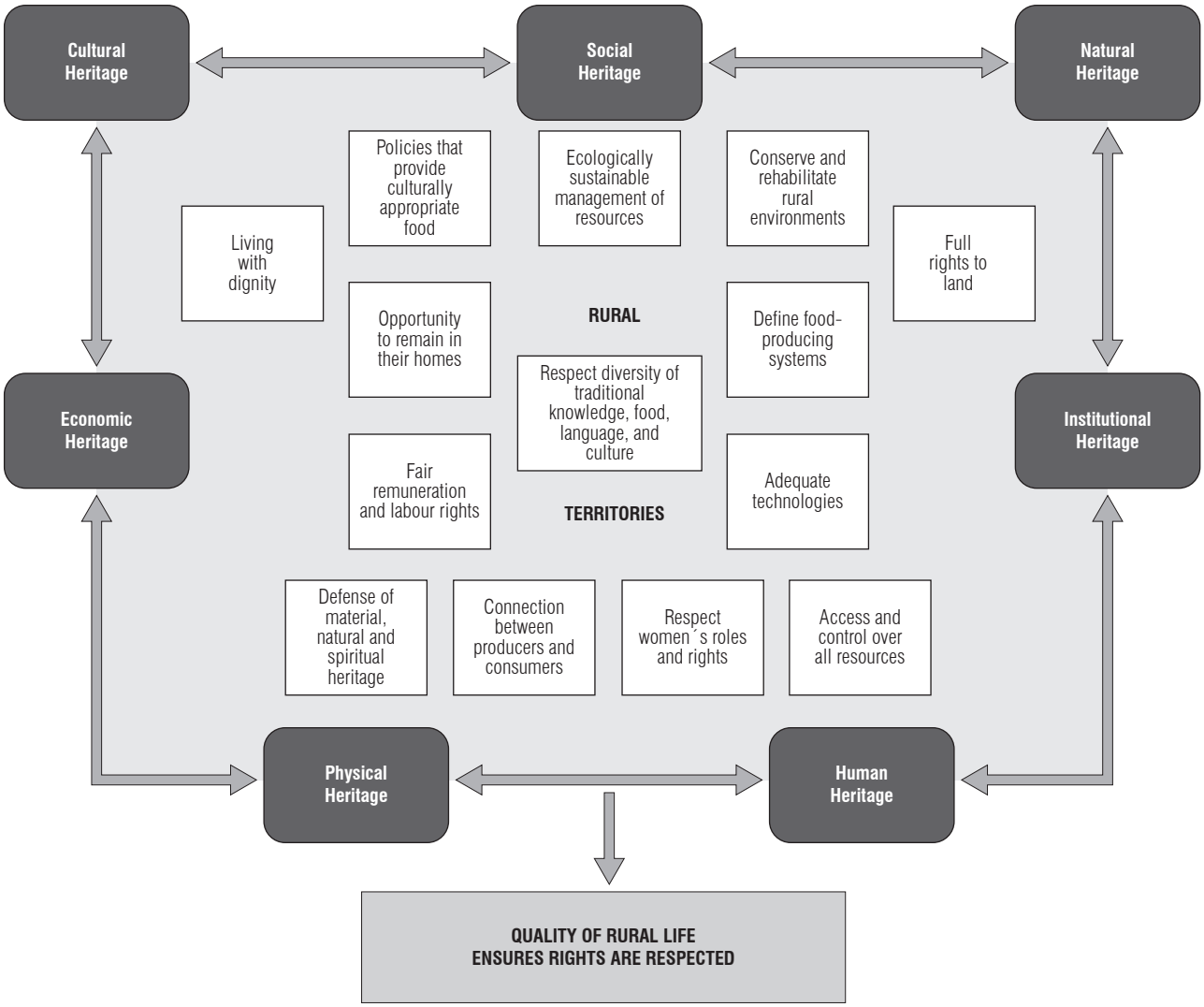


FIGURE 2. Scheme of the heritage and patrimony of the peasantry.

process is tightly linked to the meaning of the territory for its inhabitants. In this sense, rurality is a series of social networks whose inhabitants' livelihoods rely on rational use of available resources (Chambers and Conway, 1992). Furthermore, the relationships among these inhabitants are characterized by tradition and culture, the basis of rural identity. Rural territory and its inhabitants are characterized by a behavior that symbolizes an appropriation of the spaces and its resources, where the population shares feelings of identity, cooperation, and a sense of belonging (Dirven *et al*., 2011). Even though many of the members of new generations have migrated to urban places, these feelings remain deeply rooted out of respect and love for their heritage and ancestry.

Traditionally, the functionality of the rural territories has been related to the economic activities performed there. For instance, crops or livestock production can be strongly influenced by culture and tradition. However, another type of agricultural production is strongly influenced by the market (Gutierrez-Montes *et al*., 2009). That production is highly specialized, industrialized, and organized in groups of people very close to each other, or clusters by vicinity, according to the likelihood of using the natural resources, such as land and water, or the natural advantages for mining or tourism. These clusters ultimately seek to improve competitiveness and increase individual profit. The benefit of organization in clusters is its ability to facilitate the offering of technical services, inputs, and support on the assumption that the profitability could be transferred into the territory and to other inhabitants that do not participate in the cluster (Echeverri, 2011).

The environmental point of view highlights concerns related to climate change and the likelihood that rural activities mitigate the factors that increase global warming. For many years, when many people realized the consequences of global warming and the impact it has on normal lives, rural territories gained more relevance because they offered additional services compared to the traditional ones. These services are related to the likelihood of an alternative model of development based on ecosystem services, represented by environmental markets and environmental supply (Dirven *et al*., 2011).

The previous discussion emphasizes the multifunctionality and pluriactivity of rural territories. However, beyond the multifunctionality of rural areas, it is crucial to take into account more integrative ideas such as the "inter-functionality" of rural territories. "Inter-functionality" means that there should be stable relationships, close interactions, and deep integrations among all the functions and activities developed there (Florian, 2012; Kolstad, 2012). The primary goal of the "inter-functionality" is to preserve all the heritages of the peasantry present in these territories.

An example in which the inter-functionality of rural areas is not working appropriately are those territories where monoculture is predominant, undermining the possibility of producing food to feed their inhabitants. Many times, the target of the monoculture is a well-paid international market. The region of Uruapan in the State of Michoacan (Mexico) is a true archetype for this kind of production. Avocado is a widespread monoculture, mainly destined to the United States market. It is produced by peasants, small, medium and large farmers, as well as by multinational food companies. This monoculture, which is indeed well-paid, has increased the incomes of many people (input sellers, transporters, harvesters, and packers) who are directly and indirectly related to production (Pachón *et al*., 2017b).

The international peasant movement La Via Campesina and its proposal for food sovereignty through the Declaration of Nyéléni (2007) describe the principles that, according to their deliberations, are essential for the improvement of their quality of life and will guarantee that the rights of the peasantry and all rural inhabitants are respected. Figure 2 shows some of these principles (the interaction inside the rural territories plane). In the background of these principles, a political dimension can be found because, although essential, the technocratic dimension has proved to be insufficient compared to the other rural aspects. Primarily, neoliberal and neocolonialist proposals, as well as the World Trade Organization, free trade agreements, and other policies exclude the peasantry (Pachón *et al*., 2016). In this scenario, systems that allow unfair trade, such as dumping and subsidy schemes in developed countries and those that are against the likelihood of subsistence of small farmer production from developing countries are shunned (Barker, 2007).

Heritage and patrimony

The next crucial point is heritage and patrimony. At this level, seven kinds of heritage and patrimony that the peasantry must mix to improve their quality of life and ensure that their rights are respected are organized (Pachón, 2013). The first issue to discuss is the meaning of heritage followed by a description of each element in the proposed heritage. Heritage is a net of beliefs, traditions, and customs which a civilization considers significant to its history, culture, and identity (Littaye, 2016). Heritage must be understood in the scope of patrimony. They are the structures, articles,

or concepts that a civilization gets from the communities who lived before them. That means that for the current framework, heritage and patrimony could be assumed in the same way (Cominelli and Greffe, 2012). Beyond the concept, many aspects enrich and transform heritage and patrimony into one of the milestones of the current framework (Calvo *et al*., 2017).

First, we must look at the social importance of heritage and patrimony. This constitutes the traces of memories that represent a social fact legitimized as something that reflects the importance of being analyzed, preserved, and inventoried. Hence, it is socially appreciated as a cultural phenomenon such as collective memory (Criado-boado and Barreiro, 2013). Then, a heritage and a patrimony are the results of social construction. It is a symbolism for the dissemination of collective memory.

Second, we must look at the cultural importance of heritage and patrimony. This is the repository that gathers common behaviors from different societies and groups, ways to solve difficulties, knowledge, values, symbols, and socio-cultural frameworks. Heritage and patrimony are used as a means to illustrate the culture, traditions, customs, background, and landscapes (Dormaels, 2012).

Finally, we identify the importance of heritage and patrimony. The acts appreciate heritage and patrimony as something personal and distinguishable; these are impossible to separate from the admiration and respect of peoples, communities, and individuals. For that reason, heritage and patrimony are valued, managed, and conserved. Something that is poorly appreciated is no longer valued as heritage and patrimony. These are a network of paths of life, beliefs, values, emotions, and meanings that offer a resource of identity and add value to social, political, and economic claims. It is the process of unification of identities (Santos, 1993).

Heritage and patrimony are the expressions of the accumulation of knowledge through time. They are the way to understand and link the history and the traditions from our past with our present. At the same time, heritage and patrimony are the best ways to construct the future (Calvo *et al*., 2017). Figure 3 describes the heritage and patrimony of the peasantry framework in a virtuous circle. They must be, and are, appreciated and valued because they constitute the fundamental part of our lives. Venerated heritage and patrimony are protected and saved because they conserve part of our history. If heritage and patrimony are appreciated and protected, society, in general, will ponder the

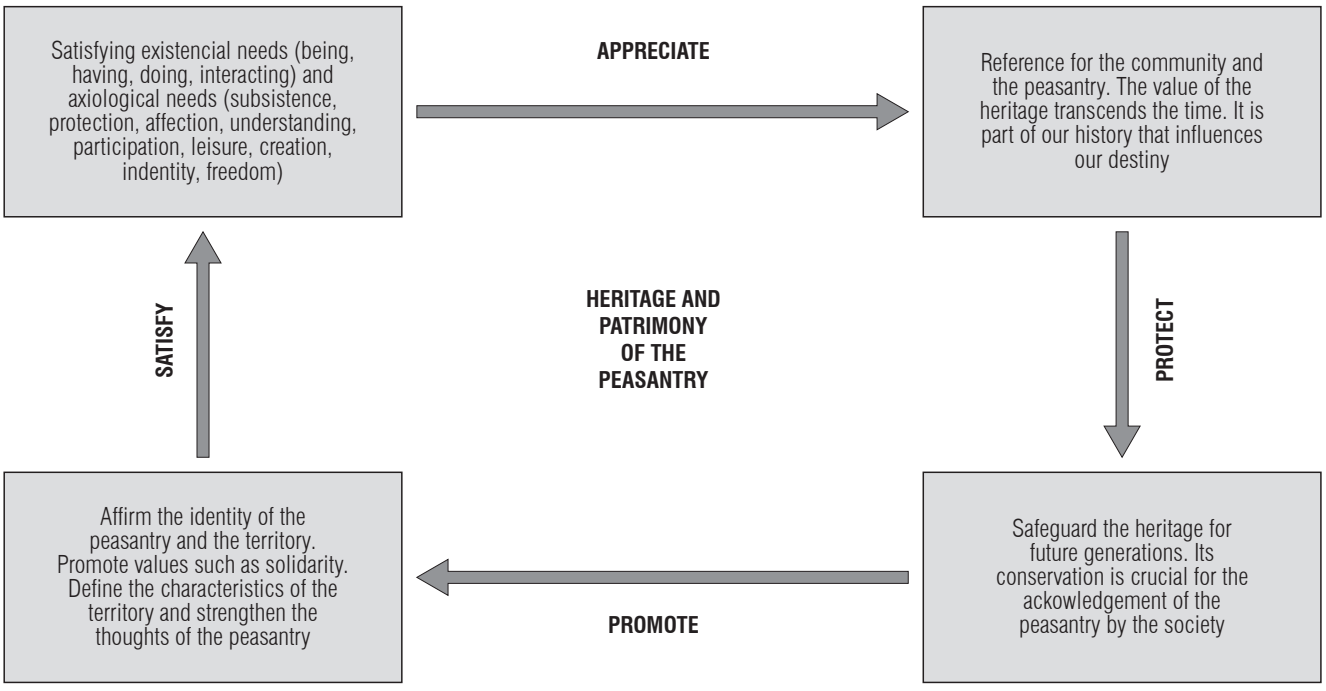


FIGURE 3. Virtuous circle of the heritage and patrimony of the peasantry.

importance of the peasantry and will encourage them in the coming generations. That promotion will inspire essential values of the peasantry. The cycle will then end but will start again when heritage and patrimony invoke the satisfaction of fundamental human needs (Max-Neef *et al*., 1994).

The circle begins with the recognition of the importance and significance of the peasantry and their customs from society as a whole. People must appreciate how rich the peasantry is, more than producing food that is vital, to maintain their rootedness (Wittman *et al*., 2010). People must also recognize that several customs of the peasantry are the best options for mitigating the consequences of climatic change. In addition, people must understand that the peasantry and their activities indirectly provide many of the products and raw materials used in urban areas. In other words, people must recognize the special qualities of the peasantry, the places where they live, and the things that they have done. If society properly appreciates the peasantry, their value would gradually increase, and, in turn, society will protect the peasantry (Patel, 2009).

The second step is the protection of the peasantry and their customs by society through collective action. For example, people must defend the peasantry from the policies that affect their customs and traditions, such as the disadvantages of free trade agreements. People can also help save the landscapes and rural environment against harm and damages to preserve them to mitigate the effects of climate change. This will help to defend the peasantry from expulsion from their lands and territories (Bebbington, 1999). When society protects the heritage and patrimony of the peasantry, society will, in turn, promote the heritage because it is important for new generations.

The third step is the promotion of the heritage and patrimony of the peasantry by society, especially among the new generations. An example of this can be, people supporting the peasantry by purchasing their products at a fair price. In this way, society helps the peasantry to reach a decent quality of life and helps to ensure respect for their human rights (Parrado and Molina, 2014).

The human scale of development defines basic measurements for human needs for both urban and rural populations. This is the last step of the circle (Max-Neef *et al*., 1994). The heritage and patrimony of the peasantry allows the rural population to satisfy their human needs because their heritage creates levels of self-reliance. It also articulates the satisfaction of human needs with environmental, technological, global and local processes, and for individuals within their communities. The human developmental scale describes two types of human needs: existential and axiological. These needs are multiple, interdependent, finite, few, and classifiable (Fig. 3). They create an interactive network whose key features are simultaneity, complementarity, and trade-offs, which characterize the process of satisfying human needs (Max-Neef *et al*., 1994).

Finally, we must treat the heritage and patrimony of the peasantry as invaluable. They are not marketable as part of their identity, as a social construction. In this scenario, the idea of 'capital' is no longer used. Capital is associated with the process of purchasing commodities in one place and selling them in another for profit (Flora *et al*., 2015). That means that the idea of the peasantry regarded just as a food supplier is excluded, forgetting its social prominence as part of the origin of the majority of societies. Because of these two different facets, patrimony can be categorized as tangible and intangible (Holt-Giménez and Altieri, 2013). Tangible patrimony is defined as those assets that are measurable, that people can touch. Intangible patrimony is the assets that are not able to be touched and which are difficult to clarify and describe (Calvo *et al*., 2017).

Tangible Patrimony

Economic Heritage and Patrimony

Clearly, this heritage refers to monetary resources available for an individual, a family, and for the society. The discussion about this issue has been carried out in two different ways. First, we analyze the origin of these funds and how they have been earned. Then, we analyze the way family/members in a household spend their money. Regarding this it is important to understand that having more income does not necessarily improve rural development (Gutierrez-Montes *et al*., 2009). Some examples of this are when the natural heritage or the environment are destroyed as a result of rural activities, or when these economic resources are the result of child labor, which impacts the social and cultural heritage. Regarding resources and the way they are spent, it is important to highlight that earning more money does not necessarily mean that the quality of life is going to improve. A household could increase its income but if the family's head spends money on alcohol consumption instead of on other aspects, such as education, rural development will not be achieved (Schultz *et al*., 2002).

In rural territories, pluriactivity has become critical. Essentially, pluriactivity in economic heritage and patrimony is understood as alternative ways to earn money for the

household. Pluriactivity can improve post-harvest activities, which add value to products and create different modes to commercialize these products (Pachón *et al*., 2016).

Monetary resources become indispensable when they are used as a way to strengthen other heritages, such as physical or human heritages. For instance, physical heritages are enhanced when the funds are spent to improve households (better floors, restrooms, and ceilings, among other things). Another example is when the funds are used as part of collective action to improve post-harvest infrastructure. Human heritage is strengthened when these funds are spent to improve education for children, healthcare, among others (World Bank, 2000).

Physical Heritage and Patrimony

Physical heritage and patrimony are imperative for improving the level of rural development. However, they have not been attended to in public policies in many developing countries due to the implementation of neoliberal dogmas. According to the neoliberal perspective, many investments in rural infrastructure must be focused on capitalist agriculture to improve competitiveness (Kay, 2009). Physical heritage and patrimony are essential elements for improving the quality of life and ensuring the respect of the rights of rural populations. For instance, roads and bridges are vital since they create access to other communities and markets. Hence, roads belong to the physical heritage, as well as health centers, schools, bridges, clean water, electricity services, among other things (Shen *et al*., 2012).

Governments of several developing countries have abandoned the construction of adequate infrastructure. According to The Global Competitiveness Report 2014-2015, the countries with the worst infrastructure are in Africa and Asia. Latin American countries, in general, are in the middle of the ranking (Corrigan *et al*., 2014). Besides the differences between developed and developing countries, the differences between rural and urban areas are significant because the preferences for investment are always prioritized for urban zones due to the population impacts.

We must also take into consideration the household infrastructure. In other words, the infrastructure that directly affects the quality of life for rural families is related to their homes, for example, access to clean water or restrooms. This aspect is narrowly related to economic heritage and patrimony because the individual use of the household incomes could improve household infrastructures (Shen *et al*., 2012).

Natural Heritage and Patrimony

Natural heritage and patrimony refer to biological resources. Some examples are water resources, landscape and land. Water sources include lakes, rivers, canals, and ponds. Landscapes consist of mountains, hills, plateaus and highlands. Finally, land comprises soil, alluvium and clay. It also includes biodiversity such as insects, birds, frogs, fish, flowers, plants, seeds, and trees as well as genetic resources and ecosystems. Weather is also taken into account through sun, rain, wind, air, and snow. Most human actions have severely damaged all these resources (Sun *et al*., 2019). This negative influence on natural patrimony has developed irreversible harm that currently impacts all of humanity.

We rely on the peasantry to manage all these shared resources and to use them based on ancestral knowledge. However, productive pressure and current policies do not support sustainable management. Recovering traditional ways to utilize these common resources will be beneficial for everyone. Natural heritage and patrimony managed with the ancestral knowledge of the peasantry could be a viable alternative for producing food for all humanity and for mitigating many effects of climatic change (Pachón *et al*., 2016).

Intangible Patrimony

Cultural Heritage and Patrimony

Cultural heritage and patrimony are centered on identity but more importantly on creativity. This patrimony is reliant on acting according to traditions. Of course, spiritual and religious practices, as part of the connection with the world, belong to this patrimony (Desmarais, 2002). Unfortunately, neglectful policies have placed priority on commercial production, opposed to peasant activities. Examples of this kind of cultural heritage are the traditional communal labor or 'minga', terrace farming, ancestral forms of cropping as polyculture, ancestral pest control, and the barter system. In many places, these practices have been a means of survival for the peasantry (Declaration of Nyéléni, 2007). However, government policies, research preferences or non-governmental organizational practices, and cultural 'capitals' from hegemony groups have been privileged over the traditions of the peasantry (Flora *et al*., 2015).

Human Heritage and Patrimony

Human heritage and patrimony could be described as the traditional knowledge of local people and the communities

to which they belong. Education, formal and informal, is possibly the best means for the construction of human heritage. As a result of instruction and experience, people and their communities obtain "know-how", skills, and abilities. Therefore, they obtain new ways to address problems (Crawshaw *et al*., 2014). Traditional knowledge is perhaps one of the most important human patrimonies, especially in rural areas, even though it has not been adequately valued in many places. However, it is essential to understand that people cannot acquire this knowledge in schools and universities (Patel, 2009). Without a doubt, human heritage and patrimony must be transferred through tradition, which needs to be taught through formal and informal education to children and adults alike.

Social Heritage and Patrimony

Social heritage and patrimony dictate belonging to a society and the ways of interacting inside that society. Many relationships build roads that establish and strengthen social collaboration. Committed relationships are the cornerstone of social patrimony. We know that trust is fundamental for creating real participation in social networks, such as communal organizations. These organizations must generate collective actions for consolidating cooperation, improving the quality of the rural life, and ensuring respect for their rights, besides pursuing individual benefits (Dormaels, 2012).

Institutional Heritage and Patrimony

The institutional heritage can be understood as the net of formal and informal institutions and stakeholders that interact in rural areas. It also takes into account the rules that they develop, agree upon, and implement for regulating access to power and resources. Of course, these rules contribute towards improving the quality of life, and hence, they lead to rural development, by providing equitable participation for all the stakeholders involved, but primarily for those who have been traditionally excluded (Kay, 2009; Pachón *et al*., 2016).

These kinds of arrangements, which many times are informal, can be carried out through the involvement and empowerment of the stakeholders. Empowerment is the result of the interaction of all heritages and patrimonies described above. This interaction maintains a virtuous circle that ensures the improvement of the other heritages, while at the same time creates the ability to improve the quality of life through respect for the rights of rural inhabitants.

Heritages and patrimonies can also be analyzed from an economic/sociological point of view (Leibenstein, 1984; Biggart and Beamish, 2003). Sometimes, institutional arrangements between different stakeholders have been constructed by custom or tradition. These habits, routines, or conventions become part of the everyday practices and ways of life for the entire community, which must be adopted as part of normal behavior. In many cases, conventions correspond to the prevailing political-economic model. However, some of these habits play out in unusual ways, meaning that these conversations can become an alternative for many rural inhabitants.

Quality of life and respect for human rights

The final key point and main goal for rural development is quality of life and respect for human rights of the rural population, which is its simplest definition. Since there is great academic discussion over the definition of quality of life and human rights, for this discussion we will use the human scale of development. Quality of life could be understood as the satisfaction of every fundamental human need. This will happen through the increase of self-reliance and the articulation of different levels among populations: the environment, technology, globalization and local processes, individuality and community. Of course, the primary focus is on people, because fundamental human needs are measured through people's involvement, prioritizing both autonomy and diversity. It aims to transform people, who are often perceived as an object, into actors of development. Participatory democracy, constructed from the bottom up, stimulates real solutions for real problems, which can satisfy all fundamental human needs (Max-Neef *et al*., 1994).

To sum up, the peasantry must combine all their heritages and patrimonies with the purpose of improving the quality of life and ensuring that their rights are respected. The interaction of heritages creates the conditions under which the peasantry will be able to identify and satisfy their own fundamental human needs. This construction must take into consideration their beliefs, ideas, and meanings in order to better satisfy all fundamental human needs. This means that the peasantry must internally identify its needs according to the particular circumstances of each community. This concern is paramount because the generalization of problems and solutions has shown poor results in many rural places (Pachón *et al*., 2017a; 2017b)

Conclusions

Rural development has many characteristics of 'wicked problems', which is why we have evaluated and examined it from different viewpoints. As a result, stakeholders often complain or disagree about the proposed alternatives. That

is why this paper considers all stakeholders' interests in rural matters. The current analytical framework, based on the idea of the heritages and patrimonies that peasantry hold, suggests a path where all heritages interact and, thereby, helps us achieve a better level of rural development.

The heritages and patrimonies of the rural small farmer interact inside the rural households, among rural families, and, finally, in rural territories. In all cases, the stakeholders must take possession of these heritages, mobilizing all their knowledge and traditions. In turn, it is important that society, as a whole, recognizes the importance of the peasantry and their heritages. When that recognition happens, reaching satisfactory rural development will be possible for all rural inhabitants.

However, the analytical framework of the heritages and patrimonies of the peasantry still has gaps to be filled. It is necessary to propose a methodology that validates the framework and measures the level of these patrimonies. The analytical framework requires some examples for the application of these indicators in rural territories with rural families. Regarding this concern, a question must be asked: What indicators can be used to measure the level of these heritages? Finally, we must ask: Do public policies allow the improvement of heritages and patrimonies? We also must take into account the involvement of all rural stakeholders while trying to tackle these concerns.

Literature cited

Anderson, J. 2003. Risk in rural development: challenges for managers and policy makers. Agricult. Syst. 75(2-3), 161-197. Doi: 10.1016/S0308-521X(02)00064-1

Andersson, C. and P. Törnberg. 2018. Wickedness and the anatomy of complexity. Futures 95, 118-138. Doi: 10.1016/j.futures.2017.11.001

Barker, D. 2007. The rise and predictable fall of globalized industrial agriculture. A report from the International Forum on Globalization. San Francisco, USA.

Bebbington, A. 1999. Capitals and capabilities: a framework for analyzing peasant viability, rural livelihoods and poverty. World Dev. 27(12), 2021-2044. Doi: 10.1016/S0305-750X(99)00104-7

Biggart, N.W. and T.D. Beamish. 2003. The economic sociology of conventions: habit, custom, practice, and routine in market order. Annu. Rev. Sociol. 29. Doi: 10.1146/annurev.soc.29.010202.100051

Bitsch, V. 2009. Grounded Theory: a research approach to wicked problems in agricultural economics. Mini-symposium qualitative Agricultural Economics at the International Conference of Agricultural Economists. 2009, August 16-22, Beijing, China.

Borras Jr., S.M. 2009. Agrarian change and peasant studies: changes, continuities and challenges-an introduction. J. Peasant Stud. 36(1), 5-31. Doi: 10.1080/03066150902820297

Brass, T. 2002. Latin American peasants - new paradigms for old? J. Peasant Stud. 29(3,4), 1-40. Doi: 10.1080/03066150412331311019c

Brown, P.H. and A. Park. 2002. Education and poverty in rural China. Econ. Edu. Rev. 21(6), 523-541. Doi: 10.1016/S0272-7757(01)00040-1

Brugue, Q., R. Canal, and P. Paya. 2015. Managerial intelligence to address "wicked problems": the case of interdepartmental committees. Gestion y Política Pública 24(1), 85-130.

Calvo, I., O. Petit, and F. Vivien. 2017. Common patrimony: a concept to analyze collective natural resource management. The case of water management in France. Ecol. Econ. 137, 126-132. Doi: 10.1016/j.ecolecon.2017.02.028

Came, H. and D. Griffith. 2018. Tackling racism as a "wicked" public health problem: enabling allies in anti-racism praxis. Soc. Sci. Med. 199, 181-188. Doi: 10.1016/j.socscimed.2017.03.028

Castro-Arce, K. and F. Vanclay. 2019. Transformative social innovation for sustainable rural development: an analytical framework to assist community-based initiatives. J. Rural Stud. Doi: 10.1016/j.jrurstud.2019.11.010

Chambers, R. 1983. Rural development: putting the last first. Routledge, London. Doi: 10.4324/9781315835815

Chambers, R. and G. Conway. 1992. Sustainable rural livelihoods: practical concepts for the 21st century. Discussion paper no. 296. Institute of Development Studies, UK.

Cominelli, F. and X. Greffe. 2012. Intangible cultural heritage: safeguarding for creativity. Convention for the safeguarding of the intangible cultural Heritage City Cult. Soc. 3(4), 245-250. Doi: 10.1016/j.ccs.2012.10.003

Corrigan, G., R. Crotti, M. Hanouz, and C. Serin. 2014. Assessing Progress toward Sustainable Competitiveness. pp. 53-84. In: Schwab, K. (ed.). The Global Competitiveness Report 2014-2015. World Economic Forum, Geneva.

Crawshaw, L., S. Fèvre, L. Kaesombath, B. Sivilai, S. Boulom, and F. Southammavong. 2014. Lessons from an integrated community health education initiative in rural Laos. World Dev. 64, 487-502. Doi: 10.1016/j.worlddev.2014.06.024

Criado-Boado, F. and D. Barreiro. 2013. El patrimonio era otra cosa. Estudios atacameños - Arqueología y antropología surandinas (45), 5-18. Doi: 10.4067/S0718-10432013000100002

Declaration of Nyeleni. 2007. Chain Reaction 100, 16.

Delgado, F. and S. Rist. 2011. La transdisciplinariedad y la investigación participativa en una perspectiva de diálogo intercultural e intercientífico. Working document, AGRUCO/CAPTURED, La Paz.

Dentoni, D. and V. Bitzer. 2015. The role(s) of universities in dealing with global wicked problems through multi-stakeholder initiatives. J. Clean. Prod. 106, 68-78. Doi: 10.1016/j.jclepro.2014.09.050

Desmarais, A. 2002. The vía campesina: consolidating an international peasant and farm movement. J. Peasant Stud. 29(2), 91-124. Doi: 10.1080/714003943

de Sousa Santos, B. 1993. Modernidade, Identidade a Cultura de Fronteira. Tempo Soc. USP 5(1-2),31-52. Doi: 10.1590/ts.v5i1/2.84940

Dewey, J. 1938. Unity of science as a social problem. pp. 29-38. In: International Encyclopedia of Unified Science, vol. 1 (1). University of Chicago Press, Chicago, USA.

Dirven, M., R. Echeverri-Perico, C. Sabalain, A. Rodríguez, D. Candia-Baeza, C. Peña, and S. Faiguenbaum. 2011. Hacia una nueva definición de "rural" con fines estadísticos en América Latina. Comisión Económica para América Latina CEPAL, Santiago de Chile.

Dormaels, M. 2012. Identidad, comunidades y patrimonio local: una nueva legitimidad social. Alteridades 22(43), 9-19.

Dutta, K. 2018. Solving wicked problems: searching for the critical cognitive trait. Int. J. Manage. Educ. 16(3), 493-503. Doi: 10.1016/j.ijme.2018.09.002

Echeverri, R. 2011. Reflexiones sobre lo rural: economía rural, economía de territorios. Hacia una nueva definición de "rural" con fines estadísticos en América Latina. Comisión Económica para América Latina CEPAL, Santiago de Chile.

Elia, G. and A. Margherita. 2018. Can we solve wicked problems? a conceptual framework and a collective intelligence system to support problem analysis and solution design for complex social issues. Technol. Forecast. Soc. Change 133, 279-286. Doi: 10.1016/j.techfore.2018.03.010

Ellis, F. and S. Biggs. 2001. Evolving themes in rural development 1950s-2000s. Dev. Policy Rev. 19(4), 448. Doi: 10.1111/1467-7679.00143

Espina, M.P. 2007. Complejidad, transdisciplina y metodología de la investigación social. Utopía y Praxis Latinoamericana 12(38), 29-43.

Farrell, R. and C. Hooker. 2013. Design, science and wicked problems. Des. Stud. 34(6), 681-705. Doi: 10.1016/j.destud.2013.05.001

Flora, C.B., J. Flora, and S. Gasteyer. 2015. Rural communities: legacy and change. Westview Press, Boulder, United States.

Florian, V. 2012. Territorial Innovation Strategies. Agricultural Economics and Rural Development 9(1), 47-60.

Gutierrez-Montes, I., M. Emery, and E. Fernández-Baca. 2009. The sustainable livelihoods approach and the community capitals framework: the importance of system-level approaches to community change efforts. Community Dev. J. 40(2), 106-113. Doi: 10.1080/15575330903011785

Holt-Giménez, E. and M. Altieri. 2013. Agroecology, food sovereignty, and the new green revolution. Agroecol. Sust. Food 37(1), 90-102.

Kay, C. 2009. Development strategies and rural development: exploring synergies, eradicating poverty. J. Peasant Stud. 36(1), 103-137. Doi: 10.1080/03066150902820339

Kolko, J. 2011. Wicked problems. pp. 96-111. In: Kolko, J. (ed.). Thoughts on interaction design. Doi: 10.1016/B978-0-12-380930-8.50007-3

Kolstad, A. 2012. Inter-functionality between mind, biology and culture: some epistemological issues concerning human psychological development. pp. 19-41. In: Seidl-De-Moura M.L. (ed.). Human development - Different perspectives. IntechOpen, London. Doi: 10.5772/2272

Gold, S., J. Muthuri, and G. Reiner. 2018. Collective action for tackling "wicked" social problems: a system dynamics model for corporate community involvement. J. Clean. Prod. 179, 662-673. Doi: 10.1016/j.jclepro.2017.11.197

Gharehgozli, A.H., J. Mileski, A. Adams, and W. von Zharen. 2017. Evaluating a "wicked problem": a conceptual framework on seaport resiliency in the event of weather disruptions. Technol. Forecast. Soc. Change 121, 65-75. Doi: 10.1016/j.techfore.2016.11.006

Gustafsson, B. and S. Li. 2004. Expenditures on education and health care and poverty in rural China. China Econ. Rev. 15(3), 292-301. Doi: 10.1016/j.chieco.2003.07.004

Head, B.W. and J. Alford. 2015. Wicked problems: implications for public policy and management. Adm. Soc. 47(6), 711-739. Doi: 10.1177/0095399713481601

Henriksen, D. 2016. The seven transdisciplinary habits of mind of creative teachers: an exploratory study of award-winning teachers. Think. Skills Creativity 22, 212-232. Doi: 10.1016/j.tsc.2016.10.007

Innes, J.E. and D.E. Booher. 2016. Collaborative rationality as a strategy for working with wicked problems. Landscape Urban Plan. 154, 8-10. Doi: 10.1016/j.landurbplan.2016.03.016

Jouini, M., J. Burte, Y. Biard, N. Benaissa, H. Amara, and C. Sinfort. 2019. A framework for coupling a participatory approach and life cycle assessment for public decision-making in rural territory management. Sci. Total Environ. 655, 1017-1027. Doi: 10.1016/j.scitotenv.2018.11.269

König, B., K. Diehl, K. Tscherning, and K. Helming. 2013. A framework for structuring interdisciplinary research management. Research Policy 42(1), 261-272. Doi: 10.1016/j.respol.2012.05.006

Kuhmonen, T. 2018. Systems view of future of wicked problems to be addressed by the common agricultural policy. Land Use Policy 77, 683-695. Doi: 10.1016/j.landusepol.2018.06.004

Leibenstein, H. 1984. On the economics of conventions and institutions: an exploratory essay. J. Inst. Theor. Econ. 74-86.

Leverenz, C.S. 2014. Design thinking and the wicked problem of teaching writing. Computers and Composition 33, 1-12. Doi. 10.1016/j.compcom.2014.07.001

Littaye, A. 2016. The multifunctionality of heritage food: the example of pinole, a Mexican sweet. Geoforum 76, 11-19. Doi: 10.1016/j.geoforum.2016.08.008

Luckey, D.S. and K.P. Schultz. 2001. Defining and coping with wicked problems: the case of Fort Ord building removal. MSc thesis, Naval Postgraduate School, Monterey, USA.

Max-Neef, M., A. Elizalde, and M. Hopenhayn. 1994. Desarrollo a escala humana: conceptos, aplicaciones y algunas reflexiones. Vol. 66. Icaria Editorial, Barcelona, Spain.

McKee, A., M. Guimarães, and T. Pinto-Correia. 2015. Social capital accumulation and the role of the researcher: an example of a transdisciplinary visioning process for the future of agriculture in Europe. Environ. Sci. Policy 50, 88-99. Doi: 10.1016/j.envsci.2015.02.006

Miguélez, M. 2009. Hacia una epistemología de la complejidad y transdisciplinariedad. Utopía y Praxis Latinoamericana 14(46), 11-31

Millar, M. 2013. Interdisciplinary research and the early career: the effect of interdisciplinary dissertation research on career placement and publication productivity of doctoral graduates in the sciences. Res. Policy 42(5), 1152-1164. Doi: 10.1016/j.respol.2013.02.004

Molina, J.P. 2010. Keys for rural territorial development. Agron. Colomb. 28(3), 421-427.

Norris, P.E., M. O'Rourke, A.S. Mayer, and K.E. Halvorsen. 2016. Managing the wicked problem of transdisciplinary team formation in socio-ecological systems. Landscape Urban Plan. 154, 115-122. Doi: 10.1016/j.landurbplan.2016.01.008

Olivé, L. 2011. Interdisciplina y transdisciplina desde la filosofía. Ludus Vitalis, XIX(35), 251-256.

Pacanowsky, M. 1995. Team tools for wicked problems. Organ. Dyn. 23(3), 36-51. Doi: 10.1016/0090-2616(95)90024-1

Pachón, F. 2013. Food sovereignty and rural development: beyond food security. Agron. Colomb. 31, 362-377.

Pachón, F., W. Bokelmann, and C. Ramirez. 2016. Rural development thinking, moving from the green revolution to food sovereignty. Agron. Colomb. 34(2), 267-276. Doi: 10.15446/agron.colomb.v34n2.56639

Pachón, F., W. Bokelmann, and C. Miranda. 2017a. Heritage and patrimony of the peasantry framework to address rural development and its application in Colombia. Acta Agron. 66, 347-359. Doi: 10.15446/acag.v66n3.60949

Pachón, F., W. Bokelmann, and C. Miranda. 2017b. Heritage and patrimony of the peasantry framework and rural development indicators in rural communities in Mexico. Rev. Econ. Sociol. Rural 55, 199-226. Doi: 10.1590/1234-56781806-94790550201

Parrado, A. and J. Molina. 2014. Mercados campesinos: modelo de acceso a mercados y seguridad alimentaria en la región central de Colombia. Oxfam, Bogota.

Patel, R. 2009. Food sovereignty. J. Peasant Stud. 36(3), 663-706. Doi: 10.1080/03066150903143079

Probst, G. and A. Bassi. 2014. Tackling complexity: a systemic approach for decision makers. Greenleaf publishing, Sheffield, UK.

Raasch, C., V. Lee, S. Spaeth, and C. Herstatt. 2013. The rise and fall of interdisciplinary research: the case of open source innovation. Res. Policy 42(5), 1138-1151. Doi: 10.1016/j.respol.2013.01.010

Rittel, H.W. and M. Webber. 1973. Dilemmas in a general theory of planning. Policy sciences 4(2), 155-169. Doi: 10.1007/BF01405730

Roberts, N. 2000. Wicked problems and network approaches to resolution. Int. Public Manage. Rev. 1(1), 1-19.

Roberts, N. 2012. Tackling wicked problems in Indonesia: a bottom-up design approach to reducing crime and corruption. Naval Postgraduate School, Monterey, USA.

Rosset, P. 2003. Food sovereignty: global rallying cry of farmer movements. Food First Backgrounder 9(4), 1-4.

Schejtman, A. and J. Berdegué. 2003. Desarrollo territorial rural. RIMISP, Santiago.

Schultz, S., S. Arndt, G. Lutz, A. Petersen, and C. Turvey. 2002. Alcohol use among older persons in a rural state. Am. J. Geriatr. Psychiatry 10(6), 750-753. Doi: 10.1097/00019442-200211000-00014

Scoones, I. 2015. Sustainable rural livelihoods and rural development. Fernwood Publishing, Winnipeg, Canada. Doi: 10.2458/v23i1.20254

Shen, L., S, Jiang, and H, Yuan. 2012. Critical indicators for assessing the contribution of infrastructure projects to coordinated urban-rural development in China. Habitat Int. 36(2), 246. Doi: 10.1016/j.habitatint.2011.10.003

Sun, W., X. Xu, Z. Lv, H. Mao, and J. Wu. 2019. Environmental impact assessment of wastewater discharge with multi-pollutants from iron and steel industry. J. Environ. Manage. 245, 210-215. Doi: 10.1016/j.jenvman.2019.05.081

Tietjen, A. and G. Jørgensen. 2016. Translating a wicked problem: a strategic planning approach to rural shrinkage in Denmark. Special Issue Working with wicked problems in socio-ecological systems: more awareness, greater acceptance, and better adaptation. Landsc. Urban Plan. 154, 29-43. Doi: 10.1016/j.landurbplan.2016.01.009

van Rijnsoever, F.J. and L. Hessels. 2011. Factors associated with disciplinary and interdisciplinary research collaboration. Res. Policy 40(3), 463-472. Doi: 10.1016/j.respol.2010.11.001

Wittman, H., A.A. Desmarais, and N. Wiebe. 2010. The origins and potential of food sovereignty. pp. 1-14. In: Wittman, H.K., A.A. Desmarais, and N. Wiebe (eds.). Food sovereignty: Reconnecting food, nature and community. Pambazuka, Oxford, United Kingdom.

World Bank. 2000. Rural Development Indicators Handbook. Washington, D.C.

Xiang, W.N. 2013. Working with wicked problems in socio-ecological systems: awareness, acceptance, and adaptation. Landsc. Urban Plan. 110, 1-4. Doi: 10.1016/j.landurbplan.2012.11.006

Zemelman, H. 2001. Pensar teórico y pensar epistémico: los retos de las ciencias sociales latinoamericanas. Instituto Pensamiento y Cultura en América Latina, A.C. (IPECAL), Mexico.

Zijp, M.C., L. Posthuma, A. Wintersen, J. Devilee, and F.A. Swartjes. 2016. Definition and use of solution-focused sustainability assessment: a novel approach to generate, explore and decide on sustainable solutions for wicked problems. Environ. Int. 91, 319-331. Doi: 10.1016/j.envint.2016.03.006

Value chain analysis of panela production in Utica, Colombia and alternatives for improving its practices

Análisis de la cadena de valor de la panela en Útica, Colombia y alternativas para mejorar sus prácticas

Natalia Martínez Zarate[1], Wolfgang Bokelmann[2], and Fabio Alberto Pachón-Ariza[3]*

ABSTRACT

Panela is mainly produced by small farmers. It is one of the most relevant agroindustries in Colombia. Traditional processing in all production phases is the main characteristic of this product. This research aimed to identify alternatives for panela farmers in the municipality of Utica (Colombia) and to improve their agricultural practices and manufacturing methods. This should help gain better access to markets while making production economically, environmentally, and socially sustainable. Face to face interviews with different stakeholders as well as an in-depth analysis of different scopes were used to identify problems of the value chain. Low incomes, environmental degradation, and lack of organization were the central issues identified. Nevertheless, traditional crop practices could be a strong argument for gaining a place in the organic food market.

Key words: food supply chain, peasantry, agricultural production, agricultural practices, jaggery.

RESUMEN

La panela es producida principalmente por pequeños agricultores y es una de las agroindustrias más importantes en Colombia. El procesamiento tradicional en todas sus fases de producción es la característica principal de este producto. La presente investigación tuvo como objetivo identificar alternativas para que los productores de panela en el municipio de Útica (Colombia) mejoren sus prácticas agrícolas y el método de fabricación, con el fin de obtener un mejor acceso a los mercados y hacer que el proceso sea sostenible desde los puntos de vista económico, ambiental y social. Se utilizaron entrevistas con diferentes actores de la cadena, así como un análisis a profundidad en diferentes ámbitos, para identificar los problemas de la cadena de valor. Los bajos ingresos, la degradación ambiental y la falta de organización fueron los problemas centrales identificados. Sin embargo, las prácticas tradicionales de cultivo podrían ser una fortaleza para ganar un lugar en el mercado de alimentos orgánicos.

Palabras clave: cadenas de abastecimiento alimentario, campesinado, producción agrícola, prácticas agrícolas, azúcar de caña.

Introduction

Panela is a traditional peasant product (Jaffé, 2015), whose production and consumption are widely distributed nationwide in 28 provinces, according to the Ministry of Agriculture and Rural Development (MADR, 2019). The province of Cundinamarca has 62,134 ha dedicated to panela production. This area corresponds to 22.64% of the total agricultural area of the province (Secretaria de Agricultura y Desarrollo Rural - Cundinamarca, 2015). Utica is one of the municipalities of Cundinamarca where panela production is an important economic activity, carried out by 272 farmers owning 165 "enramadas" or "trapiches" (Administración Municipal Útica - Cundinamarca, 2012). An "enramada" or "trapiche" is the place where mills are located and sugarcane juice is extracted. As of August 2019, Utica had 3,017 ha cultivated with sugarcane. This produced an average of 16,401 t of panela and was the fifth most productive municipality in the province and the 47th in the country (MADR, 2019).

More than 80% of the panela farmers in these areas are small and medium producers. Sugarcane is one of the crops that support their economy. In addition, panela has high cultural importance in Colombia as an energy source that is mainly consumed by low-income people. However, nowadays its consumption by individuals with greater purchasing power is increasing because of its benefits. Panela

Received for publication: 8 April, 2019. Accepted for publication: 3 December, 2019 Doi: 10.15446/agron.colomb.v37n3.78967

[1] Agronomist, MSc. Integrated Natural Resources Management.
[2] Department of Agricultural Economics, Economics of Horticultural Production, Humboldt Universität zu Berlin, Berlin (Germany).
[3] Department of Agri-food and Rural Development, Faculty of Agricultural Sciences, Universidad Nacional de Colombia, Bogota (Colombia).
* Corresponding author: fapachona@unal.edu.co

has not had an important significance in the international market beyond the producing countries (Rao *et al*., 2007); nevertheless, it has gained recognition worldwide due to its nutritional properties, which have allowed the opening of new markets in different countries.

Due to the importance of panela in Colombia, this agroindustry has been prioritized by the national research agenda. According to Martínez (2013), the key points in such a program are technological, in order to overcome critical factors in the topics that affect competitiveness. These topics are first, sanitary and phytosanitary control; second, the innovation of inputs and products; third, integral crop management; and last, postharvest and transformation.

Agreeing on the research program, the panela farmers' guild and the local governments in producer areas have joined efforts to improve productivity, product quality, and supply and to promote the consumption of panela in national and international markets. Improvement of access to markets is a key factor that will undoubtedly support small farmers because it could be a way to improve their incomes, and hence, have better conditions for producing panela of higher quality (Gereffi and Lee, 2012).

Given this context, it is essential to understand and analyze the production and marketing perspectives that panela producers have and also to recognize the potential problems, benefits, and obstacles faced in the market to be competent and keep themselves stable economically. In this scenario, value chain, global value chain, supply chain, marketing chain, or distribution chain have been popular in market analysis. Webber and Labaste (2009) consider that all these terms are used depending on the product or the target market analyzed. However, all of them describe the steps, processes and interactions required to obtain diverse products, from cultivation or manufacture in the case of food, to the moment when they reach the final consumer.

Kaplinsky and Morris (2001) define value chain as a sequence of all the activities and their connections involved in the whole process of obtaining a product or service from the production, processing, marketing, and distribution to the final consumer. In other words, Hellin and Meijer (2006) describe the concept as a full range of activities that firms and workers perform to bring a product from its conception to its end use and beyond. Among these activities, the design, production, marketing, distribution and support to the final consumer are all considered.

Since the early 1990s, the term "global value chain analysis" has appeared as a tool for understanding the dynamics of international trade and economic globalization. This approach focuses on examining the structure, actors, and dynamics of the chain. The analysis includes the identification of the role of the participants involved in the value chain, as well as the relationships between them. Additionally, it includes knowing the structure of the rewards, the allocation of the added value, and the role of norms. It also allows identifying whether such a structure facilitates or obstructs the participation of all the stakeholders. In addition, different authors and research on the value chain have focused their attention on the impacts that it has on improving the conditions of livelihood of the population characterized by poverty, vulnerability (women and children), and environmental susceptibility. That is why most of the poor people around the world live in rural areas in developing countries, and most of them engage in agricultural production as a unique or primary source of income (Riisgaard *et al*., 2010).

Value chain analysis includes all vertical links described by Dunn (2014) as a commercial relationship in bringing the product up through the value chain. In other words, it includes all the relationships at different levels between buyers and suppliers involved in the passage of goods or services from production to consumption (Riisgaard *et al*., 2010; McKague and Siddiquee, 2014). Furthermore, it includes the horizontal links that are the relationships between enterprises at the same level. These relationships include other value chains that have a connection in the provision of some goods or services and that compete or help to reduce transaction costs and access to information, increase cost-effective access to inputs and services, and empower small firms (Dunn, 2014; McKague and Siddiquee, 2014). Webber and Labaste (2009) highlight the fact that value chain analysis must include an emphasis on value creation through the innovation of products, processes, and marketing strategies.

Value chain dimensions vary depending on the authors or their influences and study objectives. According to Schneemann and Vredeveld (2015), there are four dimensions: economic, social, environmental, and institutional. These are interconnected and allow an improvement in the quality and growth of the chain. The primary challenge in value chain analysis is to identify and propose alternatives for achieving sustainable growth for all the stakeholders in the previously mentioned dimensions (Faße *et al*., 2009).

The case of panela production in Utica, Colombia is an attractive example of analysis because it is the main cash crop for farmers and is the principal source of income for almost all the population. That is why panela production plays a significant role in the livelihoods of the majority of the inhabitants of the town. Given this context and knowing the conditions of panela production in the municipality, the objective of this research was to identify the alternatives for farmers in Utica to improve the agricultural practices of the sugarcane crop and the method of manufacturing panela to gain better access to markets while making the process sustainable.

Materials and methods

Location

This research was performed in the municipality of Utica, located in the northwest of the province of Cundinamarca, in the Gualiva region (5.1878° N, 74.4815° W). The area has a mean temperature of 26°C and an altitude that varies between 400 and 1,600 m a.s.l.). The location is strategic since it is situated just a few hours from Bogota, the capital of the country. However, the road that leads from Utica to the highway to Bogota is in poor condition, and more than 60% of the route is unpaved (Alcaldía de Útica - Cundinamarca, 2016).

Statistical analysis

The current study was organized in three phases. First, the characterization of panela farmers was undertaken. Second, the role and actions of the local political stakeholders were defined. Finally, in the third phase, the information gathered was analyzed using the software ATLAS.ti, a workbench for the qualitative analysis of large bodies of textual, graphical, audio and video data. The interactions of the previously mentioned dimensions were discussed in order to achieve sustainable growth for all stakeholders.

In order to characterize the farmers, semi-structured interviews (Supplementary Material 1) were carried out to identify the most important facts of the value chain. Since the total number of panela farmers was 272, the statistical sample size was calculated using the following formula (Becerra *et al*., 2011):

$$n = \frac{p^{*}q^{*}N}{e^{2}\,(N-1) + Z^{2}\,p{\times}q} \qquad (1)$$

where:

n = Sample size

N = Population size

Z = Confidence level

e = Margin of error

p = Prior judgment of the correct *P* value

The calculated sample size had to include at least 55 farmers. However, the current research surveyed 72 producers.

For the second phase of the study, the results obtained in the characterization were contrasted with other relevant actors using face-to-face interviews (Supplementary Material 1). One of these actors was the local government, in charge of coordinating the rules in the territory as well as providing an environment for producing sugarcane and commercializing panela. At the local level, the Mayor and the Planning Secretary of Utica, and at the national level, the advisor of the panela productive chain of the Colombian Ministry of Agriculture and Rural Development were interviewed. Also, we interviewed the legal representative of the Association of Agricultural Producers of Utica and Neighboring Municipalities (Asociación de Productores Agropecuarios de Útica y Municipios Vecinos ASPRUT) and 16 employees from different areas of the National Federation of Panela Producers (Federación Nacional de Productores de Panela FEDEPANELA), including the general manager as head of the political sphere, the director of the technical division, and the director of the marketing area (a division called COMERPANELA). We also included a member of the Colombian Institute for Technical Training (Servicio Nacional de Aprendizaje SENA) Villeta campus.

Results and discussion

General characterization of panela farmers in Utica

Some of the results of the general description of panela farmers in Utica (Supplementary Material 2) showed that they are divided into three groups: medium, small, and very small producers (this last one as the largest group). Panela production is a common tradition characterized by a significant number of family members, and the traditional knowledge of panela processing has been passed from generation to generation. Nevertheless, the level of formal education of the farmers is low with 80% reaching a level of primary school and only 4% with secondary education, and 10% with no formal education, although they were literate.

Most of the farmers owned from 1-5 ha. Additionally, 90% of sugarcane crops had been producing for more than 20 years while the remaining 10% had been producing for 10 - 20 years. The crop yields were far from an optimal level, mainly due to a lack of a consistent renovation process.

Farmers in the area engage in minimal crop management. Only 5% of the farmers had conducted soil analysis, preventing the organization of a plan to cover the needs of the harvest. No prior soil preparation was performed when crops were established.

The primary source of energy for operating the "trapiches" was the bagasse resulting from the first part of the process after extracting the sugarcane juice. However, many of the mills required other sources of energy since they are not self-sufficient and use only the bagasse produced. The old burners with low technology need more time to reach dehydration of the juice so that farmers must look for an alternative source of energy. In many cases, this is firewood obtained from their farms. The consequences of requiring additional use of firewood as a source of combustion have generated deforestation in many parts of the municipality. The farmers cut down native vegetation to plant species that serve as firewood for later use in the trapiches and to sow sugarcane to increase production. The consequences of panela production with regards to this issue have been described in other studies in Colombia (Ordoñez-Díaz and Rueda-Quiñónez, 2017).

Price fluctuation was described as a big problem for farmers. In the case of Utica, an ordinary family of four people (parents with two children) with an average of 3 ha of sugarcane obtains 2.16 USD per day per person. According to the World Bank (2015), a person that earns less than 2 USD per day is considered as poor. It means that the incomes derived from panela manufacturing in Utica are barely enough to stay above the poverty line.

Given this fact, it is not clear why panela farmers continue performing this non-profitable activity. The possible reason is that it is a traditional activity in the area, and hence, it is important for the farmers to continue doing the same activities that their parents did before. As sugar cane is a traditional crop, it is an activity they learned from their ancestors, and for most of them, it is the only thing that they know how to do (Bernal *et al*., 2016). When asking farmers about this issue, the answer was straightforward in most of the cases. They said that manufacturing panela was like having a money box in the house. When they had no money, they harvested the cane, regardless of its price and quality. With panela produced on Friday, they could get paid the next Sunday. With that money, they were able to cover all their needs.

Regarding the role and actions of the local political stakeholders, according to the Mayor and the Planning Secretary, the administrative staff focused their efforts on improving panela production. They clearly understand that panela production was the basis of the local economy. Nevertheless, they agreed that such efforts are sometimes lost for various reasons. First, because the smallholders most of the time prefer to work alone and show little commitment to working together with the government. Second, even though the government has the best will to solve problems, the bureaucracy and small budgets do not allow effective decision-making. And finally, the conjunction of the two previous concerns creates skepticism and an atmosphere of discontent between the government and the farmers. This diagnosis is similar to the analysis performed by Orjuela and Colmenares (2011).

An interview carried out with the President of the ASPRUT organization concluded that ASPRUT members look forward to accomplishing two main goals: first, that the member might obtain support from the government; and second, that the members have the best access to markets. Regarding governmental support, the members requested adequate training that would allow them to improve panela manufacturing and meet the current legislative standards. Concerning access to markets, the members wish to produce the best quality panela and to reach different markets. Managing this might allow the members to avoid retailers as much as possible and to obtain consumers directly, so as to receive a fair price for their product.

Based on the interview with the staff of FEDEPANELA and SENA, both institutions seek to support panela farmers, focusing their efforts on technical processes and marketing concerns. According to the interviewed, the likelihood of offering their services in Utica was low. The most relevant explanation was that because panela farmers in the town are not interested in such support. This was evident from the low rate of affiliation with FEDEPANELA, scarcely reaching 5% of the farmers. This was probably due to the fact that most of the farmers expressed apathy for the training programs. According to the interviewed SENA members, panela farmers argued that they were too old to learn new techniques, and they preferred continuing to do things as usual.

In the marketing field, FEDEPANELA has opened new markets at the national and international levels. However, one of the most critical obstacles for panela farmers reaching such markets is fulfilling the standards requested. Overcoming this challenge required the involvement of the different stakeholders.

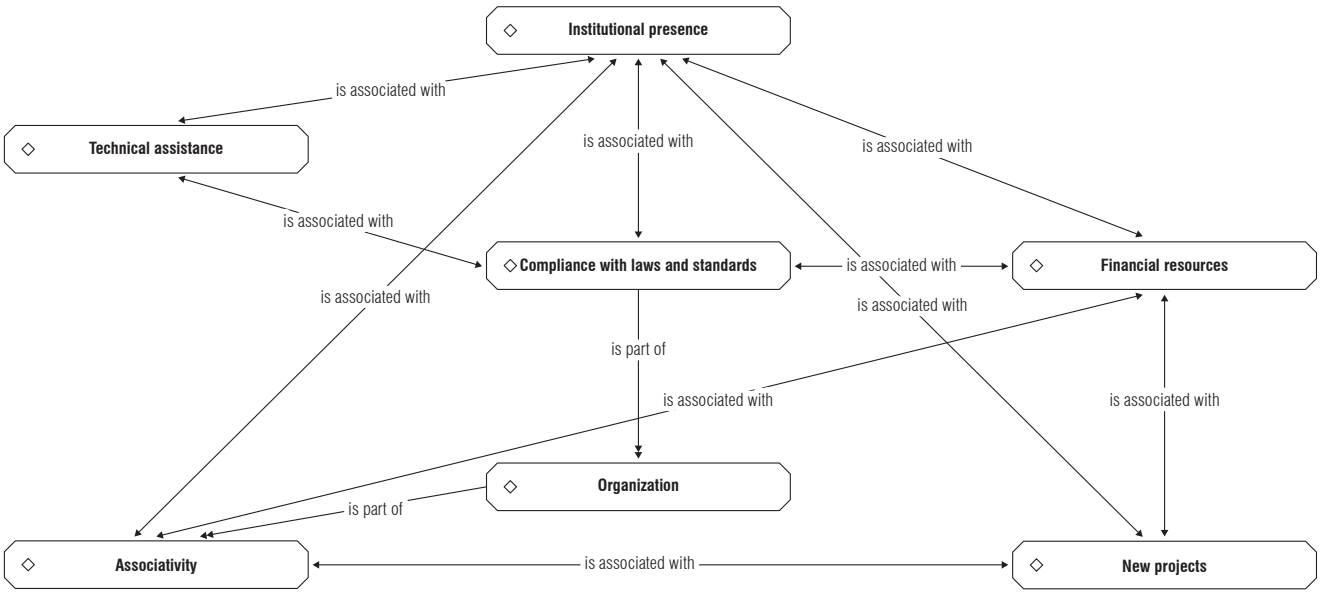


FIGURE 1. Institutional connections that support panela production in Utica, Colombia.

The most significant findings of the interviews are presented as follows. The information is organized into four different dimensions of the value chain: the institutional, environmental, social, and economic dimensions.

Institutional dimension

The institutional dimension could be understood as the axis that establishes the rules of the game in the value chain. It creates the environment regarding the laws and the coordination between the public and private sectors. However, regulation can sometimes be difficult to achieve for small farmers, so this becomes an obstacle for reaching the objectives of productive systems (Schneemann and Vredeveld, 2015). These results are shown in Figure 1.

As the leading actor in the institutional dimension, the government proposes laws and regulations. According to the Ministry of Health and Social Protection, the most important point to consider is that of sanitation. Regarding this, the Resolution 4121 of 2011 (Ministerio de Protección Social, 2011) was established to define the requirements for the production and commercialization of panela for human consumption. According to the standard the most significant changes that must be applied in the conventional mills are (1) the use of drinking water throughout the process, (2) the distribution of the mill with a sequential flow, clearly delimiting the areas for the different activities, and (3) the construction of the trapiche with easy-to-clean materials. These improvements involve significant investments that farmers are not willing to make, which is why the farmers continue manufacturing panela in conventional trapiches (Martínez, 2013).

Since the farmers do not comply with the regulations, they do not have the support of the institutions. Panela farmers have to comply with the requirements of the standards if they want to commercialize a reliable product for human consumption supported by FEDEPANELA. Given this scenario, panela farmers that cannot fulfill the requirements have to commercialize their panela through conventional channels.

The participating stakeholders agreed that technical assistance was one of the reliable tools for improving and following up the panela productive system. First of all, technical assistance controls illegal practices carried out by some farmers. Some of these practices are the addition of dyes, saturated animal fats, bleach, white sugars or any other substance that changes panela quality while putting the health of consumers at risk. Second, technical assistance helps to identify particular problems in each area and seeks to solve them through training. Finally, it allows sharing the new technological findings in the panela productive sector and keeping the growers informed and updated (Franco *et al*., 2016).

Farmers recognize the importance of technical assistance, and some of them remark on its absence in their particular cases. Nevertheless, it is important to note that many of the farmers ignored recommendations and assistance because they are reluctant to change their traditional ways of manufacturing panela (Ordoñez *et al*., 2013). Despite the criticism of some stakeholders about the pertinence and quality of the technical support, all of them agree that it is

a crucial element for arriving at an improved value chain and finding alternatives for better access to the markets.

Environmental dimension

Environmental education is central to this dimension. The absence of an environmental conscience has triggered most of the problems that currently occur in panela production in Utica. Besides a lack of commitment from farmers on this issue, a lack of knowledge of the environmental impacts caused by production and the ways to solve or mitigate them are the principal challenges concerning environmental interests in Utica (Bernal *et al*., 2016). The main connections regarding environmental matters are emphasized in Figure 2.

The impact of modern agriculture on climate change is evident, especially because of some common practices of current livestock production and monoculture (Barker, 2007). However, traditional practices also have impacts on the environment. The degradation of natural resources is tangible in Utica, and deforestation in some "veredas" (scattered rural settlements) is evidence of it. This condition is one of the consequences of logging trees to establish new crops or to use as firewood as a source of energy for the mills and boilers in panela manufacturing (Rojas, 2011). The main impact of these practices is the elimination of biodiversity that alters the balance of the natural system in the region. Similarly, soil deterioration represented by the loss of fertility, erosion, and other consequences is the result of poor management of sugarcane crops.

There are several problems related to panela production affecting the environment: first, water pollution due to the lack of treatment of wastes generated during the production of panela; second, the air pollution produced by the practice of rubber burning. Though this practice is severely sanctioned, some farmers in Utica still use car tires as a common source of energy. Also, the management of solid and liquid waste, especially by small farmers and the use of firewood for the burners implies that extensive deforestation is an important environmental concern. One of the biggest problems is the fact that wood was still used for combustion for evaporating sugarcane juice, and some farmers do not respect the legislation regarding this issue. Concerns about contamination caused by the traditional practice of burning the soil's vegetation cover to establish new crops, especially maize, is also a serious problem. The process starts as a step carried out before sowing sugarcane. This practice pollutes air significantly and affects the quality of the land since it leaves it without cover. Regarding these issues, training focused on good agricultural practices of growing sugarcane and manufacturing panela should be made available to the farmers as a partial solution for some environmental problems.

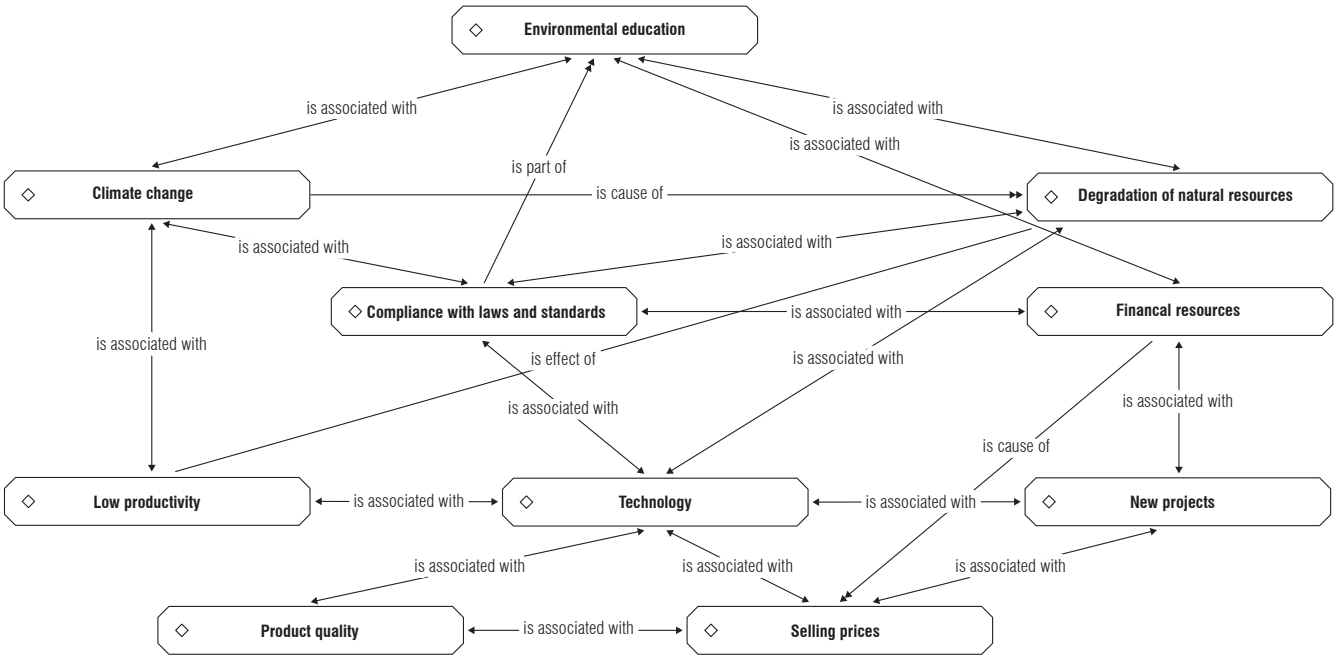


FIGURE 2. Environmental connections of panela production in Utica, Colombia.

Compliance with laws and regulations is a way to control the degradation of natural resources (Riisgaard *et al., 2010)*. The implementation of adequate technologies, especially better farming and manufacturing practices, will improve the quality and efficiency of the entire productive system. In this regard, panela farmers would be able to reduce production costs through the implementation of training projects to adopt new technologies. In this way, panela growers would make their processes more efficient and would become more environmentally friendly.

Regarding the benefits of being environmentally friendly, the implementation of Good Farming Practices (GFP) during sugarcane growing as well as Good Manufacturing Practices (GMP) in the trapiches would be useful to get the certifications for facilitating access to markets. Indeed, these kinds of good farming and manufacturing practices add value to the final product, because nowadays the consumers are willing to pay more if they realize that the product protects the environment and the wildlife.

Paradoxically, while panela manufacturing practices impact the environment, sugarcane crops are incredibly susceptible to the effects of climate change. In addition to the problems associated with the market, sugarcane crops have been severely impacted by the problems of climate variability in the last few years. Utica had a strong rainy season during 2011 and 2012 followed by extreme dry seasons during 2014 and 2015. Sugarcane crops have also been recently impacted by a strong El Niño (Southern Oscillation Cycle ENSO). Growers are becoming more sensitive to this problem. However, in their daily work, it is evident that the farmers continue doing their activities as usual.

Social dimension

The social dimension involves significant topics that go beyond agricultural production. Nevertheless, such topics are narrowly related to the economic activities carried out in the countryside. Equity, equality, the likelihood of participating, inclusiveness, access to productive and environmental resources or decent working conditions are clear examples of the topics related to this dimension. However, one of the most relevant aspects is the participation of women (Farah-Quijano, 2009). The connections among social aspects are highlighted in Figure 3.

For Alviar (2012), rural women are the pillar of the agribusiness since, without them, there would be no development in rural areas. Although the production of panela is considered a tough job, women have gained valuable spaces in the different stages of the process. Women can be more perceptive and have a greater degree of natural development so that the process can become more innovative. Three important aspects can be highlighted from these qualities:

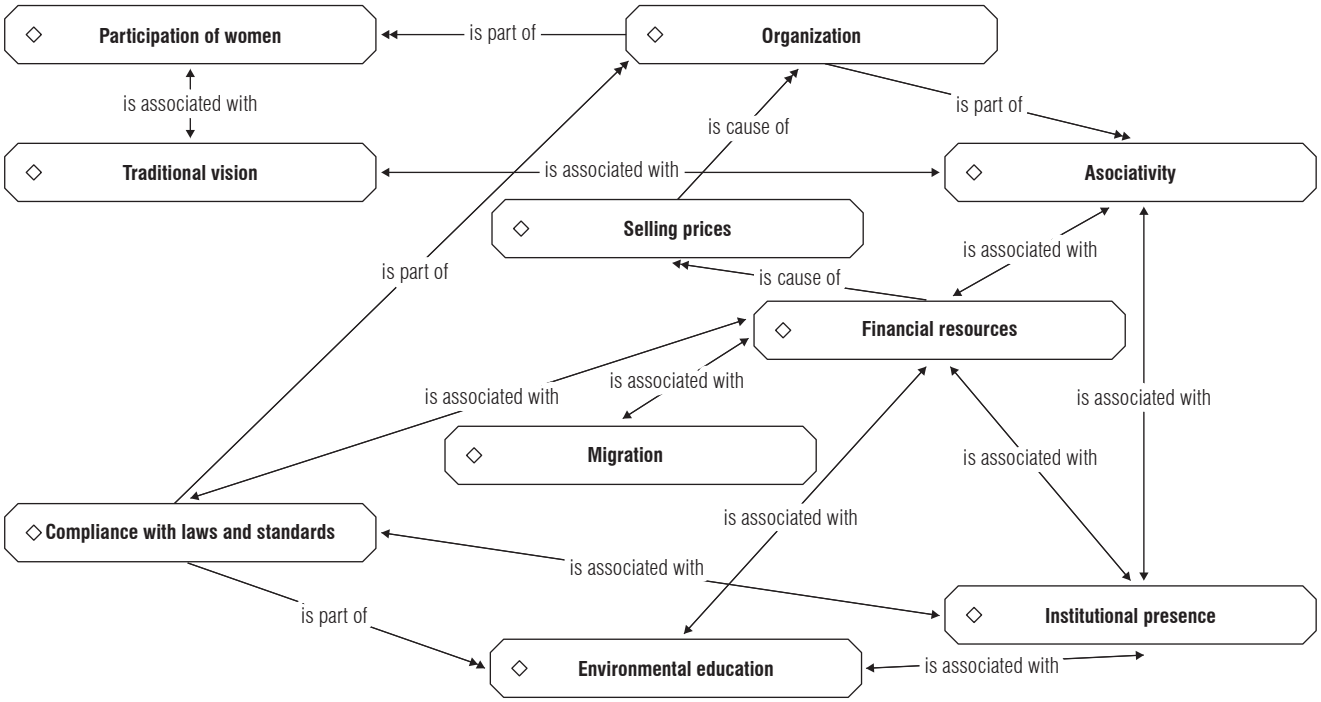


FIGURE 3. Social connections of panela production in Utica, Colombia.

first, the importance of women in the countryside; second, their strength to do particular tasks; and finally, the topic of the innovative mind (Fletschner, 2000).

Regarding these topics, women have played a prominent rural role, so their potential to share the necessities of work is immense. Linking women to the panela process would be a great success, as they tend to be more receptive, innovative, and propositional, among other qualities. The work of women is and always has been indispensable in different areas. For instance, cooking for the people manufacturing panela is a very important and labor-intensive task because these panela workers must work for long hours. Women can perform all the tasks in panela manufacturing besides cooking. As an example of the tasks that women can perform is commercializing the panela produced. However, FEDEPANELA does not currently have special programs to support or train women in order to include them in the panela manufacture or commercialization processes.

Migration is a fact in rural areas for different reasons. First of all, young people leave seeking new possibilities for access to services such as education, health, or culture. Second, they leave because governmental institutions do not offer alternatives for good jobs with decent wages or projects for improving agricultural activities. Finally, in the case of Colombia, young people leave because of rural violence forces the people to abandon their lands (Brittain, 2005). The situation of panela farmers in Utica is similar to the one in other rural localities of the country. Aside from the young population, adults are also looking for new opportunities for work, education and other activities in nearby municipalities or Bogota. According to the findings in our interviews, rural young people are looking for a new life far away from the countryside. This fact has impacted the panela producing sector because labor is becoming scarcer and, therefore, more expensive. This has increased production costs. Regarding this issue, one of the most important activities that FEDEPANELA should implement is encouraging the peasant youth to remain in the countryside, otherwise, the labor force would be scarcer every day.

Economic dimension

According to Schneemann and Vredeveld (2015), most of the stakeholders involved in value chain analysis consider the economic dimension as its baseline. They state that the economic dimension is the potential for market growth, job creation, and added value. In conjunction with the other analyzed dimensions, the highlighted topics will be useful for beginning better productive projects. The main connections are defined in Figure 4.

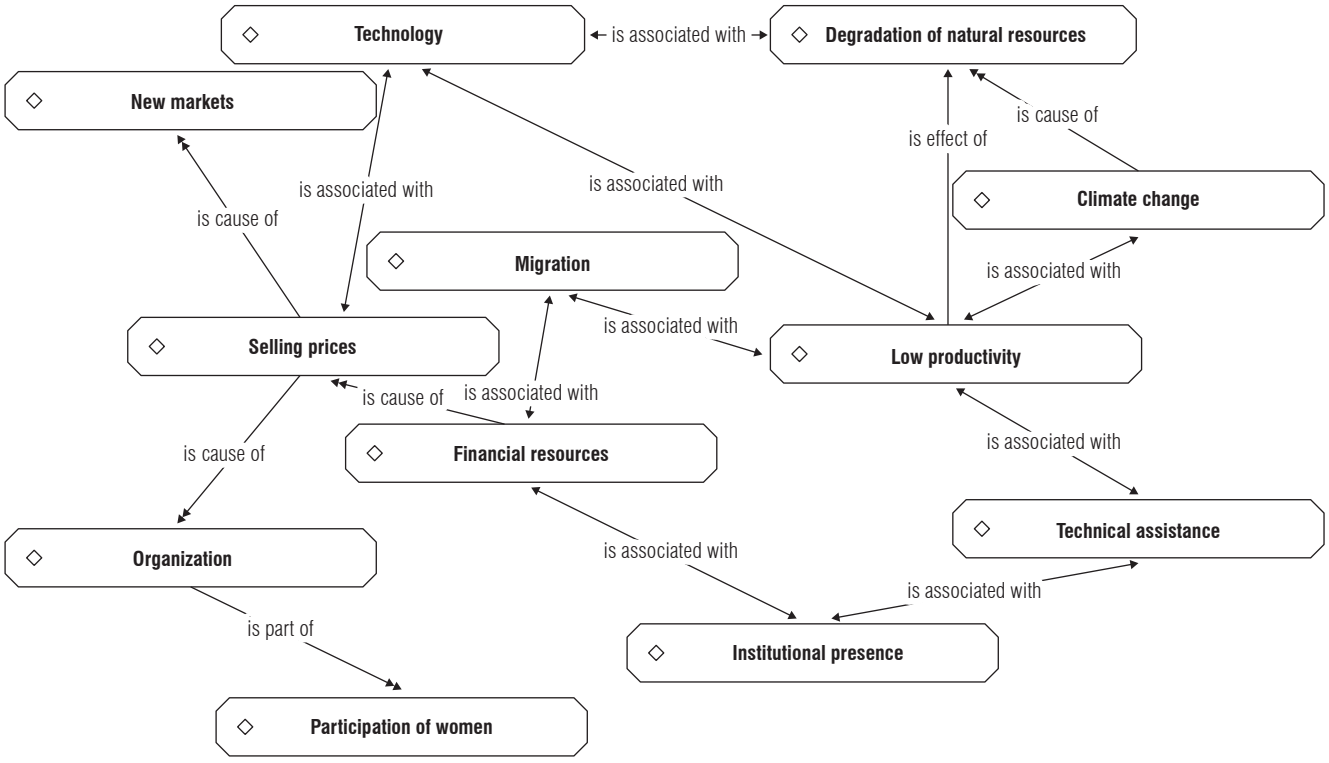


FIGURE 4. Economic connections of panela production in Utica, Colombia.

Financial resources are at the heart of any economic activity. In the current case, it is essential to take into account the scarce resources of the farmers and institutions working in Utica. As described previously, the government and the other institutions involved in the panela value chain have limited economic resources available. It means that given such scarcity, all the stakeholders must spend their funding better.

Although not always well-accepted and efficient for addressing real problems, the institutional presence offers a series of benefits that can be connected to an improvement in the conditions of all the farmers of the particular sector. Technical assistance is crucial in the panela manufacturing process because it can improve the yield without affecting or deteriorating the necessary resources (soil and water) for production. Equally, technical assistance has to prepare farmers to face or mitigate the problems caused by climate change (Feola, 2017). Similarly, the government and other institutions are essential in the process of acquiring new technologies, equipment, and infrastructures for carrying out manufacturing more efficiently, and, hence, for complying with the regulations. Nevertheless, the most critical concern that all involved stakeholders must solve is consolidating the farmers' organization and affiliation to FEDEPANELA to acquire more governmental support.

Conclusions

Panela producers suffer severe disadvantages and are threatened by many modern trends. Nevertheless, they must take advantage of their strengths as small farmers using their traditional knowledge to overcome their deficiencies. The success of different value chains of small farmers has been due to the adoption of solutions based on local advantages and experience, which offer alternatives to development. The traditional knowledge of panela production is the most reliable tool and must be reinforced and renewed based on current legislation and market demands. Otherwise, an important activity that has been carried out for generations could end.

The condition of farmers in Utica is cyclical. The panela manufactured by the farmer reaches the retailers who pay him according to his need to sell at that moment, which is generally at a low price. Retailers get better prices in other markets outside the town, where farmers usually never sell. The farmers receive money for their production, but it is barely enough to meet their basic needs. They do not receive additional income allowing them to make improvements and transform their production and manufacturing methods.

To address the value chain problems of panela in Utica, the following recommendations will be useful: first, the crops may require new management practices, such as Good Agricultural Practices that involve taking care of the environment. Another recommended transformation is to move towards an organic system, which would generate a significant added value considering the current market trends for consuming clean and healthy food. In order to do this, small farmers are required to renovate crops and to undertake practices of soil recovery. Second, the improvement of panela manufacturing facilities is a complicated situation due to the high expenses of these upgrades. However, by taking advantage of the programs proposed by the government and institutions and by working together, farmers would have to make fewer adjustments to solve this bottleneck.

The government, institutions and farmer organizations should work to find solutions to the problems. Nevertheless, without a proper organization of farmers into associations or other forms of teamwork, it is difficult to solve the individual problems of each farmer. Associated farmers can access training and new technologies more easily, and they can also obtain updated information and learn about the new challenges that the panela sector has in the country and around the world. Although the allocation of resources for new projects, new technology and financing for the poorest may be difficult because of the historical context of the country, panela production could greatly benefit from it. Then, if the aid is available, it should be assigned to those who need it most to avoid a situation in which farmers expect everything to be a gift and make use of the benefits without planning and projecting the efficient use of the resources for the future.

Literature cited

Administración Municipal Útica - Cundinamarca. 2012. Plan de desarrollo municipal 2012-2015 Útica Cundinamarca. "Por la reconciliación y la unidad de Útica". Utica, Colombia.

Alcaldía de Útica - Cundinamarca. 2016. Plan de desarrollo municipal 2016-2019 Útica Cundinamarca. "Experiencia y oportunidades para todos 2016-2019". Utica, Colombia.

Alviar, H. 2012. Searching for women and sustainable development in Colombia: restructuring the limits. Fordham Envir. Law Rev. 23(2), 22-43.

Barker, D. 2007. The rise and predictable fall of globalized industrial agriculture. The International Forum on Globalization (IFG). San Francisco, USA.

Becerra, R., N. Rueda, and M. Martínez, 2011. Diseño de experimentos en ciencias agropecuarias y biológicas con SAS, SPSS, R y STATISTIX. Fondo Nacional Universitario, Institución Auxiliar del Cooperativismo, Bogota.

Bernal, W., G. López, E. Cardozo, and J. Feo. 2016. Rescate de la identidad y la memoria cultural asociada a la molienda de caña y la producción de panela, en la provincia del Gualivá, Villeta, 2015. Tecnología y Productividad 2(2), 23-33.

Brittain, J. 2005. A theory of accelerating rural violence: Lauchlin Currie's role in underdeveloping Colombia. J. Peasant Stud. 32(2), 335-360. Doi: 10.1080/03066150500094535

De María, G. 2013. Panela: the natural nutritional sweetener. Agro Food Ind. Hi Tec. 24(6) November-December 2013.

Dunn, E. 2014. Field report No. 18: Smallholders and inclusive growth in agricultural value chains. United States Agency for International Development (USAID), USA.

Farah-Quijano, M. 2009, Social policy for poor rural people in Colombia: reinforcing traditional gender roles and identities? Soc. Policy Adm. 43, 397-408. Doi: 10.1111/j.1467-9515.2009.00670.x

Faße, A., U. Grote, and E. Winter. 2009. Value chain analysis methodologies in the context of environment and trade research. Hannover Economic Papers (HEP), Wirtschaftswissenschaftliche Fakultät, Leibniz Universität Hannover, Germany.

Fearne, A., M. Garcia Martinez, and B. Dent. 2012. Dimensions of sustainable value chains: implications for value chain analysis. Supply Chain Manage. 17(6), 575-581. Doi: 10.1108/13598541211269193

Feola, G. 2017. Adaptive institutions? Peasant institutions and natural models facing climatic and economic changes in the Colombian Andes. J. Rural Stud. 49, 117-127. Doi: 10.1016/j.jrurstud.2016.10.007

Fletschner, D. 2000. Enhancing rural women's access to capital: why it is important and how it can be done. The case of Colombia. Staff Paper No. 437. Agricultural and Applied Economics - Staff Paper Series. University of Wisconsin, Madison, USA. Doi: 10.22004/ag.econ.12640

Franco, L., N. Becerra, O. Pulido, and H. Parra. 2016. Influencia de la tecnificación panelera en los medios de vida de productores rurales en la Vereda Junco, San Benito (Santander). Revista de Antropología y Sociología Virajes 18(2), 115-137.

Gereffi, G. and J. Lee. 2012. Why the world suddenly cares about global supply chains. Supply Chain Manage. 48(3), 24-32. Doi: 10.1111/j.1745-493X.2012.03271.x

Hellin, J. and M. Meijer. 2006. Guideline for value chain analysis. Food and Agriculture Organization (FAO). URL: http://www.fao.org/fileadmin/templates/esa/LISFAME/Documents/Ecuador/value_chain_methodology_EN.pdf (accessed 13 September 2019)

Jaffé, W.R. 2015. Nutritional and functional components of non-centrifugal cane sugar: a compilation of the data from the analytical literature. J. Food Compos. Anal. 43, 194-202. Doi: 10.1016/j.jfca.2015.06.007

Kaplinsky, R. and M. Morris. 2001. A handbook for value chain research. IDRC, Ottawa.

MADR. 2019. Cadena productiva de la caña panelera - área, producción y rendimiento. URL: https://www.datos.gov.co/Agricultura-y-Desarrollo-Rural/Cadena-Productiva-Ca-a-Panelera-Area-Producci-n-Y-/n4hz-3mij/data (accessed 15 August 2019).

Martínez, D. 2013. Agenda prospectiva de investigación de la cadena productiva de la panela y su agroindustria. Tecnura 17(36), 72-86. Doi: 10.14483/udistrital.jour.tecnura.2013.2.a06

McKague, K. and M. Siddiquee. 2014. Making markets more inclusive: lessons from CARE and the future of sustainability in agricultural value chain development. Palgrave Macmillan, New York, USA. Doi: 10.1057/9781137373755

Ministerio de la Protección Social. 2011. Modificación a los requisitos sanitarios para la producción y comercialización de panela. URL: https://www.minsalud.gov.co/sites/rid/Lists/BibliotecaDigital/RIDE/DE/DIJ/RESOLUCI%C3%93N%204121%20DE%202011.pdf (accessed 15 August 2019).

Ordoñez-Díaz, M. and L. Rueda-Quiñónez. 2017. Evaluación de los impactos socioambientales asociados a la producción de panela en Santander (Colombia). Cienc. Tecnol. Agropec. 18(2), 379-396. Doi: 10.21930/rcta.vol18_num2_art:637

Ordoñez, R., F. Santa, and H. García. 2013. Proceso agroindustrial de la producción de panela. Tecnura 17(35), 47-54. Doi: 10.14483/udistrital.jour.tecnura.2013.1.a04

Orjuela, J. and I. Colmenares. 2011. Caracterización de la cadena de abastecimiento de panela para la Provincia de Bajo Magdalena - Cundinamarca. Ingeniería 16(2), 107-124.

Rao, P., M. Das, and S. Das. 2007. Jaggery - a traditional Indian sweetener. Indian J. Tradit. Knowl. 6(1), 95-102.

Riisgaard, L., S. Bolwig, S. Ponte, A. Du Toit, N. Halberg, and F. Matose. 2010. Integrating poverty and environmental concerns into value-chain analysis: a strategic framework and practical guide. Dev. Policy Rev. 28, 195-216. Doi: 10.1111/j.1467-7679.2010.00481.x

Rojas, G. 2011. La edafología en la planeación ambiental y el desarrollo territorial. Tecciencia 6(11), 53-66.

Schneemann, J. and T. Vredeveld (eds.). 2015. Guidelines for value chain selection. Integrating economic, environmental, social and institutional criteria. Deutsche Gesellschaft für Internationale Zusammenarbeit (GIZ) GmbH, Bonn and Eschborn, Germany.

Secretaria de Agricultura y Desarrollo Rural - Cundinamarca. 2015. Evaluaciones agropecuarias 2015 - Departamento de Cundinamarca. Estadísticas de Cultivos. URL: www.cundinamarca.gov.co (accessed 20 January 2017).

Webber, C. and P. Labaste. 2009. Building competitiveness in Africa's agriculture: a guide to value chain concepts and applications. Agriculture and rural development. World Bank, Washington DC. URL: http://documents.worldbank.org/curated/en/715461468003020843/Building-competitiveness-in-Africas-agriculture-a-guide-to-value-chain-concepts-and-applications. Doi: 10.1596/978-0-8213-7952-3

World Bank. 2015. The on-line tool for poverty measurement developed by the development research group of the World Bank. URL: http://iresearch.worldbank. org/PovcalNet/index.htm (accessed 20 February 2017).

Supplementary Material 1.

a) Questions for the interview with farmers.

General Information

1. Name
2. Age
3. Number of family members
4. Education level

   _____ Primary _____Secondary
   _____ Technical _____University

Crop Production

5. How did you learn about panela production?

   _____ Family _____ Extension _____Training
   _____ Learning by doing _____ Others

6. Cultivated area (ha)

   1-5 ha _____ 5-20 ha _____ 20- more ha _____

7. Which are the varieties of sugar cane used to obtain panela?

   MY 54-65 _____ RD 75-11_____ POJ 28-78 _____

8. What is the average yield of sugar cane in your plantation?
9. Which inputs do you use?

   _____Chemical inputs
   _____Organic inputs
   _____Machinery
   _____Hired Labor
   _____ Irrigation

10. How much do you pay for the inputs you need in one harvest?
11. Do you do any kind of documentation regarding farming activities?

    _____Amount of cane harvested
    _____Used pesticide, fungicide
    _____Used fertilizer

12. Which are the main pests that affect the crop?

    _____Cucacho, cornudo o cucarrón de invierno (*Podischnus agenor* Olivier)

    _____Picudo rayado de la caña (*Metamasius hemipterus Sericeus*)

    _____Barrenador del tallo (*Diatraea saccharalis* Fabricius)

    _____Barrenador gigante de la caña (*Castnia Licus* Drury)

    _____Termitas

    _____Gusano cabrito (*Caligo iIlioneus*)

    _____Hormiga loca (*Paratrechina fulva*)

13. Which are the main diseases that affect the crop?

    _____ Seed rot

    _____ Leaf lesions

    _____ Diseases caused by bacteria

    _____ Diseases caused by viruses

    _____ Diseases caused by nematodes

14. What are the main risks of crop loss?

    _____ Weather conditions
    _____ Diseases

15. What are your overall production costs for sugar cane? (calculated per 1 kg of Panela)
16. What are your revenues from panela production?
17. Do you have your own "trapiche" mill?

    a. _____ YES

       i. How old is the "trapiche" mill?

       ii. How often do you perform maintenance to the "trapiche"?

           _____ Monthly
           _____ Between 3 and 6 months
           _____ Annually

    b. _____ NO

       i. Do you have to pay for the use of the "trapiche" mill?

       ii. How much?

18. Which are the principal losses of product in the "trapiche" mill?
19. Do you obtain other products in the process of panela production?

    a. If yes, what products?

    b. Do you sell or earn any revenue for these products?

Panela Marketing

20. Which market do you supply?

    _____ Local _____ National _____ Export market

21. Where do you sell your product?

    _____ Farm gate _____ Small firms
    _____ Large firms _____Wholesalers
    _____ Association _____ Exporters
    _____ Retailers _____ Direct to consumer

22. How do you pack and sell the panela?
23. How is the relationship between you and the buyers?
24. Which requirements do wholesalers have?

    _____ Certain variety
    _____ Specific presentation
    _____ Frequent supply
    _____ Minimum supply

25. What are the best prices obtained?
26. What are the lowest prices obtained?
27. What are your main needs/opportunities in accessing markets?
28. How strong and stable is the panela market in this region?
29. Do you have some seal or quality certification for your product?
30. What standard or certification requirements does the panela market need?
31. Do you have any problems in this regard?
32. Do you have some practice to add value to your product?
33. Do you belong to an association to improve the access to markets?
34. Do you know FEDEPANELA?
35. Do you belong to this association?
36. Do you know the functions of FEDEPANELA?
37. Have you benefited from FEDEPANELA?
38. How do you transport the cane from the "trapiche"?
39. How do you transport the panela to the market?
40. What does the transport cost?

Financial and technical assistance

41. Do you usually need financial assistance to support your crop production?
42. Where do you get financial assistance?

    _____ Bank loan _____ Suppliers
    _____ Government _____ Others

43. Have you received any kind of financial support from the government?

    _____ Labels _____ Inspections
    _____ Subsidies _____ Incentives

44. Is there any public policy or regulation that is not beneficial for your business?
45. Do you receive any technical assistance?
46. Who provides this technical assistance?
47. What are the most critical infrastructure problems affecting your business, growth, and profitability?

    _____ Roads _____ Transport
    _____ Service supply _____ Crime
    _____ Storage

48. What are you doing about these problems?
49. How much of your income comes from panela production?

    _____ Nearly all _____Three quarters
    _____Half of the income _____ One quarter
    _____ Less than one quarter

b) Questions for the interview with the local government (major, planning department, agricultural department).

1. Could you please tell me about the importance of panela in this region?
2. What are the current public policies to benefit panela farmers in this region? (supports, regulations, subsidies, incentives).
3. How is the value chain structure organized? (where do inputs come from? who produces, sells to who and where?)
4. What do you think about this chain? (advantages, disadvantages).
5. What can the government do, or is going to do to improve the chain and benefit panela farmers?
6. What do you think about the future of panela production at the local level? Expectations, suggestions.

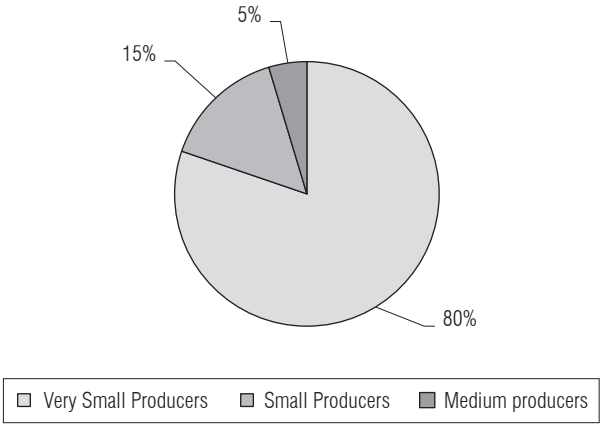
c) Questions for the interview with the leaders of associations (FEDEPANELA, MERPANELA).

1. What is the function of the association regarding panela farmers?
2. Which farmers can access the association?
3. What is the role of the association in the panela value chain?
4. How does the association help to improve the main problems of small farmers?

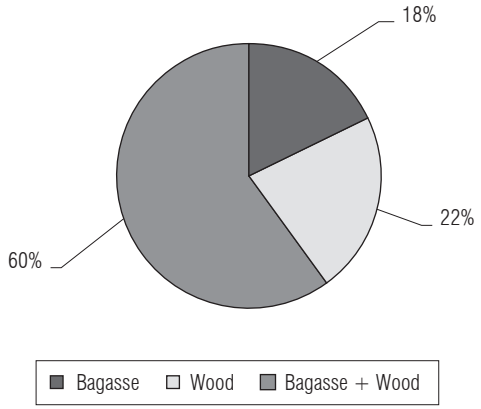
Supplementary Material 2.

Characterization of panela producers

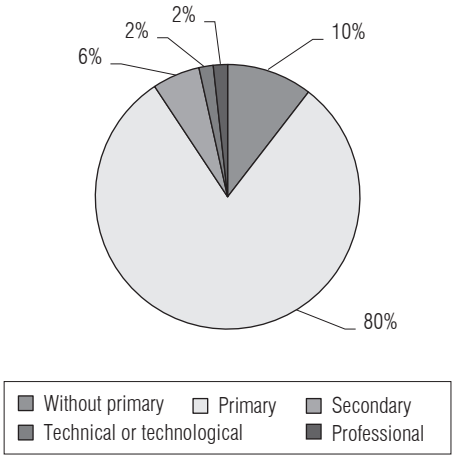
a) Types of panela producers.



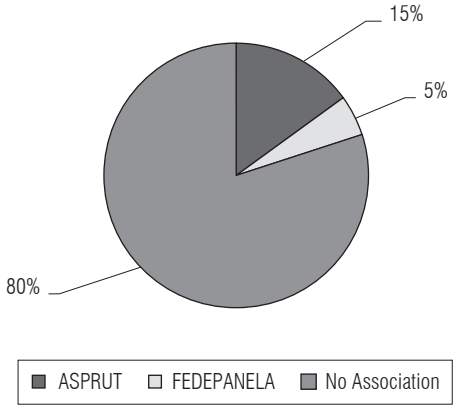
c) Source of energy in trapiches.



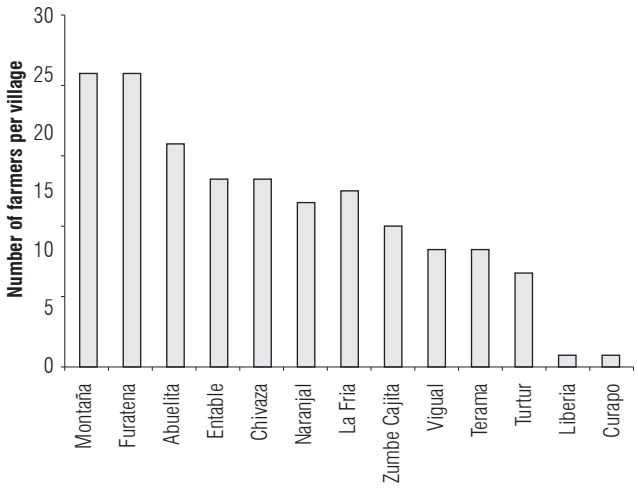
b) Educational level of panela farmers.



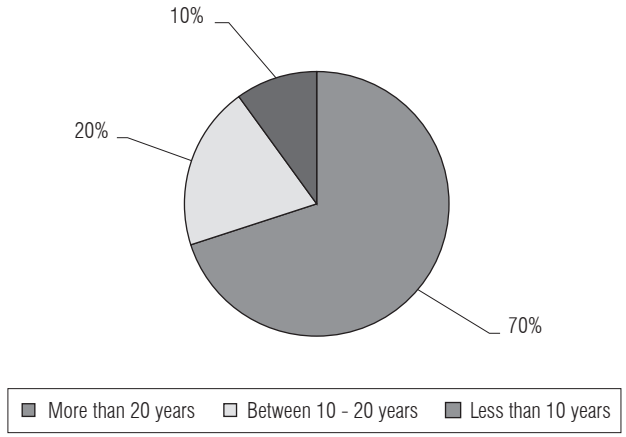
d) Associations farmers belong to.



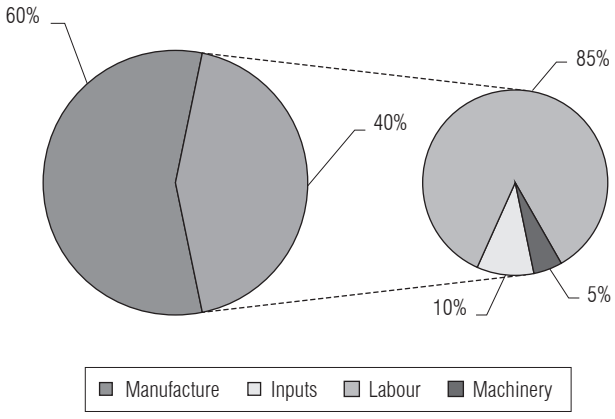
e) Number of farmers having a trapiche according to each rural settlement.



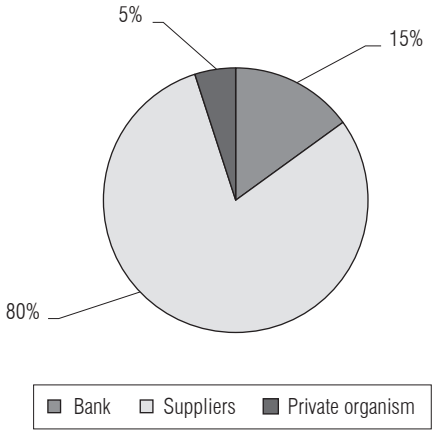
f) Ears of operation of trapiches in Utica.



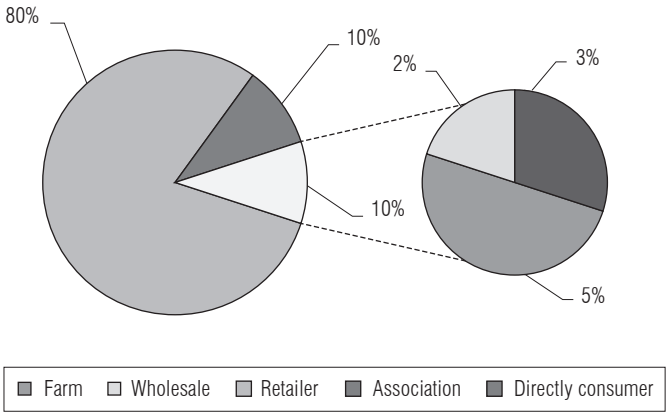
g) Distribution of production cost in the system.



h) Sources for requesting credits.



i) Panela marketing.



SCIENTIFIC NOTE

Sulfur effects on sugar content, enzyme activity and seed yield of rapeseed (*Brassica napus* L.)

Efectos del azufre sobre el contenido de azúcar, la actividad enzimática y el rendimiento de semillas de canola (*Brassica napus* L.)

Valiollah Rameeh[1*], Maryam Niakan[2], and Mohammad Hossein Mohammadi[2]

ABSTRACT

A field experiment was conducted in a randomized complete block design with four sulfur levels, S_0, S_1, S_2 and S_3, including 0, 12, 24 and 36 kg ha^{-1} (respectively) along with 115 kg N ha^{-1}, to evaluate the economic yield of the rapeseed variety (Hyola401) in Abandankash in the Central District of Sari County in Northern Iran. Parameters such as activity of leaf nitrate reductase, root nitrate content, contents of sugars in leaves and root, root peroxidase activity, and leaf catalase activity as well as seed yield were recorded. The results of the analysis of variance revealed that there were highly significant differences between characters for the majority of the traits such as contents of sugars and nitrate in leaves and root, root peroxidase activity, leaf catalase activity, and seed yield. Due to significant positive correlation between activity of root nitrate reductase and seed yield, increasing this enzyme in roots by sulfur application would have an accelerating effect on rapeseed seed yield. A highly significant positive correlation determined between leaf sugar content and seed yield (0.75**) indicated that increasing levels of sulfur had a direct effect on leaf sugar content, which had an accelerating effect on the weight of seed yield. Sulfur application significantly increased seed yield compared to the control (S_0 level), and it ranged from 2744 to 3215 kg ha^{-1} in S_0 and S_3.

Key words: correlation, fertilizer, nitrate reductase, nutrient, variation.

RESUMEN

Se realizó un experimento de campo en un diseño de bloques completos al azar con cuatro niveles de azufre, S_0, S_1, S_2 y S_3, incluyendo 0, 12, 24 y 36 kg ha^{-1} (respectivamente) junto con 115 kg N ha^{-1}, para evaluar el rendimiento económico de una variedad de canola (Hyola401) en Abandankash en el Distrito Central del condado de Sari en el norte de Irán. Se registraron parámetros tales como actividad de la nitrato-reductasa de la hoja, contenido de nitrato en raíz, contenido de azúcares en hojas y en raíz, actividades de peroxidasa en raíz y de catalasa en hojas y rendimiento de semilla. Los resultados del análisis de varianza revelaron diferencias altamente significativas entre los caracteres para la mayoría de los rasgos como contenido de nitrato y de azúcares en hojas y raíz, actividades de peroxidasa en raíz y de catalasa en hojas y rendimiento de semillas. Debido a una correlación positiva significativa entre la nitrato- reductasa de la raíz y el rendimiento de la semilla, el aumento de esta enzima en la raíz mediante la aplicación de azufre tiene un efecto acelerador en el rendimiento de la semilla de colza. Una correlación positiva altamente significativa determinada entre el contenido de azúcar en la hoja y el rendimiento de la semilla (0.75**) indica que los niveles crecientes de azufre tuvieron un efecto directo sobre el contenido de azúcar en la hoja, lo que tuvo un efecto acelerador sobre el peso del rendimiento del grano. La aplicación de azufre aumentó significativamente el rendimiento de la semilla sobre el control (nivel de S_0) y varió de 2744 a 3215 kg ha^{-1} en S_0 y S_3, respectivamente.

Palabras clave: correlación, fertilizante, nitrato reductasa, nutriente, variación.

Introduction

Sulfur (S) is considered the fourth major plant nutrient along with nitrogen, phosphorus and potassium. Sulfur is important for rapeseed production, and S deficiencies frequently constrain rapeseed yield (Jan *et al.,* 2008). Rapeseed requires about 1.5 kg of S to produce 100 kg ha^{-1} of seed (Kumar *et al*., 2002). Therefore, a 3000 kg ha^{-1} crop would require approximately 45 kg S ha^{-1}. To obtain optimum yields of high-quality rapeseed seed, S needs to be an important part of balanced fertilization along with other nutrients (Jackson, 2000; Malhi and Gill, 2002; Kandil and Gad, 2012; Sharifi, 2012). Sulfur is essential for the synthesis of amino acids including cystine and methionine (a component of vitamin A), and it activates special enzyme systems in plants (Balint and Rengel, 2009).

Received for publication: 21 April, 2018. Accepted for publication: 2 September, 2019

Doi: 10.15446/agron.colomb.v37n3.71830

[1] Agronomic and Horticultural Crops Research Department, Mazandaran Agricultural and Natural Resources Research and Education Center, AREEO Sari (Islamic Republic of Iran).

[2] Scientific member and former MSc. graduate student, Islamic Azad University, Gorgan Branch.

* Corresponding author: vrameeh@gmail.com

Regarding protein formation during rapeseed growth and development, S can also increase seed yield and improve oil content (Zhao *et al.,* 1993; Jan *et al.,* 2002; Sattar *et al.,* 2011). Sulfur is also involved in the synthesis of chlorophyll and is also required in plants of the family Cruciferae for the synthesis of volatile oils (Marschner, 2012). Castellano and Dick (1991) find that photosynthesis-related proteins such as the Rubisco protein and chlorophyll and N and S content in leaves significantly increase with sulfur levels up to 50 kg S ha^{-1} compared to 0 kg S ha^{-1}. Ahmad *et al.* (2000) report that sulfur application significantly increased acetyl-CoA concentration, acetyl-CoA carboxylase activity, and soluble protein and starch content in developing seeds. Plants take sulfur primarily in the form of $S0_4^{-2}$ by a specific transport protein (Thompson *et al.,* 1986). Shallow soils with low organic matter content are likely to provide little sulphate (Holmes, 1980). Total chlorophyll content and peroxidase activity increases with higher sulfur levels (Khanpara *et al.,* 1993). Abiotic stresses contribute to the formation of reactive oxygen species (ROS), superoxide radical (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH^-), with the last one as the most cytotoxic. These ROS cause perturbation of basic metabolic pathways and damage membranes and organic molecules, mainly proteins, DNA, and pigments (Fridovich, 1986; Imlay and Linn, 1988) as well as sulfur containing amino acids in proteins (Hernandez *et al.,* 2000). Plants use different strategies to solve this problem. For instance, one strategy is that plants increase the activity of antioxidant enzymes. The toxic superoxide radical is rapidly dismutated by superoxide dismutase (SOD) to H_2O_2, a product that is relatively stable and can be detoxified by catalase (CAT) and guaiacol peroxidases (Grant and Loake, 2000). Increased SOD activity is known to confer oxidative stress tolerance (Bowler *et al.,* 1992). The balance between ROS production and activities of antioxidative enzymes determines whether oxidative signaling and/or damage will occur (Moller *et al.,* 2007). The activities of these antioxidant enzymes are reported to increase under various environmental stresses (Hernandez *et al.,* 1995; Hernandez *et al.,* 2000). There are many reports in the literature that underline the intimate relationship between enhanced or constitutive antioxidant enzyme activities and increased resistance to environmental stresses in *Brassica* and other plant species (Sreenivasulu *et al.,* 2000; Rameeh *et al.,* 2004; Khanna-Chopra and Selote, 2007). Sulfur application will improve seed and oil quality and is also a key factor for oil formation. In the present study, four sulfur levels: 0, 12, 24 and 36 kg ha^{-1} along with 115 kg N ha^{-1} were applied to evaluate economic yield and also enzyme activity of rapeseed under rainfed conditions.

Materials and methods

A field experiment was carried out in a farm in Abendankash located in Sari, Iran (53°7' E, 36°32" N, 60 m a.s.l.) during the 2006-2007 cropping seasons. The soil was classified as a deep loam soil (Typic Xerofluents, USDA classification), which maintained an average of 280 g clay kg^{-1}, 560 g silt kg^{-1}, 160 g sand kg^{-1}, and 22.4 g organic matter kg^{-1} with a pH of 7.3. Soil samples were found to have 45 kg ha^{-1} of mineral nitrogen (N) in the upper 30-cm profile. The experiment received 50 kg P ha^{-1} and 75 kg K ha^{-1}. The average temperature was around 15.5°C and the average rainfall was 36.7 mm. Seeds of the rapeseed cultivar Hyola401 were planted on October 18, 2006. Seeds were sown with a uniform seed rate of 5 kg ha^{-1} in all plots with the help of a hand hoe in straight rows. The experiment was set in a randomized complete block design with four replicates. The treatments under study included different amounts of ammonium sulfate (containing 21% nitrogen and 24% sulfur) and urea fertilizer (containing 46% nitrogen): S_0: 250 kg ha^{-1} urea; S_1: 227 kg ha^{-1} urea+50 kg ha^{-1} ammonium sulfate; S_2: 204 kg ha^{-1} urea + 100 kg ha^{-1} ammonium sulfate; and S_3: 182 kg ha^{-1} urea + 150 kg ha^{-1} ammonium sulfate. S_0, S_1, S_2 and S_3 included 0, 12, 24 and 36 kg ha^{-1} S, respectively, and all treatments maintained 115 kg N ha^{-1}. All cultural practices were uniformly applied to all plots. All plant protection measures were adopted to keep the crop free from insect pests. Seed yield (adjusted to kg ha^{-1}) was recorded based on three middle rows in each plot. For enzyme assays, frozen leaves were ground to fine powder with liquid nitrogen and extracted with ice-cold 0.1 M Tris-HCl buffer (pH 7.5) containing 5% (w/v) sucrose and 0.1% 2- mercaptoethanol (3:1 buffer volume/FW). The homogenate was centrifuged at 10,000 g for 20 min, at 4°C, and the supernatant was used for enzyme activity. Regarding the enzyme assay, superoxide dismutase activity which has the ability to inhibit the phothochemical reduction of nitroblue tetrazolium (NBT) (Beauchamp and Fridovich, 1971), was determined according to the method by Dhindsa *et al.* (1980). For the SOD assay, the reaction mixture contained 50 mM K-phosphate buffer (pH 7.8), 13 mM methionine, 75 µM NBT, 0.1 µM EDTA, 4 µM riboflavin and the required amount of enzyme extract. The reaction was initiated by adding riboflavin and placing the tubes under two 15 W fluorescent lamps for 15 min. A complete reaction mixture without enzyme, which gave the maximal color, served as control. Peroxidase activity was assayed adopting the method by Polle *et al.* (1994). According to this method, Peroxidase activity was determined at 436 nm by its ability to convert guaiacol to tetraguaiacol ($E = 26.6\ mM^{-1}\ cm^{-1}$) The reaction mixture contained 100 mM K-phosphate

buffer (pH 7.0), 20.1 mM guaiacol, 10 mM H_2O_2, and the enzyme extract. The increase in absorbance was recorded by the addition of H_2O_2 at 436 nm for 5 min. CAT activity was determined by monitoring the disappearance of H_2O_2 at 240 nm ($E = 40\ mM^{-1}\ cm^{-1}$) according to the method by Aebi (1984). The reaction mixture contained 50 mM K-phosphate buffer (pH 7.0), 33 mM H_2O_2 and the enzyme extract. To estimate sugar and starch contents in leaves, chopped leaves were fixed in boiling 80% ethanol. Sugar contents were estimated colorimetrically by the method described by Nelson (1994) at 500 nm. Data recorded were analyzed statistically, using analysis of variance (ANOVA) with appropriate techniques for randomized complete block design (Steel and Torrie, 1980). For the analysis, an Excel worksheet was programmed. All the analyses were performed using the software SAS version 9 (SAS Institute Inc., 2004).

Results and discussion

Significant mean square, which indicates significant different effects of sulfur levels, were found for the traits including leaf nitrate reductase, root nitrate reductase, leaf and root sugars, root peroxidase, leaf catalase, and seed yield (Tab. 1). Leaf peroxidase activity was not affected by sulfur levels. Sulfur application along with seed yield that improves oil quality is a critical factor for oil formation. Sulfur shortage adversely decreases yield as well as protein and enzyme synthesis (Scherer, 2001).

Means comparisons of leaf nitrate reductase as influenced by different levels of sulfur is presented in Table 2 and Figure 1. Sulfur application induced a significant increase in activity of leaf nitrate reductase. Leaf nitrate reductase activity varied from 0.82 to 2.90 mM $No_2^-\ g^{-1}$ in S_0 (control) and S_3 (36 kg S ha^{-1}), respectively. Mean values of leaf nitrate reductase activity were classified into two statistical groups for the application of four S levels. This trait related to S_0 and S_1 determined the same group, and for S_2 and S_3 it was also classified in the same statistical group. Ahmad *et al*. (2000) report that sulfur application significantly increases acetyl-CoA concentration acetyl-CoA carboxylase activity, soluble protein and starch content in developing seeds. A significant positive correlation was determined between leaf nitrate reductase activity and sugar content in leaf and root of rapeseed (Tab. 3). Therefore, any variation for this enzyme will have considerable effect on leaf and root sugar contents. Root nitrate reductase activity ranged from 0.33 to 1 mM $No_2^-\ g^{-1}$ in S_0 and S_3, respectively. A significant positive correlation between root nitrate reductase activity and sugar content in leaf and root of rapeseed was observed (Tab. 3).

Due to a significant positive correlation between activity of root nitrate reductase and seed yield, increasing this enzyme in the roots followed by sulfur application will have considerable effect on rapeseed seed yield. Leaf sugar was positively affected by sulfur levels, and it varied from 0.72 to 2.29 g g^{-1} DW for S_0 and S_3, respectively. A highly significant

TABLE 1. Randomized complete block (RCBD) analysis of variance for the studied traits.

S.O.V.	df	MS							
		Leaf nitrate reductase	Root nitrate reductase	Leaf sugar	Root sugar	Leaf peroxidase	Root peroxidase	Leaf catalase	Seed yield
Replicate	2	0.08	0.12	0.06*	0.05	0.001	0.001**	6.52**	3912
Treatments	3	2.65**	0.24*	1.39**	0.41**	0.0001	0.0002*	1.57**	365024**
Error	6	0.23	0.03	0.01	0.04	0.001	0.00003	0.08	85432
Coefficient of variation (C.V.) %		27.4	25.7	6.67	24.5	34.7	11.4	5.9	11.56

S.O.V.: source of variance, df: degree of freedom, MS: mean squares.
*, ** Significant at $P<0.05$ and 0.01, respectively.

TABLE 2. Mean comparison of yield components, seed yield and oil percentage.

Sulfur (kg ha^{-1})	Leaf nitrate reductase (mM $NO_2^-\ g^{-1}$)	Root nitrate reductase (mM $NO_2^-\ g^{-1}$)	Leaf sugar (g g^{-1} DW)	Root sugar (g g^{-1} DW)	Leaf peroxidase (OD g^{-1} FW min^{-1})	Root peroxidase (OD g^{-1} FW min^{-1})	Leaf catalase (μM H_2O_2 d min^{-1})	Seed yield (kg ha^{-1})
S_0=0	0.82b	0.33c	0.72d	0.40c	0.053a	0.036b	3.87b	2744b
S_1=12	1.18b	0.63bc	1.07c	0.63bc	0.056a	0.041b	4.50b	2844ab
S_2=24	2.12a	0.77ab	1.55b	0.91ab	0.052a	0.047ab	4.87ab	3190ab
S_3=36	2.90a	1.00a	2.29a	1.26a	0.053a	0.057a	5.60a	3215a

S_0, S_1, S_2 and S_3 included 0, 12, 24 and 36 Kg S ha^{-1}, respectively, and all treatments maintained 115 Kg N ha^{-1}.

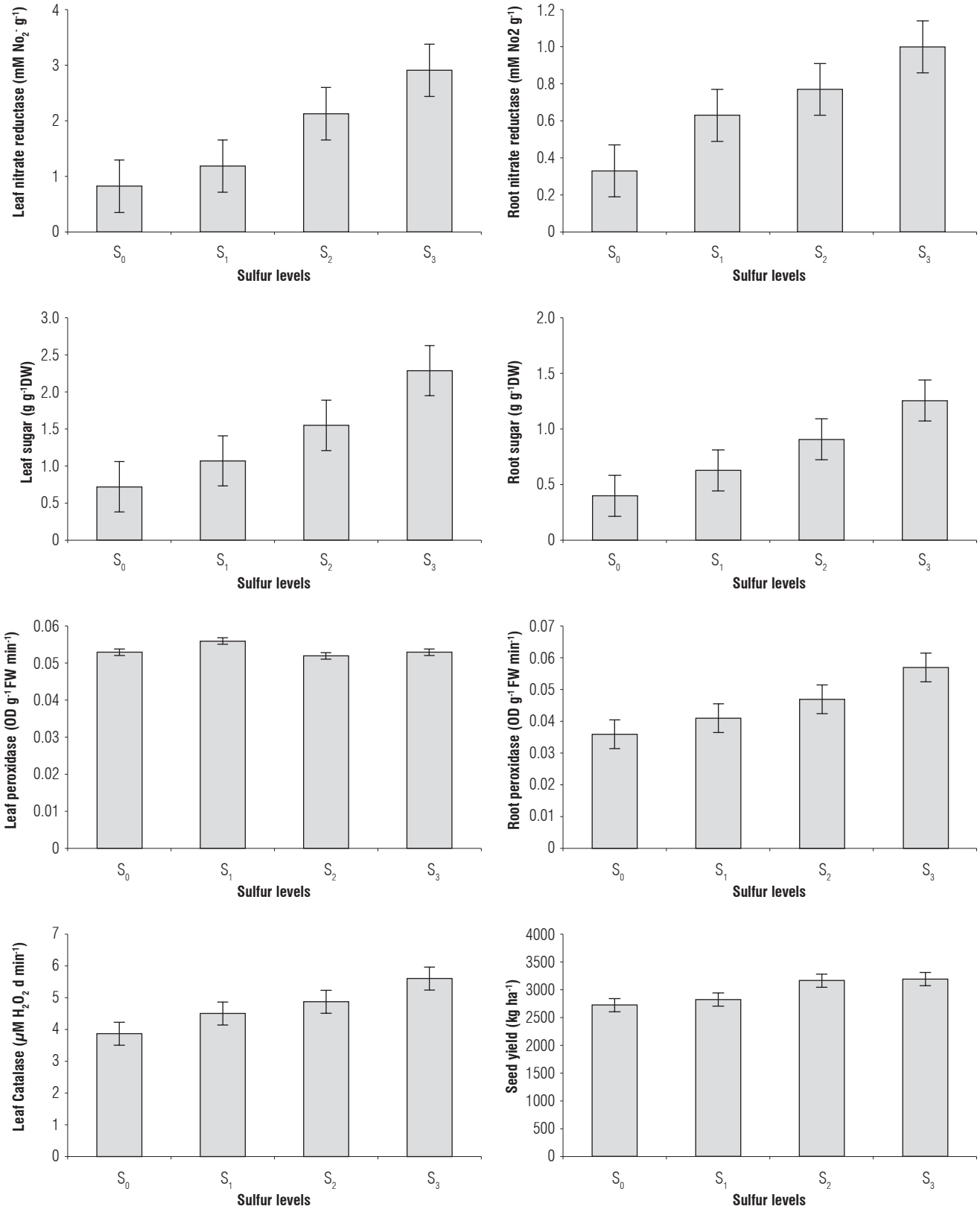


FIGURE 1. Means of plant heights, yield components, seed yields and oil contents of rapeseed var. Hyola401 under different sulfur levels (S_0, S_1, S_2 and S_3 including 0, 12, 24 and 36 kg S ha^{-1}, respectively). Error bars indicate 95% confidence intervals.

TABLE 3. Pearson correlation coefficient estimates of biochemical traits and seed yield in different levels of sulfur used on a rapeseed variety.

Traits	Leaf nitrate reductase	Root nitrate reductase	Leaf sugar	Root sugar	Leaf peroxidase	Root peroxidase	Leaf catalase	Seed yield
Leaf nitrate reductase	1							
Root nitrate reductase	0.67*	1						
Leaf sugar	0.88**	0.74**	1					
Root sugar	0.78**	0.76**	0.86**	1				
Leaf peroxidase	-0.15	0.35	0.06	-0.09	1			
Root peroxidase	0.21	-0.18	-0.07	-0.19	-0.19	1		
Leaf catalase	0.12	-0.24	0.01	0.25	0.25	0.65*	1	
Seed yield	0.53	0.71*	0.75**	0.27	0.27	-0.36	-0.18	1

*, ** Significant at $P<0.05$ and 0.01, respectively.

positive correlation (0.75**) was determined between leaf sugar content and seed yield; therefore, increasing sulfur levels had a direct increasing effect on leaf sugar content, which had an escalating effect on seed yield. Root sugar content was positively affected by sulfur levels and changed from 0.40 to 1.26 g g^{-1} DW for S_0 and S_3, respectively (Tab. 2). Sulfur levels had an increasing effect on root peroxidase activity and this enzyme ranged from 0.036 to 0.057 OD g^{-1} FW min^{-1} in roots. Leaf catalase activity was significantly affected by sulfur levels, and the mean value of this enzyme activity varied from 3.87 to 5.60 μM H_2O_2 d min^{-1} for S_0 and S_3. Bashir *et al.* (2015) indicate that the activity of ascorbate peroxidase, glutathione reductase and catalase declined in Cd-treated and S-deficient plants, but it was upregulated in the presence of sulfur.

Sulfur application significantly increased seed yield compared to control (S_0 level) and it ranged from 2744 to 3215 kg ha^{-1} in S_0 and S_3 (Tab. 3). S_3 (36 kg ha^{-1} S) increased seed yield on 17%. Sulfur application improves seed yield quantity and oil quality and also sulfur shortage adversely decreases yield, protein and enzyme synthesis (Scherer, 2001; Rehmanuh *et al.,* 2013).

Conclusion

All the traits except leaf peroxidase activity were significantly affected by sulfur levels. A significant positive correlation between leaf nitrate reductase and sugar content in rapeseed leaves and roots was found. Therefore, any variation of this enzyme will have considerable effect on leaf and root sugar contents. Due to a significant positive correlation between root nitrate reductase activity and seed yield, increasing this enzyme in roots by sulfur application will have a considerable effect on rapeseed seed yields. Leaf sugar was positively affected by sulfur levels and its high mean value was observed at level S_3. A highly significant positive correlation determined between leaf sugar content and seed yield (0.75**) was observed. Thus, increasing sulfur levels had a direct increasing effect on leaf sugar content, which had an accelerating effect on seed yield. Sulfur application significantly increased seed yield compared to S_0 level, and it ranged from 2744 to 3215 kg ha^{-1} in S_0 and S_3.

Literature cited

Aebi, H. 1984. Catalase in vitro. Methods Enzymol. 105, 121-126.

Ahmad, A. and M.Z. Abdin. 2000. Interactive effect of sulphur and nitrogen on the oil and protein contents and on the fatty acid profiles of oil in the seeds of rapeseed (*Brassica campestris* L.) and mustard (*Brassica juncea* L. Czern. and Coss.). J. Agron. Crop Sci. 185(1), 49-54. Doi: 10.1046/j.1439-037X.2000.00401.x

Ahmad, G., A. Jan, I. Arif, and M. Arif. 2006. Phenology and physiology of canola as affected by nitrogen and sulphur fertilization. J. Agron. 5, 555-562. Doi: 10.3923/ja.2006.555.562

Balint, T. and Z. Rengel. 2009. Differential sulphur efficiency in canola genotypes at vegetative and grain maturity stage. Crop Past. Sci. 60, 262-270. Doi: 10.1071/CP08224

Bashir, H., M.M. Ibrahim, R. Bagheri, J. Ahmad, I.A. Arif, M.A. Baig, and M.I. Qureshi. 2015. Influence of sulfur and cadmium on antioxidants, phytochelatins and growth in Indian mustard. AoB Plants 7(1), 1-13. Doi: 10.1093/aobpla/plv001

Beauchamp, C. and I. Fridovich. 1971. Superoxidase dismutase: improved assays and an assay applicable to acrylamide gels. Anal. Bioch. 44, 276-286. Doi: 10.1016/0003-2697(71)90370-8

Bowler, C., M.W. Montagu, and D. Inze. 1992. Superoxide dismutase and stress tolerance. Ann. Rev. Plant Physiol. Plant Mol. Biol. 43, 83-116. Doi: 10.1146/annurev.pp.43.060192.000503

Castellano, S.D. and R.P. Dick. 1991. Cropping and sulphur fertilization influence on sulphur transformation in soil. Soil Sci. Soc. Am. J. 55, 1, 114-121.

Chen, X.J., Z.J. Zhu, X.L. Ni, and Q.Q. Qlan. 2006. Effect of nitrogen and sulfur supply on glucosinolates in *Brassica campestris* ssp. *chinensis*. Agric. Sci. China 5(8), 603-608.

Fridovich, I. 1986. Biological effects of superoxide radical. Arch. Biochem. Biophysic. 247(1), 1-11. Doi: 10.1016/0003-9861(86)90526-6

Giannopolitis, C.N. and S.K. Ries. 1977. Superoxide dismutases: I. occurrence in higher plants. Plant Physiol. 59, 309-314.

Grant, J.J. and G.J. Loake. 2000. Role of reactive oxygen intermediates and cognate redox signaling in disease resistance. Plant Physiol. 124, 21-29. Doi: 10.1104/pp.124.1.21

Hernandez, J.A., A. Jimenez, P. Mullineaux, and F. Sevilla. 2000. Tolerance of pea (*Pisum sativum* L.) to long-term salt stress is associated with induction of antioxidant defences. Plant Cell Environ. 23, 853-862. Doi: 10.1046/j.1365-3040.2000.00602.x

Hernandez, J.A., E. Olmos, F.J. Corpas, F. Sevilla, and L.A. Del Rio. 1995. Salt-induced oxidative stress in chloroplasts of pea plants. Plant Sci. 105, 151-167. Doi: 10.1016/0168-9452(94)04047-8

Holmes, M.R.J. 1980. Nutrition of the oilseed rape crop. Applied Science Publishers ltd., London.

Imlay, J.A. and S. Linn. 1998. DNA damage and oxygen radical toxicity. Science 240, 1302-1309. Doi: 10.1126/science.3287616

Jackson, G.D. 2000. Effects of nitrogen and sulfur on canola yield and nutrient uptake. Agron. J. 92(4), 644-649. Doi: 10.2134/agronj2000.924644x

Jan, A., G. Ahmad, T. Jan, M. Jamal, and F. Subhan. 2008. Oil yields of Canola as affected by N and S levels and methods of application under rainfed condition. Sarhad J. Agric. 24(1), 1-10.

Jan, A., N. Khan, I.A. Khan, and B. Khattak. 2002. Chemical composition of canola as affected by nitrogen and sulphur. Asian J. Plant Sci. 1, 519-521. Doi: 10.3923/ajps.2002.519.521

Kandil, H. and N. Gad. 2012. Growth and oil production of canola as affected by different sulphur sources. J. Basic. Appl. Sci. Res. 2, 5196-5202.

Kayupova, G.A. and L.K. Klyshev. 1984. Superoxide dismutase of pea root under the influence of high NaCl concentrations. Plant Physiol. 31, 441-445.

Khanna-Chopra, R. and D.S. Selote. 2007. Acclimation to drought stress generates oxidative stress tolerance in drought-resistant than -susceptible wheat cultivar under field conditions. Environ. Exp. Bot. 60, 276-283. Doi: 10.1016/j.envexpbot.2006.11.004

Khanpara, V.N., B.L. Porwal, and J.E. Patel. 1993. Effect of levels and modes of sulphur application on biochemical changes in mustard *(Brassica juncea)* leaves. Indian J. Agron. 38(3), 410-413.

Kumar, R., D. Singh, and H. Singh. 2002. Growth and yield of *Brassica* species as influence by sulphur application and sowing dates. Indian J. Agron. 47(3), 418-421.

Malhi, S., Y. Gan, and J. Raney. 2007. Yield, seed quality and sulphur uptake of *Brassica* oil seed crops in response to sulphur fertilization. Agron. J. 99, 570-577. Doi: 10.2134/agronj2006.0269

Malhi, S.S. and K.S. Gill. 2002. Effectiveness of sulphate-S fertilization at different growth stages for yield, seed quality and S uptake of canola. Can. J. Plant Sci. 82, 665-674. Doi: 10.4141/P01-184 Doi: 10.4141/P01-184

Marschner, P. 2012. Mineral nutrition of higher plants. 3rd ed. Academic Press, London.

Moller, I.M., P.E. Jensen, and A. Hansson. 2007. Oxidative modifications to cellular components in plants. Ann. Rev. Plant Biol. 58, 459-481. Doi: 10.1146/annurev.arplant.58.032806.103946

Nelson, N. 1994. A photometric adaptation of the Somogyi method for determination of glucose. J. Biol. Chem. 53, 375-378.

Polle, A., T. Otter, and F. Seifert. 1994. Apoplastic peroxidases and lignification in needles of Norway Spruce (*Picea abies* L.). Plant Physiol. 106, 53-60. Doi: 10.1104/pp.106.1.53

Rameeh, V., A. Rezai, and G. Saeidi. 2004. Study of salinity tolerance in rapeseed. Commun. Soil Sci. Plant Anal. 35, 2849-2866. Doi: 10.1081/CSS-200036472

Rehmanuh, Q., M. Iqbal, I. Farooq, and S.M.A. Afzal. 2013. Sulphur application improves the growth, seed yield and oil quality of canola. Acta Physiol. Plant. 35(10), 1331-339. Doi: 10.1007/s11738-013-1331-9

SAS Institute Inc. 2004. SAS/STAT user's guide. Version 9. Fourth Edition. Statistical Analysis Institute Inc. Cary, North Carolina, USA.

Sattar, A., M.A. Cheema, M.A. Wahid, M.F. Saleem, and M. Hassan. 2011. Interactive effect of sulphur and nitrogen on growth, yield and quality of canola. Crop Environ. 2, 32-37.

Scherer, H.W. 2001. Sulphur in crop production. Eur. J. Agron. 14(2), 81-111. Doi: 10.1016/S1161-0301(00)00082-4

Sharifi, R.S. 2012. Sulphur fertilizer effects on grain yield and the sum of physiological indices of canola (*Brassica napus* L.). Ann. Biol. Res. 3(11), 5034-5041.

Sreenivasasula, N., B. Grimm, U. Wobus, and W. Weschke. 2000. Differential response of antioxidant compounds to salinity stress in salt-tolerant and salt-sensitive seedlings of foxtail millet (*Setaria italica*). Physiol. Plant. 109, 435-442. Doi: 10.1034/j.1399-3054.2000.100410.x

Steel, R.G.D. and J.H. Torrie. 1980. Principles and procedures of statistics: a biometrical approach. 2nd ed. McGraw Hill Book Co., New York, USA.

Thompson, J.F., I.K. Smith, and J.T. Madison. 1986. Sulfur metabolism in plants. In: Tabatabai, M.A. (ed.). Sulfur in Agriculture. Agronomy Society of America, Madison, USA.

Zhao, F., P.E. Bilsborrow, E.J. Evans, and J.K. Syers. 1993. Sulphur turnover in the developing pods of single and double low varieties of oilseed rape (*Brassica napus* L.). J. Sci. Food Agric. 62(2), 111-119. Doi: 10.1002/jsfa.2740620203

SCIENTIFIC NOTE

Edible coatings based on sodium alginate and ascorbic acid for application on fresh-cut pineapple (*Ananas comosus* (L.) Merr)

Recubrimientos comestibles a base de alginato de sodio y ácido ascórbico para aplicación sobre piña (*Ananas comosus* (L.) Merr) fresca cortada

Alex López-Córdoba[1,2]* and Andrea Aldana-Usme[1]

ABSTRACT

The demand for healthy and ready-to-eat products, such as freshly-harvested fruits, has been growing steadily over the years. However, these products are very susceptible to spoilage and have a short shelf-life. In this research, edible coatings based on sodium alginate and its blends with ascorbic acid (a natural antioxidant and anti-browning agent) were applied on fresh-cut pineapple samples, and the changes in their physicochemical properties were monitored during 10 d of storage at 4ºC. Initially, the surface of the coated fruits was brighter and statistically significant differences were not found between uncoated and coated samples (P<0.05); similar values were obtained in the parameters of soluble solids (~11 ºBrix), pH (~3.74) and titratable acidity (~0.64%). During storage, coated samples were more protected against changes in appearance compared to uncoated fresh-cut pineapple samples. The current results will be beneficial for further research that focuses on the preservation of minimally processed fruits such as pineapple.

Key words: minimally processed foods, storage losses, food preservation, added value.

RESUMEN

La demanda de productos saludables y listos para consumir, tales como las frutas frescas cortadas, ha venido creciendo sostenidamente en los últimos años. Sin embargo, estos productos son muy susceptibles al deterioro y tienen una corta vida útil. En el presente trabajo, se aplicaron recubrimientos comestibles a base de alginato de sodio y sus mezclas con ácido ascórbico (un agente antioxidante y anti-pardeamiento natural) sobre muestras de piña fresca cortada y se monitorearon los cambios en sus características fisicoquímicas durante 10 d de almacenamiento a 4ºC. Inicialmente, la superficie de las frutas recubiertas fue más brillante y no se observaron diferencias estadísticamente significativas entre las muestras sin y con recubrimiento (P<0.05), obteniendo valores similares en los parámetros de contenido de solidos solubles (~11 ºBrix), pH (~3.74) y acidez titulable (~0.64%). Durante el almacenamiento, las muestras recubiertas estuvieron mejor protegidas frente a cambios en la apariencia en comparación con las muestras de piña fresca cortada sin recubrir. Estos resultados serán muy útiles para futuras investigaciones que se centren en la conservación de frutas mínimamente procesadas, tales como piña.

Palabras clave: alimentos mínimamente procesados, pérdidas por almacenamiento, preservación de alimentos, valor añadido.

Introduction

Pineapple (*Ananas comosus* (L.) Merr) is one of the most-produced tropical fruits worldwide (Ancos *et al*., 2016). It is recognized as an important source of sugars, organic acids, fiber, minerals, and vitamins (Ancos *et al*., 2016; Montero-Calderón and Martín-Belloso, 2016). In addition, pineapple is rich in health-promoting bioactive compounds such as ascorbic acid, carotenoids, and flavonoids (Ancos *et al*., 2016; Montero-Calderón and Martín-Belloso, 2016).

Pineapple is commonly consumed fresh or used to make jam, jellies, desserts, and other products. Minimally processed pineapples are gaining popularity in developed countries due to their convenience and freshness (Montero-Calderón and Martín-Belloso, 2016). However, the typical minimal processing operations, such as peeling and cutting, increases the surface area of the fruit promoting browning, softening, decay, off-flavor and microbial growth that in turn shortens the shelf life of fresh-cut pineapple (Azarakhsh *et al*., 2012). Therefore, additional innovative strategies should be developed, such as using

Received for publication: 11 November, 2018. Accepted for publication: 8 August, 2019 Doi: 10.15446/agron.colomb.v37n3.76173

[1] Instituto Universitario de La Paz UNIPAZ. Centro de Investigaciones Santa Lucia, Barrancabermeja (Colombia).
[2] Escuela de Administración de Empresas Agropecuarias. Facultad Seccional Duitama, Universidad Pedagógica y Tecnológica de Colombia, Boyaca (Colombia).
* Corresponding author: alex.lopez01@uptc.edu.co

edible coatings, in order to extend the shelf life of fresh-cut fruits and vegetables.

Coatings are defined as mixtures of film-forming materials plus solvents and other additives (e.g., plasticizers) which, when applied to a surface and cured or dried, yield a solid protective, decorative and/or functional adherent in a thin layer (Bierwagen, 2016). Moreover, antioxidants, antimicrobial agents, oxygen scavengers, or moisture absorbers, among others can be added to coatings to obtain active coatings (Yousuf *et al*., 2018).

Over the past years, the application of coatings has become more and more important in the food field (Tharanathan, 2003; Versino *et al*., 2016). The application of coatings on food products allows an extension of shelf life of perishable and sensitive products, such as fruits and vegetables, since these materials act as an external protective layer. These coatings slow the respiration rate, reduce moisture and solute migration, gas exchange, oxidative reaction rates, and suppress physiological disorders of fresh-cut fruits (Robles-Sánchez *et al*., 2013; Yousuf *et al*., 2018).

Several polymers of natural origin have been used for the fabrication of food-edible coatings including starch (Praseptiangga *et al*., 2017), alginate (Mannozzi *et al*., 2017), chitosan (Mannozzi *et al*., 2018), pectin (Mannozzi *et al*., 2017), cellulose derivatives (Gunaydin *et al*., 2017), etc. Among these polymers, sodium alginate is well-known for its excellent film-forming properties and functionalities (López Córdoba *et al*., 2013; Mannozzi *et al*., 2017).

Alginates are a family of unbranched binary copolymers of (1-4)-linked β-D-mannuronic acid (M) and α-L-guluronic acid (G) residues of widely varying composition and sequential structure (Wüstenberg, 2015). The food industry uses alginates for specific gelling, thickening, and stabilizing applications. Sodium alginate films and coatings with food additives, such as antioxidants, antibrowning agents, antimicrobials, probiotic bacteria, etc., have also been prepared (Robles-Sánchez *et al*., 2013; Valero *et al*., 2013; Salvia-Trujillo *et al*., 2015). One of the most used additives in the fabrication of edible coating is ascorbic acid, because it has excellent antibrowning and antioxidant properties (Bierhals *et al*., 2011; Robles-Sánchez *et al*., 2013). Alginate-based edible coatings have been useful in maintaining postharvest quality of tomatoes, peaches, sweet cherries, plums, etc. (Valero *et al*., 2013; Cakmak *et al*., 2017). Recently, Mannozzi *et al*. (2017) studied the efficacy of edible coatings based on sodium alginate on the quality of blueberry fruits during 14 d of storage at 4°C. They found that the application of sodium alginate coating improved the firmness of blueberry samples as compared to the uncoated ones. Moreover, changes in the surface reflection properties in coated blueberries induced a general lower lightness and a more intense blue hue color than the control sample. The microbiological results indicated that the alginate-coated blueberry samples significantly reduced the growth kinetics of yeasts and mesophilic aerobic bacteria (Mannozzi *et al*., 2017).

Several authors have reported that alginate-based edible coatings constitute a useful tool for maintaining the quality and shelf-life of fresh-cut pineapples. Montero-Calderón *et al*. (2008) studied the effects of edible coatings on the quality of fresh-cut pineapples, based on blends of sodium alginate and glycerol emulsified with sunflower oil. The sodium alginate-based coatings were crosslinked in a calcium chloride batch containing ascorbic acid and citric acid. The use of edible coatings significantly reduced juice leakage of fresh-cut pineapples increasing their shelf-life (Montero-Calderón *et al*., 2008). Azarakhsh *et al.* (2012) optimized alginate and gellan-based edible coating formulations for fresh-cut pineapples finding that weight loss and respiration rate were significantly lower, and firmness was maintained in both optimized coated samples as compared to a control. Mantilla (2012) used the layer-by-layer technique to obtain coated fresh-cut pineapples. Coating formulation consisted of sodium alginate, trans-cinnamaldehyde (antimicrobial agent), pectin, and calcium chloride. Mantilla (2012) observed that the coating improved the shelf-life of fresh-cut pineapple up to 12 d compared to uncoated samples.

In this research, we studied the effect of edible coatings during storage, based on sodium alginate and its blends with ascorbic acid on the behavior of the physicochemical properties of fresh-cut pineapple. Uncoated and coated pineapple samples were prepared and stored at 4°C for 10 d. The physicochemical properties of the fruits were periodically monitored, and the changes that occurred due to the effects of the treatment were statistically analyzed. As far as the authors are aware, there are no studies on the effect of similar coating formulations on cut pineapples.

Materials and methods

Pineapples (*Ananas comosus* (L.) Merr) were obtained from a local supermarket from the same batch. Visual inspection was conducted to ensure consistent ripeness and absence of significant defects or physical damages that could interfere with the experiments. The fruits were washed in a 50 mg L^{-1} NaClO solution and then peeled manually and cut in pieces.

Sodium alginate (Saporiti, Argentina) and glycerol (JT Baker, Mexico) were used as coating material and plasticizer, respectively. Ascorbic acid was used as an antioxidant and antibrowning agent.

Preparation and application of coating materials

Coating solutions without ascorbic acid (AlgNa) were prepared by dissolving sodium alginate powder (1% w/v) in distilled water, heating the solution under constant stirring until the mixture became clear. Then, 0.5% (w/v) glycerol was added to the sodium alginate solution and stirred for 5 min. Coating solutions with 0.1% w/v ascorbic acid (AlgNa+AA) and sodium alginate/glycerol blends were mixed.

Fresh-cut pineapple samples were randomly divided into three groups: the control group (uncoated fruit), fruit coated with sodium alginate, and fruit coated with the sodium alginate/ascorbic acid blend. Fruits were dip-coated by immersion in the coating solutions for 90 s, drained of excess coating, and air-dried at room temperature during 24 h. Uncoated and coated samples were packed in separate polyethylene plastic bags and stored at 4°C. Changes in the fruit quality parameters (visual appearance, soluble solid content (°Brix), pH and titratable acidity (%)) were monitored after 3, 7 and 10 d of storage at 4°C according to AOAC standards (AOAC, 1998).

Fruit samples were crushed using a blender and filtered through filter paper to obtain fruit juice. The soluble solid content was measured in the fruit juice using a refractometer (Zhifong®, Changhua, Taiwan) and expressed as °Brix (AOAC 932.12). Titratable acidity (%) was determined by titration with 0.1 N NaOH up to pH 8.1 using 1 g of sample in 10 ml of distilled water (AOAC 942.15). The results were expressed in citric acid percentage. The pH of fruit samples was assessed using a digital pH meter (Oakton Instruments, Vernon Hills, IL, USA) (AOAC 981.12).

Statistical analysis

The statistical analysis was performed using the Systat Inc. software (Evanston, USA). Analysis of variance (ANOVA) and Tukey pairwise comparisons were carried out using a 95% confidence level. The experiments were performed at least in duplicate, and data were reported as mean ± standard deviation.

Results and discussion

Coating-forming solutions based on sodium alginate (AlgNa) and its blends with ascorbic acid (AlgNa+AA) were obtained. They were easy to prepare and handle and yielded a transparent and homogenous coating on the fresh-cut pineapple samples. Moreover, the coatings adhered well to the fruit and gave its surface a bright appearance (Fig. 1).

General appearance of uncoated and coated fresh-cut pineapple samples

The general appearance of uncoated and coated fresh-cut pineapple samples was visually evaluated before and after storage. On day 0, the surface of the coated fruits was bright and shiny (Fig. 1).

During storage, uncoated samples showed visible symptoms of decay, whereas fruit samples coated with sodium alginate and its blends with ascorbic acid had a better appearance during the assay, looking fresher and more attractive, mainly in AlgNa samples (Fig. 2). Robles-Sánchez *et al*. (2013) studied the influence of alginate edible coatings carrying ascorbic and citric acid as antibrowning agents

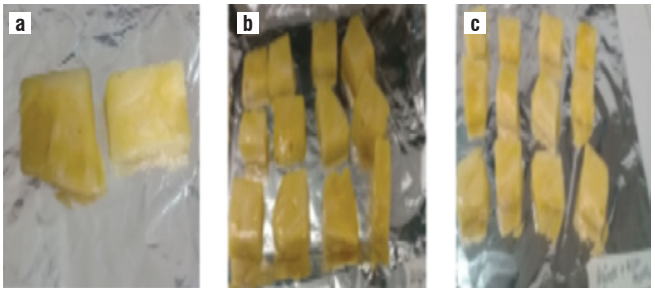


FIGURE 1. Digital images of uncoated and coated samples on day 0 of the assay. a) uncoated sample, b) coated sample with sodium alginate and c) coated sample with sodium alginate and ascorbic acid.

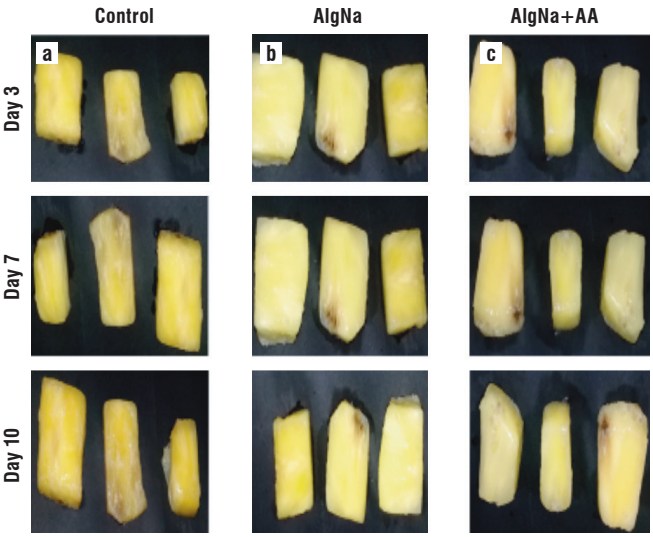


FIGURE 2. Digital images of uncoated and coated samples taken during storage. a) Uncoated sample, b) coated sample with sodium alginate and c) coated sample with sodium alginate and ascorbic acid.

on quality parameters of fresh-cut Kent mangoes stored at 4°C. They found that the combination of alginate and antibrowning agent preserved the color of fresh-cut mangoes. This was attributed to antibrowning agents such as ascorbic and citric acids, which inhibit the polyphenol oxidase activity functioning as reducing agents (o-quinones to diphenols) and pH reducers (Robles-Sánchez *et al*., 2013).

Behavior of physicochemical properties of uncoated and coated cut pineapple samples during storage

Behavior of the pH and titratable acidity (%)

The behavior of the pH and titratable acidity (%) of uncoated and coated cut pineapple samples is shown in Figures 3 and 4, respectively. At the initial time, statistically significant differences were not found between uncoated and coated samples (P<0.05); similar values of pH (~3.74) and titratable acidity (~0.64%) were obtained. These results suggest that the application of edible coatings based on sodium alginate and its blends with ascorbic acid allow the preservation of coated fresh-cut pineapple with similar characteristics to the uncoated fruit. Values of pH and titratable acidity (%) of 3.5 and 0.18, respectively, were reported by Dussan-Sarria *et al*. (2014) when working with the pineapple (*Ananas comosus*) variety Manzana.

Figure 3 shows the behavior of pH of cut pineapple samples during storage. All samples showed a gradual increase in the pH with the passing of time until reaching pH values of around 4.11 at the end of storage (P<0.05). A decrease of around 38% in the initial values of titratable acidity was observed for all samples, obtaining titratable acidity values of 0.4% for control and AlgNa samples and 0.3% for the AlgNa+AA samples (P<0.05) (Fig. 4).

The decrease of the titratable acidity (%) in both uncoated and coated pineapple samples agreed with the increase in pH of the samples (Figs. 3 and 4). In a recent study, Pizato *et al*. (2019) evaluated the effect of a different gum-based edible coating on the shelf life of fresh-cut pineapple (variety Smooth Cayenne). They also found a decrease in the acidity of the minimally processed pineapple and an increase in the pH with the passing of storage days. According to Valero *et al*. (2013), the decrease in total acidity is typical during postharvest storage and has been attributed to the use of organic acids as substrates for the respiratory metabolism in detached fruit.

Behavior of the soluble solid content

The behavior of the soluble solid content of uncoated and coated cut pineapple samples is shown in Figure 5. At the initial time, statically significant differences were not found between uncoated and coated samples (P<0.05) with values around 11 °Brix observed. With the passing of storage days, the uncoated and the coated samples showed soluble solid contents fluctuating between 10 and 12 °Brix during storage. At the end of storage, control and AlgNa samples exhibited a slight decrease in the values of soluble solid content, while in the AlgNa+AA coated samples, this parameter was similar to the initial one. It is well known that sugars are the most important components in soluble solids of fruits and that their concentration varies with the species, cultivar, maturation stage, and climate. Soluble solid contents from 7 to 14% have been reported for several

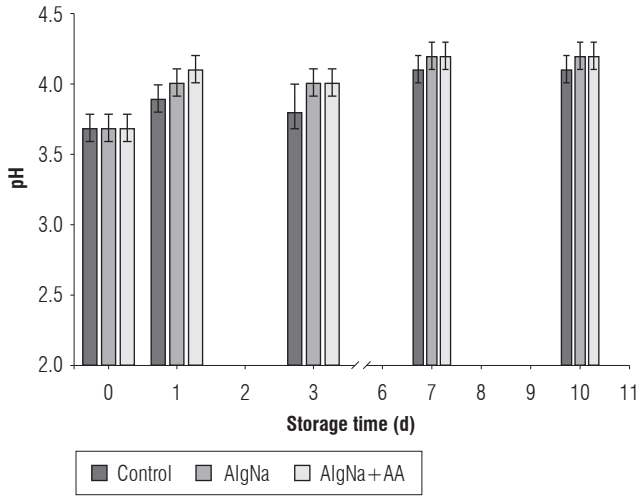


FIGURE 3. Behavior of pH during storage of uncoated (control) and coated (AlgNa and AlgNa+AA) cut pineapple samples.

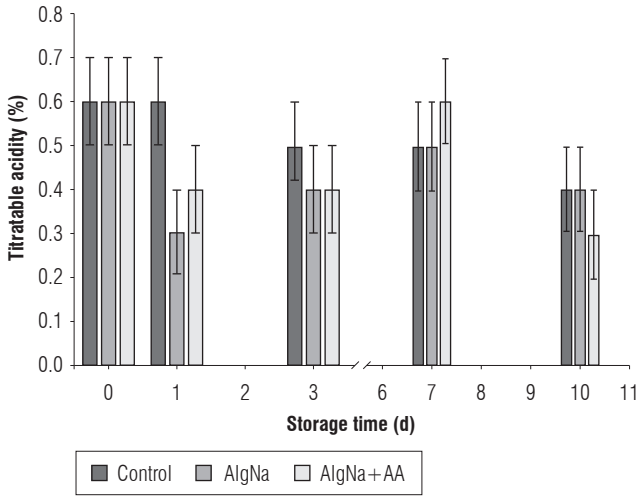


FIGURE 4. Behavior of the titratable acidity percentage during storage of uncoated (control) and coated (AlgNa and AlgNa+AA) cut pineapple samples.

pineapple cultivars (Dussan-Sarria *et al*., 2014; Pizato *et al*., 2019), and different behaviors have been observed in the soluble solid content of cut pineapple samples during refrigerated storage. For instance, Pizato *et al*. (2013, 2019), who worked with minimally processed peaches and pineapples, observed an increase in the soluble solid content of fruits with the passing of storage days. This result was attributed to the significant water loss suffered by the fruit, resulting in a concentration of total soluble solids.

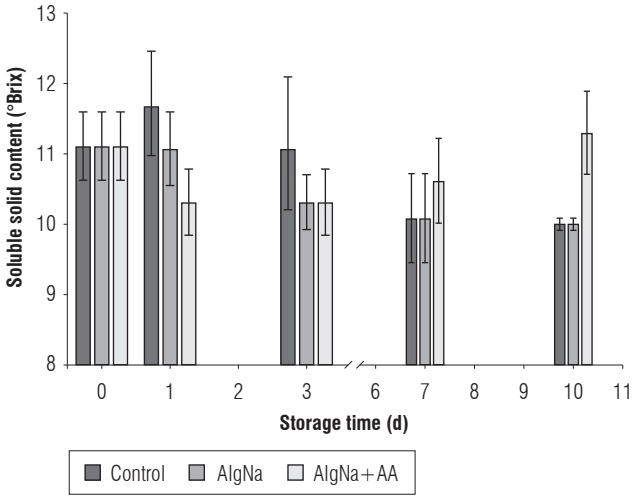


FIGURE 5. Behavior of the soluble solid content during storage of uncoated (control) and coated (AlgNa and AlgNa+AA) cut pineapple samples.

Conclusions

The application of edible coatings based on sodium alginate and its blends with ascorbic acid have proven to be a useful strategy for the preservation of the visual appearance of minimally processed pineapple under refrigerated storage. These coatings formed a good film on the surface of fresh-cut pineapple samples giving the fruit a bright, translucent, fresh-like appearance. Coated samples showed better appearance during storage when compared to the uncoated ones. Overall, these results suggest that the use of sodium alginate coatings for the preservation of fresh-cut pineapple is a promising strategy that could find useful applications in postharvest technology.

Acknowledgments

The authors would like to thank the Instituto Universitario de La Paz and Universidad Pedagógica y Tecnológica de Colombia (Proyecto SGI 2461) for their financial support, facilities and equipments.

Literature cited

Ancos, B., C. Sánchez-Moreno, and G.A. González-Aguilar. 2016. Pineapple composition and nutrition. pp. 221-239. In: Lobo, M.G. and R.E. Paull (eds.). Handbook of pineapple technology. Wiley-Blackwell, Chichester, UK. Doi: 10.1002/9781118967355.ch12

AOAC. 1998. Official methods of analysis, 16th ed. AOAC INTERNATIONAL, Rockville, USA.

Azarakhsh, N., A. Osman, H.M. Ghazali, C.P. Tan, and N. Mohd Adzahan. 2012. Optimization of alginate and gellan-based edible coating formulations for fresh-cut pineapples. Int. Food Res. J. 19(1), 279-285.

Bierhals, V.S., M. Chiumarelli, and M.D. Hubinger. 2011. Effect of cassava starch coating on quality and shelf life of fresh-cut pineapple (*Ananas comosus* L. Merril Cv "Pérola"). J. Food Sci. 76(1), E62–E72. Doi: 10.1111/j.1750-3841.2010.01951.x

Bierwagen, G.P. 2016. Surface coating. Encyclopædia Britannica. URL: https://www.britannica.com/technology/surface-coating (accessed 13 November 2017).

Cakmak, H., S. Kumcuoglu, and S. Tavman. 2017. Production of edible coatings with twin-nozzle electrospraying equipment and the effects on shelf-life stability of fresh-cut apple slices. J. Food Process Eng. 2017, e12627. Doi: 10.1111/jfpe.12627

Dussan-Sarria, S., P.M. Reyes-Calvache, and J.I. Hleap-Zapata. 2014. Efecto de un recubrimiento comestible y diferentes tipos de empaque en los atributos físico-químicos y sensoriales de piña *manzana* mínimamente procesada. Inf. Tecnol. 25, 41-46. Doi: 10.4067/S0718-07642014000500007

Gunaydin, S., H. Karaca, L. Palou, B. de la Fuente, and M.B. Pérez-Gago. 2017. Effect of hydroxypropyl methylcellulose-beeswax composite edible coatings formulated with or without antifungal agents on physicochemical properties of plums during cold storage. J. Food Qual. 2017, 1-9. Doi: 10.1155/2017/8573549

López Córdoba, A., L. Deladino, and M. Martino. 2013. Effect of starch filler on calcium-alginate hydrogels loaded with yerba mate antioxidants. Carbohydr. Polym. 95(1), 315-323. Doi: 10.1016/j.carbpol.2013.03.019

Mannozzi, C., J.P. Cecchini, U. Tylewicz, L. Siroli, F. Patrignani, R. Lanciotti, P. Rocculi, M. Dalla Rosa, and S. Romani. 2017. Study on the efficacy of edible coatings on quality of blueberry fruits during shelf-life. LWT - Food Sci. Technol. 85, 440–444. Doi: 10.1016/j.lwt.2016.12.056

Mannozzi, C., U. Tylewicz, F. Chinnici, L. Siroli, P. Rocculi, M. Dalla Rosa, and S. Romani. 2018. Effects of chitosan based coatings enriched with procyanidin by-product on quality of fresh blueberries during storage. Food Chem. 251, 18-24. Doi: 10.1016/j.foodchem.2018.01.015

Mantilla, N.V. 2012. Development of an alginate-based antimicrobial edible coating to extent the shelf-life of fresh-cut pineapple. MSc thesis, Texas A&M University, Texas, USA.

Montero-Calderón, M. and O. Martín-Belloso. 2016. Fresh-cut pineapple. pp. 153-174. In: Lobo, M.G. and R.E. Paull (eds.). Handbook of pineapple technology. Wiley-Blackwell, Chichester, UK. Doi: 10.1002/9781118967355.ch9

Montero-Calderón, M., M.A. Rojas-Graü, and O. Martín-Belloso. 2008. Effect of packaging conditions on quality and shelf-life of fresh-cut pineapple (*Ananas comosus*). Postharvest Biol. Technol. 50(2), 182-189. Doi: 10.1016/j.postharvbio.2008.03.014

Pizato, S., R.C. Chevalier, M.F. Dos Santos, T.S. Da Costa, R. Arévalo Pinedo, and W.R. Cortez Vega. 2019. Evaluation of the shelf-life extension of fresh-cut pineapple (Smooth Cayenne) by application of different edible coatings. Br. Food J. 121(7), 1592-1604. Doi: 10.1108/BFJ-11-2018-0780

Pizato, S., W.R. Cortez-Vega, J.T.A. de Souza, C. Prentice-Hernández, and C.D. Borges. 2013. Effects of different edible coatings in physical, chemical and microbiological characteristics of minimally processed peaches (*Prunus persica* L. Batsch). J. Food Saf. 33(1), 30-39. Doi: 10.1111/jfs.12020

Praseptiangga, D., R. Utami, L.U. Khasanah, I.P. Evirananda, and Kawiji. 2017. Effect of cassava starch-based edible coating incorporated with lemongrass essential oil on the quality of papaya MJ9. IOP Conf. Ser. Mater. Sci. Eng. 176, 012054. Doi: 10.1088/1757-899X/176/1/012054

Robles-Sánchez, R.M., M.A. Rojas-Graü, I. Odriozola-Serrano, G. González-Aguilar, and O. Martin-Belloso. 2013. Influence of alginate-based edible coating as carrier of antibrowning agents on bioactive compounds and antioxidant activity in fresh-cut Kent mangoes. LWT - Food Sci. Technol. 50(1), 240-246. Doi: 10.1016/j.lwt.2012.05.021

Salvia-Trujillo, L., M.A. Rojas-Graü, R. Soliva-Fortuny, and O. Martín-Belloso. 2015. Use of antimicrobial nanoemulsions as edible coatings: impact on safety and quality attributes of fresh-cut *Fuji* apples. Postharvest Biol. Technol. 105, 8-16. Doi: 10.1016/j.postharvbio.2015.03.009

Tharanathan, R.N. 2003. Biodegradable films and composite coatings: Past, present and future. Trends Food Sci. Technol. 14(3), 71-78. Doi: 10.1016/S0924-2244(02)00280-7

Valero, D., H.M. Díaz-Mula, P.J. Zapata, F. Guillén, D. Martínez-Romero, S. Castillo, and M. Serrano. 2013. Effects of alginate edible coating on preserving fruit quality in four plum cultivars during postharvest storage. Postharvest Biol. Technol. 77, 1-6. Doi: 10.1016/j.postharvbio.2012.10.011

Versino, F., O.V. Lopez, M.A. Garcia, and N.E. Zaritzky. 2016. Starch-based films and food coatings: an overview. Starch/Stärke 68, 1026-1037. Doi: 10.1002/star.201600095

Wüstenberg, T. 2015. General overview of food hydrocolloids. pp. 1-68. In: T. Wüstenberg (ed.). Cellulose and cellulose derivatives in the food industry fundamentals and applications. Wiley-VCH, Weinheim, Germany. Doi: 10.1002/9783527682935.ch01

Yousuf, B., O.S. Qadri, and A.K. Srivastava. 2018. Recent developments in shelf-life extension of fresh-cut fruits and vegetables by application of different edible coatings: a review. LWT - Food Sci. Technol. 89, 198-209. Doi: 10.1016/j.lwt.2017.10.051

Evaluation of β-glucan content, viscosity, soluble dietary fiber and processing effect in grains of Ecuadorian barley genotypes

Evaluación del contenido del β-glucano, viscosidad, fibra dietética soluble y efecto del procesamiento en granos de genotipos de cebada ecuatoriana

Elena Villacrés[1]*, Diego Campaña[2], Javier Garófalo[2], Esteban Falconí[3], María Quelal[1], Janet Matanguihan[4], and Kevin Murphy[4]

ABSTRACT

We analyzed seventy barley accessions from Ecuador to determine the content of mixed-linkage β-glucan in seeds. Twelve of these materials showed a higher content than the population average 2.10% (w/w), and they were chosen to determine the relationship among β-glucan, viscosity and dietary fiber as well as the effect of scarification, cooking, roasting and malting on its content. In the 12 accessions, the content of β-glucan showed a high degree of correlation (r=0.86) with soluble dietary fiber but a low correlation with viscosity (r=-0.17). In most accessions, β-glucan increased in roasted or scarified grains. The roasting process increased the content by 35.51% (w/w) and scarification by 26.53% (w/w). Cooking decreased content by 39.92% and malting by 77.90%. The megazyme kit was used to determine the content of (1→3) (1→4)-β-D-glucan (Mixed-linkage). Results of this study show that Ecuadorian barley genotypes with a β-glucan content greater than 2.1% are suitable for human consumption and those with a lower value than 2.1% are suitable for the beer industry.

Key words: cooking, scarification, germination, malting, roasting.

RESUMEN

Setenta accesiones de cebada provenientes de Ecuador se analizaron para determinar el contenido de β-glucano en semillas. Doce de estos materiales presentaron un contenido superior a la media poblacional 2.10% (w/w) y fueron seleccionados para determinar la relación del β-glucano con la viscosidad y la fibra dietética, así como el efecto de la escarificación, cocción, tostado y malteo en su contenido. En las 12 accesiones el contenido de β-glucano presentó un alto grado de correlación (r=0.86) con la fibra dietética soluble pero baja correlación con la viscosidad (r=-0.17). En la mayoría de accesiones el β-glucano aumentó en los granos tostados o escarificados. El proceso de tostado incrementó el contenido en 35.51% (w/w) y la escarificación en 26.53% (w/w). La cocción disminuyó el contenido en 39.92% y el malteo en 77.90%. Se utilizó el kit megazyme para determinar el contenido de (1→3) (1→4)-β-D-glucano (Mixed-linkage). Resultados de este estudio muestran que los genotipos de cebada ecuatoriana con un contenido de β-glucano mayor a 2.10% son adecuados para la alimentación humana y aquellos con menor valor al indicado son adecuados para la industria cervecera.

Palabras clave: cocción, escarificación, germinación, malteo, tostado.

Introduction

Cereals including barley (*Hordeum vulgare* L.) are the most widely consumed food group in the world, particularly in developing countries, due to their low cost and high energy content (López *et al*., 2008). Indigenous groups of the inter-Andean region of Ecuador have cultivated barley as far back as the third quarter of the sixteenth century. Barley was quickly accepted among the indigenous people of the Sierra and, although they had very good native crops, the barley grain was planted for use as food and for brewing (Patiño, 1963). Most of the barley grain is used for human consumption, including traditional *machica* (toasted and ground barley flour) to make *coladas* (beverages) and *pinol* (a traditional hot drink of Ecuador made from a mixture of *machica*, panela and some sweet species), flour and baked bread, and as grain for preparing soups and desserts. In addition, barley is used in the malting industry for brewing and to a lesser extent as fodder for cattle (Villacrés, 2008).

Barley is an important source of vitamins, minerals, proteins, antioxidants and dietary fiber (Baik and Ullrich,

Received for publication: 1 January, 2019. Accepted for publication: 20 November, 2019 Doi: 10.15446/agron.colomb.v37n3.77101

[1] Departamento de Nutrición y Calidad, Estación Experimental Santa Catalina, INIAP, Mejia (Ecuador).
[2] Programa de Cereales, Estación Experimental Santa Catalina, INIAP, Mejia (Ecuador).
[3] Programa de Cereales, Estación Experimental Santa Catalina, INIAP, Mejia (Ecuador) (until January 2016).
[4] Sustainable Seed Systems Lab, Department of Crop and Soil Sciences, Washington State University, Pullman, WA (USA).
* Corresponding author: elena.villacres@iniap.gob.ec

2008; Havrlentová *et al*., 2011). Dietary fibers in cereals are complex carbohydrates with ten or more monomeric units. These fibers are cellulose, hemicellulose, pectin, and β-glucans, among other compounds, and their structural differences determine their physical, chemical and physiological properties (FAO, 2010). β-glucans constitute approximately 75% of the cell wall of the endosperm of the barley grain (Sarwar *et al*., 2013). The structure of β-glucan has a significant impact on human health, specifically its effect of lowering total cholesterol and reducing the risk of cardiovascular diseases (Havrlentová e*t al*., 2011). β-glucan has been shown to reduce postprandial glucose by increasing the viscosity in the upper digestive tract and providing a feeling of satiety sooner. A balanced diet rich in dietary fiber provides immense benefits to cardiovascular health, reducing between 10-20% the low-density lipoproteins (LDL). In addition, the rheological properties of β-glucans have promoted dietary fiber incorporation into different food with the aim of improving the stability, texture and shelf life of food products (Wood, 2007; Alminger and Eklund-Jonsson, 2008; Regand *et al*., 2011; Pizarro *et al*., 2014). The objectives of this study were to measure the β-glucan content of accessions in the seeds of Ecuadorian elite barley collection of the Instituto Nacional de Investigaciones Agropecuarias (INIAP), guide the use of accessions, establish the relationship among β-glucan, viscosity and dietary fiber and determine the effect of several industrial processes on β-glucan content.

Materials and methods

Plant material and raw sample processing

This research was conducted at the Santa Catalina Research Station in the laboratories of the Nutrition and Quality Department of INIAP, Ecuador. Initial characterizations were based on the β-glucan content of seventy barley accessions from elite lines and varieties of yield trials grown in 2017 at Santa Catalina Research Station. A complete block design was applied with three replicates. One sample of each repetition was taken for the analysis (Supplementary material 1) for a total of 70 materials. The grains were ground in an analytical mill (Retsch Zn 200®, Hann, Germany) with a 42-mesh sieve (355 μm) to obtain a homogeneous powder. To express the results on a dry basis, the moisture content of the flour was determined by weight difference drying.

β-glucan analysis

The Megazyme kit (Megazyme International, Ireland) was used specifically for the mixed-linkage (1→3) (1→4)-β-D-glucan assay. This enzyme assay allows for the direct analysis of β-glucan in raw and cooked flour slurries in which the fine particles are suspended and hydrated in a buffer solution at pH 6.5 (Doehlert *et al*., 1997). The suspension was incubated with a lichenase enzyme to depolymerize into tri-, tetra-, and higher degree oligosaccharides, which were then hydrolyzed to glucose using β-D-glucosidase. The D-glucose produced was quantified using the reagent glucose/oxidase/peroxidase (Mcclear and Glennie-Holmes, 1985). Three replicates from each sample were analyzed in a completely randomized design (CRD) using ANOVA with the statistical software InfoStat (Di Rienzo *et al*., 2015). The LSD (0.05) test was used to perform means separation.

Viscosity determination

Viscosity was determined using a Cannon Frenskey glass capillary viscometer following the method by Alvarado and Aguilera (2001).

Soluble dietary fiber quantification

The soluble dietary fiber was quantified using a modified Method 991.42 and Method 993.19 of AOAC (AOAC, 1995). The modification consisted in the dispersion of the sample in a pH 7.4 phosphate buffer and the addition of bile and pancreatic enzymes. An ANOVA was conducted to analyze the data which was organized under a completely randomized design (CRD) using InfoStat (Di Rienzo *et al*., 2015). An LSD test (0.05) was applied to conduct means separation. Twelve accessions with a β-glucan content greater than 2.48 were selected for further assays to determine viscosity, soluble dietary fiber, and the effect of four industrial processes on β-glucan content.

Effect of processing on β-glucan content of grain

Twelve barley accessions with high β-glucan content in the initial tests were further analyzed to determine the effect on the β-glucan content of four different industrial food processes listed here: scarification, roasting, cooking, and malting. One kilogram of grain was scarified in a Strong-Scott 07810 abrasive system (Strong-Scott, Minnesota, USA) for 1 min. A second set of grains (1 kg) was roasted in a Barber Colman, 471 P model oven (Rockford, Illinois, USA) at 120°C for 10 min at a speed of 10 rpm. A third set of grains (1 kg) was cooked in an open pot using an electric stovetop at 91°C for 40 min, maintaining a grain:water ratio of 1:3. At the end of the set time, the cooking water was removed and the grain was dried in an oven (Memmert, Schwabach, Germany) at 60°C for 5 h. A fourth set of grains (1 kg) was malted, a process which includes soaking the grains in water at 16°C for 12 h, then draining the water and allowing the grains to germinate in a Barber-Colman oven (Rockford, Illinois, USA) at 90% humidity at 16°C for 72 h. At this point in the malting process, the

grains were dried in a Barber-Colman dryer to stop the germination process. After processing, the grains were milled and analyzed for β-glucan content, with the results expressed as a percentage of sample dry weight. There were three replicates per treatment, and the data were analyzed in a completely randomized design (CRD) with factorial structure (AxB) using InfoStat (Di Rienzo *et al*., 2015). An LSD (0.05) test was conducted for the means separation of significant factors.

Results and discussion

β-glucan content in seventy accessions of raw barley

Barley accessions showed significant variability in β-glucan content with mean values from 0.38% to 3.74% (w/w) (Supplementary material 1). Twenty-eight of the accessions had a β-glucan content which ranged from 1.82% to 2.30% (w/w) and 86% of the evaluated accessions had β-glucan content below 2.78% (Fig. 1). The accessions with a β-glucan content lower than 2.10% (w/w) have the potential to be used in the brewing industry and in the animal feed industry for monogastric animals. In studies with chickens and rats, the main deleterious component of barley grain is the hydrocolloidal β-D-glucan. Probably because of the increase in the viscosity of the intestinal contents this causes poor performance for chicks (Griffey *et al*., 2010). β-glucans decreases the activity of endogenous enzymes, alters intestinal morphology and modifies microflora composition and activity, reducing nutrient digestion and absorption and increasing the susceptibility to disease (Jacob and Pescatore, 2014). The β-glucan contents of the remaining 32 cultivars and accessions were higher than 2.10% (w/w). Those in the range of 2% - 4% (w/w) can be used for animal feed, while those with β-glucan content higher than 3% can be used for human consumption, either consumed directly as cooked or roasted grain or processed as ingredients for different food products. Barley accessions with high β-glucan content can also be used as raw material for obtaining soluble dietary fiber (SDF) to enrich different foods. Barley bran, which is rich in β-glucan, can be incorporated into oriental noodles and pasta products. Consumption of cereal fiber, such as that from barley, has been shown to have an effect on cardiovascular health by reducing low-density lipoprotein (LDL) cholesterol by about 10-20%. In addition, cereal fiber helps to reduce blood glucose levels and provides a greater sense of satiety (Wood, 2007).

Based on the low β-glucan content, the following varieties are used as checks for malted barley accessions: Metcalfe, Scarlett, Clipper and Harrington. According to this, accessions with β-glucan content of 2.10% (w/w) or lower are appropriate for the brewing industry, since high levels of β-glucan in a malt means incomplete cell wall degradation, diminished mobilization of starch and proteins for hydrolysis, and lower malt extract values, hence, less available nutrients for fermentation during brewing. Moreover, β-glucans, in association with polyphenols, proteins and other polysaccharides, can be components of chill hazes, which form during beer storage and give the first visual impression to the consumer of beer quality (Jadhav *et al*., 1998). The amount and molecular weight of β-glucans in malt affects the viscosity of must and beer. Barley β-glucans also negatively influence turbidity in beer (Speers *et al*., 2005).

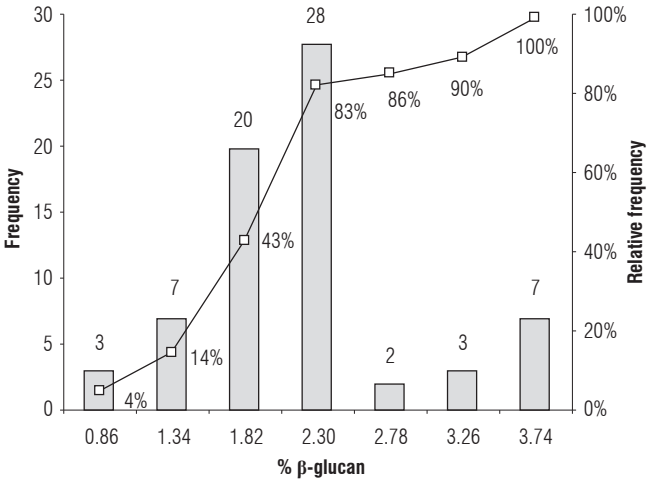


FIGURE 1. Histogram of frequencies of β-glucan percentage in 70 barley accessions.

There were no significant differences when hulled barley accessions were compared to hulless barley accessions for β-glucan content. No statistical differences were found for β-glucan content in the 27 two-row accessions with relation to the 43 six-row accessions with mean values of 2.0% and 1.9% (w/w), respectively. The barley accessions with the highest β-glucan content were INIAP-Guaranga and barley breeding lines CD-09-009 and CM-09-007 with β-glucan contents of 3.74% (w/w), 3.72% (w/w) and 3.63% (w/w), respectively.

β-glucan, viscosity and soluble dietary fiber in selected barley cultivars

The twelve accessions with the highest β-glucan content were selected for further assays to determine viscosity, soluble dietary fiber, and the effect of four industrial processes on β-glucan content.

The correlation coefficient showed a low dependence of the β-glucan content on viscosity (Fig. 2), suggesting that

in addition to β-glucan, viscosity is also affected by other components. These results are similar to those reported by Knutsen and Holtekjølen (2007), who analyzed 16 barley cultivars from Norway, Canada and Denmark and did not find significant differences in the β-glucan content. Their results confirm that β-glucan is located mainly in the aleurone layer surrounding the endosperm, rather than in the grain husk (Knutsen and Holtekjølen, 2007). β-glucan content is influenced by genotypic and environmental factors (Doehlert *et al*., 1997; Jadhav *et al*., 1998), though the genetic background of barley is considered more important than environmental conditions as a determinant of the final β-glucan content of the grain (Doehlert *et al*., 1997). Both hulled and hulless (naked) barley have a higher percentage of Fe, P, Zn and K in their grains compared to the other cereals commonly grown in Ecuador. However, the mineral concentration in hulled barley decreases by approximately 32% after the pearling process (Villacrés and Rivadeneira, 2005), suggesting that an emphasis on breeding hulless or naked types will be beneficial to the overall nutrition of people who consume barley.

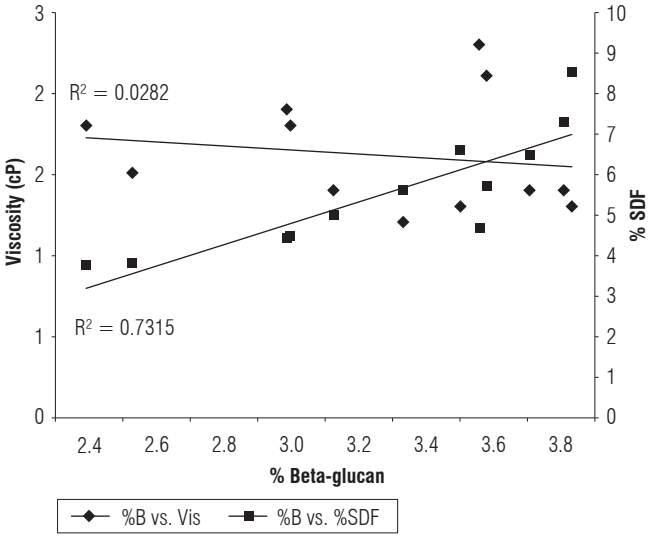


FIGURE 2. β-glucan viscosity and soluble dietary fiber percentages in barley cultivars.

The correlation coefficient demonstrated a lack of a positive relationship between the β-glucan content and viscosity. Barley accessions with β-glucan content higher than 2.10% showed a high degree of correlation with soluble dietary fiber (SDF) concentration, suggesting that in addition to β-glucan, viscosity is also affected by other components. These can include arabinoxylans or pentosans which are not hydrolysed by β-glucanases (Fig. 2). Other authors indicate that water extracts of ground barley contain gums with low-molecular weight (about 200,000 D) β-D-glucans and pentosans (Prentice, 2000). Several studies indicate that the molecular structure and structural organization of β-glucan and arabinoxylan in oats and barley are important in determining their physical properties such as water solubility, viscosity (gelation) and digestibility (Lazaridou and Biliaderis, 2007; Izydorczyk *et al*., 2008; Skendi and Biliaderis, 2016). This correspondence is due to the fact that β-glucans are the major components of the SDF in barley (Yoon *et al.,* 1995; Bunzel *et al*., 2001; Mudgil and Barak, 2013).

Effect of processing on β-glucan content of grain

The aqueous cooking of barley grains caused a decrease of 39.9% in the β-glucan content of the 12 accessions evaluated compared to the β-glucan content of raw grain (Tab. 4). The accessions that had the greatest reduction compared to the raw grain were CH-09-011, CD-09-013, and CD-09-008, with a decrease of 58.5, 55.4 and 52.2%, respectively (Fig. 2). This could be attributed to the solubility of β-glucan in hot water. In contrast, the grain roasting process increased β-glucan content by 35.5% compared to the raw grain (Tab. 4). The accessions that showed a significant increase in β-glucan content with the roasting process were CD-09-013, CM-09-007 and CH-09-011, with a 60.9, 55.6 and 54.8% increase compared to the raw grain (Fig. 2). Roasting involves heat and mechanical shearing which may have affected the molecular and structural characteristics of β-glucans and their interaction with other barley components, thus having an impact on β-glucan content (Lazaridou and Biliaderis, 2007). The high temperatures during the roasting process may have also inhibited the activity of β-glucanases, so that β-glucan was not hydrolyzed and was present in higher concentrations (Lazaridou and Biliaderis, 2007).

In the scarified or polished grain, the β-glucan content increased by 26.5% compared to the raw grain. Three accessions (CH-09-006, INIAP-CAÑICAPA and CM-09-007) showed a significant increase in β-glucan content in relation to the raw grain, with increases of 69.23, 48.49, and 46.01% (Fig. 2). This effect could be due to the removal of the external peel adhering to the pericarp while retaining the outer cell layer where β-glucans are concentrated. Regarding this, Knutsen and Holtekjølen (2007) and Marconi *et al*. (2000) suggest that β-glucan content is not significantly affected by the polishing and scarifying process, though, in general, there could be an increase in the β-glucan content. Additionally, abrasion removes the seed coat, the aleurone and subaleurone layers, and the germ, resulting in polished grain rich in starch, β-glucans and protein (Jadhav *et al*., 1998).

TABLE 1. Effect of industrial food processing on the β-glucan content of barley accessions.

Accession	Percentage β-glucan content of different accessions					Percentage loss or gain of β-glucans in relation to crude grain			
	Raw grain	Cooked	Roasted	Scarified	Malted	Cooked	Roasted	Scarified	Malted
CD-09-008	3.12 ± 0.01	1.49 ± 0.10	4.07 ± 0.05	3.88 ± 0.26	0.88 ± 0.03	-52.24	30.45	24.36	-71.79
CD-09-009	3.72 ± 0.03	1.88 ± 0.16	4.53 ± 0.18	4.35 ± 0.12	1.15 ± 0.23	-49.46	21.77	16.94	-69.09
CD-09-013	3.4 5 ± 0.08	1.54 ± 0.14	5.55 ± 0.22	4.79 ± 0.62	0.42 ± 0.14	-55.36	60.87	38.84	-87.83
CH-09-006	2.60 ± 0.02	1.84 ± 0.18	3.73 ± 0.04	4.40 ± 0.08	1.09 ± 0.18	-29.23	43.46	69.23	-58.08
CH-09-009	3.00 ± 0.30	2.23 ± 0.59	3.25 ± 0.09	2.91 ± 0.38	1.16 ± 0.23	-25.67	8.33	-3.00	-61.33
CH-09-010	3.52 ± 0.01	1.73 ± 0.30	4.57 ± 0.56	3.72 ± 0.15	1.16 ± 0.03	-50.85	29.83	5.68	-67.05
CH-09-011	3.01 ± 0.41	1.25 ± 0.05	4.66 ± 0.38	4.15 ± 0.74	0.62 ± 0.49	-58.47	54.82	37.87	-79.40
CH-09-012	3.50 ± 0.35	3.00 ± 0.11	3.68 ± 0.35	3.03 ± 0.22	0.22 ± 0.10	-14.29	5.14	-13.43	-93.71
CH-09-014	2.48 ± 0.01	1.29 ± 0.16	3.51 ± 0.11	3.41 ± 0.38	0.27 ± 0.14	-47.98	41.53	37.50	-89.11
CM-09-007	3.63 ± 0.01	2.87 ± 0.18	5.65 ± 0.50	5.30 ± 0.20	0.75 ± 0.11	-20.94	55.65	46.01	-79.34
INIAP CAÑICAPA	3.30 ± 0.07	2.28 ± 0.02	4.42 ± 0.21	4.90 ± 0.18	0.37 ± 0.13	-30.91	33.94	48.48	-88.79
INIAP GUARANGA	3.74 ± 0.02	2.11 ± 0.37	5.25 ± 0.10	4.11 ± 0.07	0.40 ± 0.08	-43.58	40.37	9.89	-89.30
Mean ± SD	3.26 ± 0.42	1.96 ± 0.57	4.75 ± 1.51	4.15 ± 0.80	0.71 ± 0.30	-39.92	35.51	26.53	-77.90

The malting process decreased the β-glucan content by as much as 78.3% compared to the raw grain. The accessions that showed a greater reduction in β-glucan content due to the malting process were CH-09-012, INIAP-GUARANGA, and CH-09-014, with a decrease of 93.71, 89.30 and 89.11% (Tab. 4) (Fig. 2), which can be attributed to the enzymatic activity during grain germination. The degradation of endosperm cell walls and the subsequent change in β-glucan levels during malting are largely related to the activity of β-glucanases, which depolymerize β-glucans and influence their reduction during the malting process (Zhang *et al*., 2002). Therefore, the content of β-glucan in the malted grain results from the initial content of each accession and the effect of the β-glucanases. However, the two characteristics have independent genetic effects.

Conclusions

An analysis of β-glucan in 70 barley accessions showed variations between 0.38% and 3.74% (w/w). Twelve of these materials had a β-glucan content greater than 2.10% (w/w) and were subjected to four industrial processes to determine their effect on the β-glucan content. This compound was increased in the grain by roasting and scarification, while cooking and malting caused a decrease in β-glucan content, helping to guide the use of the grain. Thus, accessions with a content higher than 3% can be used for human consumption, either consumed directly as cooked or roasted grain, or processed as ingredients for various food products. Accessions with a content of less than 2.10% have the potential to be used in the brewing and animal feed for monogastric animal industries.

Literature cited

Alminger, M. and C. Eklund-Jonsson, 2008. Whole-grain cereal products based on a high-fibre barley or oat genotype lower post-prandial glucose and insulin responses in healthy humans. Eur. J. Nutr. 47, 294. Doi: 10.1007/s00394-008-0724-9

Alvarado, J. de D. and J.M. Aguilera, 2001. Métodos para medir propiedades físicas en industrias de alimentos. Editorial Acribia, Zaragoza, Spain.

AOAC. 1995. Official methods of analysis of the association of official analytical chemists international. Method 991.43. Arlington, Virginia, USA.

Baik, B.K. and S.E. Ullrich. 2008. Barley for food: characteristics, improvement, and renewed interest. J. Cereal Sci. 48, 233-242. Doi: 10.1016/j.jcs.2008.02.002

Bunzel, M., J. Ralph, J.M. Marita, R.D. Hatfield, and H. Steinhart. 2001. Diferulates as structural components in soluble and insoluble cereal dietary fibre. J. Sci. Food Agric. 81, 653-660. Doi: 10.1002/jsfa.861

Di Rienzo, J., F. Casanoves, M.G. Balzarini, L. González, A. Julio, M. Tablada, and C.W. Robledo. 2015. InfoStat. InfoStat Group, Facultad de Ciencias Agropecuarias, Universidad Nacional de Córdoba, Argentina.

Doehlert, D.C., D. Zhang, M.S. McMullen, and W.R. Moore. 1997. Estimation of mixed linkage beta-glucan concentration in oat and barley from viscosity of whole grain flour slurry. Crop Sci. 37, 235-238.

FAO. 2010. Codex Alimentarius. Guidelines on nutrition labelling CA/GL 2-1985 as last amended 2010. Rome.

Griffey, C., W. Brooks, M. Kurantz, W. Thomason, F. Taylor, D. Obert, R. Moreau, R. Flores, M. Sohn, and K. Hicks. 2010. Grain composition of Virginia winter barley and implications for use in feed, food, and biofuels production. J. Cereal Sci. 51, 41-49. Doi: 10.1016/j.jcs.2009.09.004

Havrlentová, M., Z. Petruláková, A. Burgárová, F. Gago, A. Hlinková, and E. Šturdík. 2011. Cereal β-glucans and their significance for the preparation of functional foods: a review. Czech J. Food Sci. 29, 1-4.

Izydorczyk, M.S., T.L. Chornick, F.G. Paulley, N.M. Edwards, and J.E. Dexter. 2008. Physicochemical properties of hull-less barley fibre-rich fractions varying in particle size and their potential as functional ingredients in two-layer flat bread. Food Chem. 108, 561-570. Doi: 10.1016/j.foodchem.2007.11.012

Jacob, J.P. and A.J. Pescatore. 2014. Barley β-glucan in poultry diets. Ann. Transl. Med. Doi: 10.3978/j.issn.2305-5839.2014.01.02

Jadhav, S.J., S.E. Lutz, V.M. Ghorpade, and D.K. Salunkhe. 1998. Barley: Chemistry and value-added processing. Crit. Rev. Food Sci. 38, 123-171. Doi: 10.1080/10408699891274183

Knutsen, S.H. and A.K. Holtekjølen. 2007. Preparation and analysis of dietary fibre constituents in whole grain from hulled and hull-less barley. Food Chem. 102, 707-715. Doi: 10.1016/j.foodchem.2006.06.006

Lazaridou, A. and C.G. Biliaderis. 2007. Molecular aspects of cereal *β*-glucan functionality: physical properties, technological applications and physiological effects. J. Cereal Sci. 46, 101-118. Doi: 10.1016/j.jcs.2007.05.003

López, E.C., J.J. Ordaz, T.H. Madrigal, J.A. Morga, and R.B. Hernández. 2008. Composición química de cebadas cultivadas bajo diferentes condiciones de labranza en tres localidades del estado de Hidalgo, México. Bioagro 20, 201-208.

Marconi, E., M. Graziano, and R. Cubadda. 2000. Composition and utilization of barley pearling by-products for making functional pastas rich in dietary fiber and β-glucans. Cereal Chem. 77, 133-139. Doi: 10.1094/CCHEM.2000.77.2.133

Mcclear, B.V. and M. Glennie-Holmes. 1985. Enzymic quantification of (1→3) (1→4)-β-*D*-glucan in barley and malt. J.I. Brewing 91, 285-295. Doi: 10.1002/j.2050-0416.1985.tb04345.x

Mudgil, D. and S. Barak. 2013. Composition, properties and health benefits of indigestible carbohydrate polymers as dietary fiber: a review. Int. J. Biol. Macromol. 61, 1-6. Doi: 10.1016/j.ijbiomac.2013.06.044

Patiño, V.M. 1963. Plantas cultivadas y animales domésticos en América equinoccial I: frutales. Imprenta departamental, Cali, Colombia

Pizarro, S., A.M. Ronco, and M. Gotteland. 2014. β-glucanos: ¿qué tipos existen y cuáles son sus beneficios en la salud? Rev. Chil. Nutr. 41, 439-446. Doi: 10.4067/S0717-75182014000400014

Prentice, N. 2000. Brewers' spent grain in high fiber muffins. Baker's Digest 52(5), 22-23.

Regand, A., Z. Chowdhury, S.M. Tosh, T.M. Wolever, and P. Wood. 2011. The molecular weight, solubility and viscosity of oat beta-glucan affect human glycemic response by modifying starch digestibility. Food Chem. 129, 297-304. Doi: 10.1016/j.foodchem.2011.04.053

Sarwar, M.H., M.F. Sarwar, M. Sarwar, N.A. Qadri, and S. Moghal. 2013. The importance of cereals (Poaceae: Gramineae) nutrition in human health: a review. J. Cereals Oilseeds 4, 32-35. Doi: 10.5897/JCO12.023

Skendi, A. and C.G. Biliaderis. 2016. Gelation of wheat arabinoxylans in the presence of Cu^{+2} and in aqueous mixtures with cereal β-glucans. Food Chem. 203, 267-275. Doi: 10.1016/j.foodchem.2016.02.063

Speers, R.A., Y.L. Jin, A.T. Paulson, and R.J. Stewart. 2005. Los Beta-Glucanos de cebada y su degradación durante el malteado y la fabricación de cerveza. Cerveza y Malta 168, 23-35.

Villacrés, E. 2008. La cebada: un cereal nutritivo (50 recetas para preparar). Editorial Grafistas, Quito.

Villacrés, E. and M. Rivadeneira. 2005. Barley in Ecuador: Production, grain quality for consumption, and perspectives for improvement. pp. 127-137. In: Grando, S. and H. Gómez (eds.). Food barley importance, uses local knowledge. ICARDA, Aleppo, Siria.

Wood, P.J. 2007. Cereal *β*-glucans in diet and health. J. Cereal Sci. 46, 230-238. Doi: 10.1016/j.jcs.2007.06.012

Yoon, S.H., P.T. Berglund, and C.E. Fastnaught. 1995. Evaluation of selected barley cultivars and their fractions for β-glucan enrichment and viscosity. Cereal Chem. 72, 187-190.

Zhang, G., W. Junmei, and C. Jinxin. 2002. Analysis of β-glucan content in barley cultivars from different locations of China. Food Chem. 79, 251-254. Doi: 10.1016/S0308-8146(02)00127-9

SUPPLEMENTARY MATERIAL 1. Ecuadorian elite barley collection used in the study.

Nº	Code Accession	Pedigree	% β-glucan
1	INIAP GUARANGA*	JAZMIN/CARDO//TOCTE	3.74 ± 0.02
2	CD-09-009**	CAMELOT/ALELI	3.72 ± 0.04
3	CM-09-007**	LEGACY*2/5ATACO/BERMEJO/HIGO/3/CLN-B/80.5138//GLORIA-BAR/COPAL/4/CHEVRON-BAR	3.63 ± 0.01
4	CH-09-010**	OPTIMA-BAR//BLLU//LA MOLINA 94	3.52 ± 0.01
5	CH-09-012**	M94060003	3.50 ± 0.35
6	CD-09-013**	MSEL/AZAF	3.45 ± 0.08
7	INIAP CAÑICAPA*	INIAP-SHYRI 89/3/GAL/PI6384//ESC-II-72-607-1E-1E-1E-5E	3.30 ± 0.07
8	CD-09-008**	INIAP SHYRI 89/GRIT 43	3.12 ± 0.01
9	CH-09-011**	MJA/BRB2//QUINA/5/DC-B/SEN/3/AGAVE/YANALA//TUMBO/4/CEN.B/2*CALI92/6/TOCTE/3/GAL/PI6384//ESC.II.72.607.1E.4E.5E	3.01 ± 0.41
10	CH-09-009**	CIRUELO	3.00 ± 0.30
11	CH-09-006**	MJA/BRB2//QUINA/5/DC-B/SEN/3/AGAVE YANALA//TUMBO/4/CEN.B*CALI92/6/TOCTE/3/GAL/PI6384//ESC.II.72.607.1E.4E.5E	2.60 ± 0.02
12	CH-09-014**	PFC9202/LA MOLINA94	2.48 ± 0.01
13	CH-09-016**	TOCTE/JUGL//SUCHE	2.23 ± 0.18
14	CN-09-003**	H.V-III.37.E1-0E-0E-0E	2.23 ± 0.22
15	CD-09-012**	INIAP SHYRI 89/GRIT 8	2.22 ± 0.12
16	CH-09-013**	ZIGZIG/BLLUP//PETUNIA1	2.21 ± 0.10
17	CD-09-002**	ANDESS297.91/BSRD1.72	2.17 ± 0.22
18	CN-09-011**	H.V-III.37.E1-0E-0E-0E	2.17 ± 0.02
19	CN-09-005**	H.V-IV.65.E1-0E-0E-0E	2.15 ± 0,09
20	CN-09-016**	H.V-I.20.E1-0E-0E-0E	2.14 ± 0.06
21	CD-09-004**	INIAP SHYRI 89/GRIT 9	2.12 ± 0.12
22	CD-09-006**	INIAP SHYRI 89/GRIT 3	2.11 ± 0.12
23	CH-09-001**	PETUNIA 2/3/GAL/PI6384//ESC.II.72.607.1E.4E.5E	2.11 ± 0.13
24	CH-09-008**	MJA/BRB2//QUINA/5/DC-B/SEN/3/AGAVE YANALA//TUMBO/4/CEN.B*CALI92/6/TOCTE/3/GAL/PI6384//ESC.II.72.607.1E.4E.5E CBSS99M00383D-8B-1Y-2B-1Y-0B-0E-0E	2.10 ± 0.08
25	CLIPPER***	PROCTOR/PRIOR A	2.06 ± 0.74
26	RITA PELADA** *	Variedad Criolla	2.05 ± 0.18
27	CN-09-015**	H.V-I.11.E2-0E-0E-0E	2.00 ± 0,30
28	CH-09-017**	TOCTE/JANE//TOCTE/SUCHE	1.98 ± 0.12
29	CN-09-004**	H.V-IV.52.E4-0E-0E-0E	1.97 ± 0.27
30	CD-09-007**	INIAP SHYRI 89/GRIT 19	1.95 ± 0.09
31	CN-09-007**	H.V-1.2.E1-0E-0E-0E	1.95 ± 0,04
32	CH-09-005**	ATAH92/GOB//F101.78/3/ARUPO/K8755//MORA	1.93 ± 0.08
33	CH-09-015**	MN BRITE/LEGACY	1.92 ± 0.16
34	CM-09-001**	CAMELOT/ALELI	1.89 ± 0.05
35	METCALFE***	ACOxbow/Manley	1.89 ± 0.12
36	CD-09-011**	INIAP SHYRI 89/GRIT 17	1.86 ± 0.20
37	CN-09-012**	H.V-1.2.E1-0E-0E-0E	1.84 ± 0.07
38	CN-09-009**	H.V-IV.61.E1-0E-0E-0E	1.84 ± 0.11
39	CM-09-004**	CALI92/KASOTA/CALI92/ROBUST	1.83 ± 0.09
40	CN-09-006**	H.V-III.37.E1-0E-0E-0E	1.83 ± 0.14
41	CN-09-001**	H.V-I.16.E3-0E-0E-0E	1.82 ± 0.11
42	CN-09-018**	H.V-II.31.E5-0E-0E-0E	1.81 ± 0,12

Continue

Nº	Code Accession	Pedigree	% β-glucan
43	SCARLET***	03C0602426	1.70 ± 0.20
44	CH-09-003**	EBC(A)/PALTON//CABUYA	1.75 ± 0.01
45	INIAP ATAHUALPA*	SUTTER/GLORIA“S”/COMES“S” /3/PI6384/CAPUCHONA	1.74 ± 0.78
46	CD-09-005**	INIAP SHYRI 89/GRIT 20	1.72 ± 0.10
47	CN-09-008**	H.V-I.20.E1-0E-0E-0E	1.70 ± 0.09
48	CD-09-001**	INIAP SHYRI 89/GRIT 7	1.68 ± 0.30
49	CH-09-004**	QUINN/ALOE//CARDO/3/CIRU	1.68 ± 0.05
50	CN-09-010**	H.V-III.37.E1-0E-0E-0E	1.68 ± 0.10
51	CD-09-010**	INIAP SHYRI 89/GRIT 10	1.66 ± 0.02
52	INIAP QUILOTOA*	LIGNEE527/4/MCU33/FZA//TIB/3/PI356456/5/LIGNEE527/F7 70077	1.59 ± 0.20
53	INIAP PACHA*	NIAP-SHYRI 89/GRIT	1.54 ± 0.16
54	CN-09-002**	H.V-I.16.E3-0E-0E-0E	1.54 ± 0.13
55	CM-09-006**	STANDER-BAR/CABUYA	1.52 ± 0.06
56	CN-09-014**	H.V-I.20.E3-0E-0E-0E	1.52 ± 0.30
57	CH-09-002**	PETUNIA 2/3/TOCTE/TOCTE//BERROS/4/CABUYA	1.50 ± 0.06
58	CM-09-003**	STANDER-BAR/CALI92/ROBUST	1.45 ± 0.16
59	FRANSISCANA***	Variedad criolla	1.45 ± 0.05
60	CH-09-007**	ENCINO/CIRU//CABUYA	1.36 ± 0.02
61	CN-09-013**	H.V-IV.62.E1-0E-0E-0E	1.22 ± 0.04
62	CM-09-008**	IBTA MALTERA/3/PUEBLA/CARDO/TOCTE	1.21 ± 0.05
63	CM-09-009**	HARRINGTON	1.17 ± 0.17
64	INIAP DORADA*	CI9650 Col. M, 1963	1.14 ± 0.14
65	CM-09-005**	CALI92/ROBUST/PENCO/CHEVRONBAR/3/SLLO/ROBUST/QUINA	1.17 ± 0.17
66	CM-09-002**	STANDER-BAR/IBTA MALTERA	0.97 ± 0.05
67	CN-09-017**	H.V-IV.65.E1-0E-0E-0E	0.94 ± 0.02
68	INIAP TERAN*	SELEC. Abyssinian 669 Col. M. 1970	0.53 ± 0.07
69	CM-09-011**	NIOBE	0.49 ± 0.03
70	CM-09-010**	CAMELOT	0.38 ± 0.06

* INIAP improved varieties, ** material from ICARDA, *** local samples/brewers.

AUTHOR INDEX OF *AGRONOMÍA COLOMBIANA* VOLUME 37, 2019

Author name	Article title	Location
Alcântara Rodrigues Aliny Heloísa	Different soaking times and niacin concentrations affect yield of upland rice under water deficit conditions	No. 2, pp. 166-172
Aldana-Usme Andrea	Edible coatings based on sodium alginate and ascorbic acid for application on fresh-cut pineapple (*Ananas comosus* (L.) Merr)	No. 3, pp. 317-322
Álvarez-Herrera Javier	Water potential in cape gooseberry (*Physalis peruviana* L.) plants subjected to different irrigation treatments and doses of calcium	No. 3, pp. 274-282
Andres Adriana Noemi	Cross-species transfer of SSR markers in *Setaria sphacelata* and *Trichloris crinita* sp.	No. 2, pp. 112-119
Antwi Micheal Akwasi	Prioritization on cultivation and climate change adaptation techniques: a potential option in strengthening climate resilience in South Africa	No. 1, pp. 62-72
Aranda-Camacho Yesid	An analytical model to evaluate the performance of associative seed producer organizations in the framework of Plan Semilla - Agrosavia (Colombia)	No. 2, pp. 173-188
Araújo de Freitas Gilson	Phosphate fertilization on soils with improved fertility in the Brazilian Cerrado	No. 1, pp. 39-46
Araya Mario	Effect of nematicide rotation on banana (*Musa* AAA cv. Williams) root nematode control and crop yield	No. 2, pp. 153-165
Arce-Barboza Blanca	Effect of climate variability on *Collaria scenica* (Hemiptera: Miridae) on the Bogotá plateau	No. 1, pp. 47-61
Argentel-Martínez Leandris	Global warming is reducing the tillering capacity and grain yield of wheat in Yaqui Valley, Mexico	No. 1, pp. 90-96
Argüelles-Cárdenas Jorge	Yield and physicochemical quality of *Physalis peruviana* L. fruit related to the resistance response against *Fusarium oxysporum* f. sp. *physali*	No. 2, pp. 120-128
Ariza-Nieto Claudia	Yield and physicochemical quality of *Physalis peruviana* L. fruit related to the resistance response against *Fusarium oxysporum* f. sp. *physali*	No. 2, pp. 120-128
Barrero Luz Stella	Yield and physicochemical quality of *Physalis peruviana* L. fruit related to the resistance response against *Fusarium oxysporum* f. sp. *physali*	No. 2, pp. 120-128
Barreto-Triana Nancy	Effect of climate variability on *Collaria scenica* (Hemiptera: Miridae) on the Bogotá plateau	No. 1, pp. 47-61
Barreto-Triana Nancy	*Leptostylus hilaris* Bates, 1872 (Coleoptera: Cerambycidae) on Tahiti lime (*Citrus latifolia* Tanaka, Rutaceae) in Colombia	No. 2, pp. 193-196
Bokelmann Wolfgang	Heritage and Patrimony of the Peasantry: an analytical framework to address rural development	No. 3, pp. 283-296
Bokelmann Wolfgang	Value chain analysis of panela production in Utica, Colombia and alternatives for improving its practices	No. 3, pp. 297-310
Boshell-Villamarin Francisco	Effect of climate variability on *Collaria scenica* (Hemiptera: Miridae) on the Bogotá plateau	No. 1, pp. 47-61
Botero Juan Pablo	*Leptostylus hilaris* Bates, 1872 (Coleoptera: Cerambycidae) on Tahiti lime (*Citrus latifolia* Tanaka, Rutaceae) in Colombia	No. 2, pp. 193-196
Calderón Jhon	Variability of P26 and P10 genes in Colombian isolates of *Potato yellow vein virus* (PYVV)	No. 2, pp. 129-143
Campaña Diego	Evaluation of □-glucan content, viscosity, soluble dietary fiber and processing effect in grains of Ecuadorian barley genotypes	No. 3, pp. 323-330
Carabajal Paladino Leonela	Cross-species transfer of SSR markers in *Setaria sphacelata* and *Trichloris crinita* sp.	No. 2, pp. 112-119
Castilla-Campos Carlos E.	Physicochemical characteristics of cacao (*Theobroma cacao* L.) soils in Colombia: Are they adequate to improve productivity?	No. 1, pp. 28-38
Castillo Jorge	The importance of non-monetary cost in start-up and annual cacao (*Theobroma cacao* L) production activities in Santander, Colombia	No. 1, pp. 73-83
Castro-Espinoza Luciano	Global warming is reducing the tillering capacity and grain yield of wheat in Yaqui Valley, Mexico	No. 1, pp. 90-96
Cayón Daniel	Effect of pollen doses on fruit formation and oil production in two hybrid palm genotypes (*Elaeis oleifera* H.B.K. Cortes x *Elaeis guineensis* Jacq.)	No. 1, pp. 12-17
Chaparro-Giraldo Alejandro	Design of expression cassettes using the *Cry*1Ba1 gene for potato (*Solanum tuberosum* L.) varieties	No. 2, pp. 103-111
Chaves Gabriel	Effect of pollen doses on fruit formation and oil production in two hybrid palm genotypes (*Elaeis oleifera* H.B.K. Cortes x *Elaeis guineensis* Jacq.)	No. 1, pp. 12-17
da Costa Leite Robson	Phosphate fertilization on soils with improved fertility in the Brazilian Cerrado	No. 1, pp. 39-46
da Costa Leite Rubson	Phosphate fertilization on soils with improved fertility in the Brazilian Cerrado	No. 1, pp. 39-46
da Motta Eder Alexandre	Crude protein contents in hybrids of *Paspalum* evaluated in the Pampa Biome of Southern Brazil	No. 2, pp. 189-192

Continued

Author name	Article title	Location
da Silva Carneiro Jefferson Santana	Phosphate fertilization on soils with improved fertility in the Brazilian Cerrado	No. 1, pp. 39-46
Dall'Agnol Miguel	Crude protein contents in hybrids of *Paspalum* evaluated in the Pampa Biome of Southern Brazil	No. 2, pp. 189-192
Darghan Aquiles	Effect of nitrogen and potassium on plant height and stem diameter of *Jatropha curcas* L. in Colombian tropical dry forest	No. 3, pp. 203-212
Deaquiz-Oyola Yuli	Physiological performance of quinoa (*Chenopodium quinoa* Willd.) under agricultural climatic conditions in Boyaca, Colombia	No. 2, pp. 144-152
Erira Natalyth	Design of expression cassettes using the *Cry*1Ba1 gene for potato (*Solanum tuberosum* L.) varieties	No. 2, pp. 103-111
Falconi Esteban	Evaluation of β-glucan content, viscosity, soluble dietary fiber and processing effect in grains of Ecuadorian barley genotypes	No. 3, pp. 323-330
Ferreira de Lima Sebastião	Different soaking times and niacin concentrations affect yield of upland rice under water deficit conditions	No. 2, pp. 166-172
Ferri Andrea Matilde	Cross-species transfer of SSR markers in *Setaria sphacelata* and *Trichloris crinita* sp.	No. 2, pp. 112-119
Fischer Gerhard	Water potential in cape gooseberry (*Physalis peruviana* L.) plants subjected to different irrigation treatments and doses of calcium	No. 3, pp. 274-282
Fuentes Juan	The importance of non-monetary cost in start-up and annual cacao (*Theobroma cacao* L) production activities in Santander, Colombia	No. 1, pp. 73-83
Gaigl Andreas	Potential of a *Cladosporium cladosporioides* strain for the control of *Tetranychus urticae* Koch (Acari: Tetranychidae) under laboratory conditions	No. 1, pp. 84-89
Gámez-Guzmán Angie	Potential of a *Cladosporium cladosporioides* strain for the control of *Tetranychus urticae* Koch (Acari: Tetranychidae) under laboratory conditions	No. 1, pp. 84-89
García-Molano José	Physiological performance of quinoa (*Chenopodium quinoa* Willd.) under agricultural climatic conditions in Boyaca, Colombia	No. 2, pp. 144-152
Garcia-Parra Miguel	Physiological performance of quinoa (*Chenopodium quinoa* Willd.) under agricultural climatic conditions in Boyaca, Colombia	No. 2, pp. 144-152
Garibaldi-Chávez Juan	Global warming is reducing the tillering capacity and grain yield of wheat in Yaqui Valley, Mexico	No. 1, pp. 90-96
Garófalo Javier	Evaluation of β-glucan content, viscosity, soluble dietary fiber and processing effect in grains of Ecuadorian barley genotypes	No. 3, pp. 323-330
Gómez-Caro Sandra	Effect of thermal and in vitro fungicide treatments on pathogens of the genus *Fusarium* associated with maize seeds	No. 3, pp. 228-238
González Hernán	Water potential in cape gooseberry (*Physalis peruviana* L.) plants subjected to different irrigation treatments and doses of calcium	No. 3, pp. 274-282
González-Almario Carolina	Yield and physicochemical quality of *Physalis peruviana* L. fruit related to the resistance response against *Fusarium oxysporum* f. sp. *physali*	No. 2, pp. 120-128
Gutiérrez-Coronado Marco	Global warming is reducing the tillering capacity and grain yield of wheat in Yaqui Valley, Mexico	No. 1, pp. 90-96
Hernández Ionel	Rhizospheric rhizobia identification in maize (*Zea mays* L.) plants	No. 3, pp. 255-262
Ingala Lorena Romina	Cross-species transfer of SSR markers in *Setaria sphacelata* and *Trichloris crinita* sp.	No. 2, pp. 112-119
Jaramillo Julio	Effect of nematicide rotation on banana (*Musa* AAA cv. Williams) root nematode control and crop yield	No. 2, pp. 153-165
Lázaro-Palacio Leisdy	An analytical model to evaluate the performance of associative seed producer organizations in the framework of Plan Semilla - Agrosavia (Colombia)	No. 2, pp. 173-188
León-Moreno Clara Esperanza	Physicochemical characteristics of cacao (*Theobroma cacao* L.) soils in Colombia: Are they adequate to improve productivity?	No. 1, pp. 28-38
Ligarreto Gustavo	Effect of pollen doses on fruit formation and oil production in two hybrid palm genotypes (*Elaeis oleifera* H.B.K. Cortes x *Elaeis guineensis* Jacq.)	No. 1, pp. 12-17
López-Córdoba Alex	Edible coatings based on sodium alginate and ascorbic acid for application on fresh-cut pineapple (*Ananas comosus* (L.) Merr)	No. 3, pp. 317-322
López-Pazos Silvio	Design of expression cassettes using the *Cry*1Ba1 gene for potato (*Solanum tuberosum* L.) varieties	No. 2, pp. 103-111
Magnitskiy Stanislav	Effect of nitrogen and potassium on plant height and stem diameter of *Jatropha curcas* L. in Colombian tropical dry forest	No. 3, pp. 203-212
Maldonado-Archila Germán	Effect of thermal and in vitro fungicide treatments on pathogens of the genus *Fusarium* associated with maize seeds	No. 3, pp. 228-238
Mariussi Luís Marcelo	Phosphate fertilization on soils with improved fertility in the Brazilian Cerrado	No. 1, pp. 39-46
Martínez Zárate Natalia	Value chain analysis of panela production in Utica, Colombia and alternatives for improving its practices	No. 3, pp. 297-310
Martins dos Santos Antônio Carlos	Phosphate fertilization on soils with improved fertility in the Brazilian Cerrado	No. 1, pp. 39-46

Continued

Author name	Article title	Location
Matanguihan Janet	Evaluation of β-glucan content, viscosity, soluble dietary fiber and processing effect in grains of Ecuadorian barley genotypes	No. 3, pp. 323-330
Mayorga-Cubillos Franklin	Yield and physicochemical quality of *Physalis peruviana* L. fruit related to the resistance response against *Fusarium oxysporum* f. sp. *physali*	No. 2, pp. 120-128
Mohammadi Mohammad Hossein	Sulfur effects on sugar content, enzyme activity and seed yield of rapeseed (*Brassica napus* L.)	No. 3, pp. 311-316
Monje Buenaventura	*Leptostylus hilaris* Bates, 1872 (Coleoptera: Cerambycidae) on Tahiti lime (*Citrus latifolia* Tanaka, Rutaceae) in Colombia	No. 2, pp. 193-196
Montenegro Omar	Effect of nitrogen and potassium on plant height and stem diameter of *Jatropha curcas* L. in Colombian tropical dry forest	No. 3, pp. 203-212
Morales Juan Gonzalo	Polyphasic identification of preharvest pathologies and disorders in avocado cv. Hass	No. 3, pp. 213-227
Morales-Coronado Denisse	Global warming is reducing the tillering capacity and grain yield of wheat in Yaqui Valley, Mexico	No. 1, pp. 90-96
Moreno-Fonseca Liz	Physiological and antioxidant responses of cape gooseberry (*Physalis peruviana* L.) seedlings to phosphorus deficiency	No. 1, pp. 3-11
Mosquera Vásquez Teresa	Variability of P26 and P10 genes in Colombian isolates of *Potato yellow vein virus* (PYVV)	No. 2, pp. 129-143
Murphy Kevin	Evaluation of β-glucan content, viscosity, soluble dietary fiber and processing effect in grains of Ecuadorian barley genotypes	No. 3, pp. 323-330
Nápoles María	Rhizospheric rhizobia identification in maize (*Zea mays* L.) plants	No. 3, pp. 255-262
Niakan Maryam	Sulfur effects on sugar content, enzyme activity and seed yield of rapeseed (*Brassica napus* L.)	No. 3, pp. 311-316
Oduniyi Oluwaseun Samuel	Prioritization on cultivation and climate change adaptation techniques: a potential option in strengthening climate resilience in South Africa	No. 1, pp. 62-72
Ortiz-Enríquez José	Global warming is reducing the tillering capacity and grain yield of wheat in Yaqui Valley, Mexico	No. 1, pp. 90-96
Oudot Maxime	Rhizospheric rhizobia identification in maize (*Zea mays* L.) plants	No. 3, pp. 255-262
Pachón-Ariza Fabio Alberto	Value chain analysis of panela production in Utica, Colombia and alternatives for improving its practices	No. 3, pp. 297-310
Pachón-Ariza Fabio Alberto	Heritage and Patrimony of the Peasantry: an analytical framework to address rural development	No. 3, pp. 283-296
Peñaranda-Rolón Andrea	Diversity of arbuscular mycorrhizal fungi communities associated with cape gooseberry (*Physalis peruviana* L.) crops	No. 3, pp. 239-254
Pérez-Benavides Alba Lucía	A first approach to chalcid wasps (Hymenoptera, Chalcididae) of the entomological museum UNAB with new records for Colombia	No. 1, pp. 18-27
Pérez-Martínez Simón	Rhizospheric rhizobia identification in maize (*Zea mays* L.) plants	No. 3, pp. 255-262
Pérez-Moncada Urley	Diversity of arbuscular mycorrhizal fungi communities associated with cape gooseberry (*Physalis peruviana* L.) crops	No. 3, pp. 239-254
Pérez-Pérez Reneé	Rhizospheric rhizobia identification in maize (*Zea mays* L.) plants	No. 3, pp. 255-262
Piñeros-Guerrero Natalia	Effect of thermal and in vitro fungicide treatments on pathogens of the genus *Fusarium* associated with maize seeds	No. 3, pp. 228-238
Pradi Vendruscolo Eduardo	Different soaking times and niacin concentrations affect yield of upland rice under water deficit conditions	No. 2, pp. 166-172
Quelal María	Evaluation of β-glucan content, viscosity, soluble dietary fiber and processing effect in grains of Ecuadorian barley genotypes	No. 3, pp. 323-330
Rameeh Valiollah	Sulfur effects on sugar content, enzyme activity and seed yield of rapeseed (*Brassica napus* L.)	No. 3, pp. 311-316
Ramírez-Gil Joaquín Guillermo	Polyphasic identification of preharvest pathologies and disorders in avocado cv. Hass	No. 3, pp. 213-227
Ramírez-Gómez Margarita	Diversity of arbuscular mycorrhizal fungi communities associated with cape gooseberry (*Physalis peruviana* L.) crops	No. 3, pp. 239-254
Ramírez-Miranda César	Heritage and Patrimony of the Peasantry: an analytical framework to address rural development	No. 3, pp. 283-296
Randazzo Cecilia Paola	Cross-species transfer of SSR markers in *Setaria sphacelata* and *Trichloris crinita* sp.	No. 2, pp. 112-119
Ribeiro da Silva Rubens	Phosphate fertilization on soils with improved fertility in the Brazilian Cerrado	No. 1, pp. 39-46
Roa-Ortiz Santiago	The importance of non-monetary cost in start-up and annual cacao (*Theobroma cacao* L) production activities in Santander, Colombia	No. 1, pp. 73-83
Rockenbach de Ávila Mariana	Crude protein contents in hybrids of *Paspalum* evaluated in the Pampa Biome of Southern Brazil	No. 2, pp. 189-192
Rodríguez Alia	Diversity of arbuscular mycorrhizal fungi communities associated with cape gooseberry (*Physalis peruviana* L.) crops	No. 3, pp. 239-254
Rodríguez-Roa Andrea	Effect of climate variability on *Collaria scenica* (Hemiptera: Miridae) on the Bogotá plateau	No. 1, pp. 47-61

Continued

Author name	Article title	Location
Rodríguez-Velásquez Edwin	Yield and physicochemical quality of *Physalis peruviana* L. fruit related to the resistance response against *Fusarium oxysporum* f. sp. *physali*	No. 2, pp. 120-128
Rojas-Molina Jairo	Physicochemical characteristics of cacao (*Theobroma cacao* L.) soils in Colombia: Are they adequate to improve productivity?	No. 1, pp. 28-38
Rosa Correa Sávio	Different soaking times and niacin concentrations affect yield of upland rice under water deficit conditions	No. 2, pp. 166-172
Roveda-Hoyos Gabriel	Physiological and antioxidant responses of cape gooseberry (*Physalis peruviana* L.) seedlings to phosphorus deficiency	No. 1, pp. 3-11
Roveda-Hoyos Gabriel	Diversity of arbuscular mycorrhizal fungi communities associated with cape gooseberry (*Physalis peruviana* L.) crops	No. 3, pp. 239-254
Rubio David	Effect of nematicide rotation on banana (*Musa* AAA cv. Williams) root nematode control and crop yield	No. 2, pp. 153-165
Salas Eduardo	Effect of nematicide rotation on banana (*Musa* AAA cv. Williams) root nematode control and crop yield	No. 2, pp. 153-165
Saraiva de Ávila Karla	Crude protein contents in hybrids of *Paspalum* evaluated in the Pampa Biome of Southern Brazil	No. 2, pp. 189-192
Schneider-Canny Raquel	Crude protein contents in hybrids of *Paspalum* evaluated in the Pampa Biome of Southern Brazil	No. 2, pp. 189-192
Seleguini Alexander	Different soaking times and niacin concentrations affect yield of upland rice under water deficit conditions	No. 2, pp. 166-172
Serna Francisco	A first approach to chalcid wasps (Hymenoptera, Chalcididae) of the entomological museum UNAB with new records for Colombia	No. 1, pp. 18-27
Serralde-Ordoñez Diana	Diversity of arbuscular mycorrhizal fungi communities associated with cape gooseberry (*Physalis peruviana* L.) crops	No. 3, pp. 239-254
Serrano Lizette	Rhizospheric rhizobia identification in maize (*Zea mays* L.) plants	No. 3, pp. 255-262
Socha Jéssica	Effect of pollen doses on fruit formation and oil production in two hybrid palm genotypes (*Elaeis oleifera* H.B.K. Cortes x *Elaeis guineensis* Jacq.)	No. 1, pp. 12-17
Sosa Daynet	Rhizospheric rhizobia identification in maize (*Zea mays* L.) plants	No. 3, pp. 255-262
Soto Gonzalo	Effect of nematicide rotation on banana (*Musa* AAA cv. Williams) root nematode control and crop yield	No. 2, pp. 153-165
Tekana Sibongile Sylvia	Prioritization on cultivation and climate change adaptation techniques: a potential option in strengthening climate resilience in South Africa	No. 1, pp. 62-72
Tobar Mauricio	Effect of nematicide rotation on banana (*Musa* AAA cv. Williams) root nematode control and crop yield	No. 2, pp. 153-165
Torres-Rojas Esperanza	Potential of a *Cladosporium cladosporioides* strain for the control of *Tetranychus urticae* Koch (Acari: Tetranychidae) under laboratory conditions	No. 1, pp. 84-89
Valenzuela-Balcázar Ibonne Geaneth	Impact of soil use on aggregate stability and its relationship with soil organic carbon at two different altitudes in the Colombian Andes	No. 3, pp. 263-273
Vargas Ángela María	Variability of P26 and P10 genes in Colombian isolates of *Potato yellow vein virus* (PYVV)	No. 2, pp. 129-143
Vergara-Navarro Erika Valentina	*Leptostylus hilaris* Bates, 1872 (Coleoptera: Cerambycidae) on Tahiti lime (*Citrus latifolia* Tanaka, Rutaceae) in Colombia	No. 2, pp. 193-196
Villacrés Elena	Evaluation of β-glucan content, viscosity, soluble dietary fiber and processing effect in grains of Ecuadorian barley genotypes	No. 3, pp. 323-330
Vintimilla Marlon	Effect of nematicide rotation on banana (*Musa* AAA cv. Williams) root nematode control and crop yield	No. 2, pp. 153-165
Visconti-Moreno Efraín Francisco	Impact of soil use on aggregate stability and its relationship with soil organic carbon at two different altitudes in the Colombian Andes	No. 3, pp. 263-273
Zabala Andrés	The importance of non-monetary cost in start-up and annual cacao (*Theobroma cacao* L) production activities in Santander, Colombia	No. 1, pp. 73-83

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 XXXVII, No. 3 SEPTEMBER-DECEMBER 2019 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