AGRONOMIA COLOMBIANA

Doi: 10.15446/agron.colomb

VOLUME XXXIX, No. 3 SEPTEMBER-DECEMBER 2021 ISSN 0120-9965

Centro Editorial
Facultad de Ciencias Agrarias
Sede Bogotá

UNIVERSIDAD
NACIONAL
DE COLOMBIA

AGRONOMIA COLOMBIANA

VOLUME XXXIX No. 3 SEPTEMBER-DECEMBER 2021 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.revistas.unal.edu.co/index.php/agrocol

http://www.scielo.org.co/scielo.php?script=sci_serial&pid=0120-9965

ISSN: 0120-9965 (Print)

ISSN: 2357-3732 (Online)

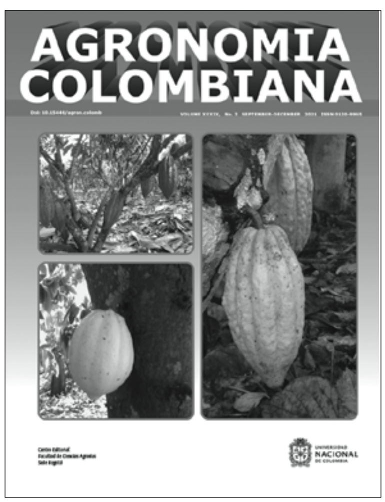
Published: Triannual
Number of copies: 20

Editorial assistance
Juan Carlos Anduckia Ávila
Ana María Díaz
Luz Grass Bernal
Mauricio Parra Quijano

Translators and English proofreaders
Thomas Defler
Paul Priolet

Electronic Design and Development
Isabel Sandoval

Printer
DGP Editores



Our cover:

Photographs (cacao plants) courtesy of Stanislav Magnitskiy

Agronomía Colombiana is a technical-scientific publication classified by Colciencias in category C 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, and is ranked Q3 in the Scimago Journal Rank (SJR). 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 XXXIX No. 3 SEPTEMBER-DECEMBER 2021 ISSN (print): 0120-9965 / ISSN (online): 2357-3732

TABLE OF CONTENTS

Parental selection and descriptor evaluation in the identification of superior cowpea genotypes

Selección de padres y evaluación de descriptores en la identificación de genotipos superiores de frijol caupí

Francisca Reijane Gadelha de Alencar[1], Charles Lobo Pinheiro[1], Francisco Linco de Souza Tomaz[2*], Cândida Hermínia Campos de Magalhães Bertini[1], and Ana Kelly Firmino da Silva[1]

ABSTRACT

The objective of this study was to evaluate and quantify the genetic divergence between accessions of cowpea, aiming to identify the characters that most contribute to diversity and select superior parents. Seventy-six cowpea accessions were evaluated using 19 descriptors that were also utilized to estimate genetic diversity using the Shannon-Weaver index. Phenology and production data were analyzed using descriptive statistics and were subjected to Pearson's correlation test. The descriptors flower color, pod color, and seed brightness showed greater variability in the quantification of genetic diversity. The genotypes Das Almas, Galanjão, selected from CE-13, 7907-Purple Hull and Caupi Branco should be incorporated into cowpea breeding programs as they exhibit desirable agronomic characters. Crossings involving the genotype 7907-Purple Hull with Das Almas and Galanjão are indicated, which could result in obtaining superior genotypes in terms of precocity and grain yield.

Key words: *Vigna unguiculata* (L.) Walp., diversity, dendrogram.

RESUMEN

El objetivo de este estudio fue evaluar y cuantificar la divergencia genética entre accesiones de fríjol caupí, para identificar los caracteres que más contribuyen a la diversidad y seleccionar padres superiores. Se evaluaron 76 accesiones de fríjol caupí utilizando 19 descriptores, que también fueron usados para estimar la diversidad genética utilizando el índice de Shannon-Weaver. Los datos de fenología y producción se analizaron mediante estadística descriptiva y se sometieron a la prueba de correlación de Pearson. Los descriptores color de flor, color de vaina y brillo de semilla presentaron mayor variabilidad en la cuantificación de la diversidad genética. Los genotipos Das Almas, Galanjão, seleccionado de CE-13, 7907-Purple Hull y Caupi Branco deben incorporarse a los programas de mejoramiento del frijol caupí ya que presentan características agronómicas deseables. Los cruces que involucran el genotipo 7907-Purple Hull con Das Almas y Galanjão están indicados con el objetivo de obtener genotipos superiores en términos de precocidad y rendimiento de grano.

Palabras clave: *Vigna unguiculata* (L.) Walp., diversidad, dendrograma.

Introduction

The cowpea [*Vigna unguiculata* (L.) Walp.] represents one of the most relevant and strategic food sources for the tropical and subtropical regions of the planet. In Brazil, the cultivation of cowpea has traditionally been concentrated in the Northern and Northeastern regions. However, it has been expanding to other regions, mainly to the Midwest, due to its wide adaptability to tropical conditions, low production costs, and intense breeding approaches applied to the cultivation (Freire Filho *et al*., 2011).

The cowpea breeding programs are usually based on the selection of parents followed by hybridization to form a base population and an advanced generation. This occurs with simultaneous selection of more than one characteristic, such as productivity, size, resistance to pests and diseases, and grain quality (Bertini *et al*., 2009). Therefore, the previous characterization of the available germplasm becomes indispensable since it allows the breeder to explore the existing genetic diversity and evaluate its potential for use in the breeding program (Oliveira *et al*., 2017).

Studies on genetic divergence are relevant because they provide estimates for the correct choice of parents that, when crossed, result in a high heterotic effect on the progenies, maximizing the chances of obtaining superior genotypes for the characters of interest (Rotili *et al*., 2012). In this sense, several multivariate techniques can be applied in the study of genetic divergence. Among them, the most

Received for publication: 10 May, 2021. Accepted for publication: 17 November, 2021. Doi: 10.15446/agron.colomb.v39n3.95660

[1] Federal University of Ceará, Fortaleza (Brazil).
[2] Universidade Federal Rural do Semi-Árido - UFERSA, Mossoró (Brazil).
[*] Corresponding author: francisco.tomaz@alunos.ufersa.edu.br

used are those involving canonical variables and main components, in addition to the grouping methods, whose application depends on the calculation of the previously estimated dissimilarity measures (Cruz *et al*., 2014).

In recent years, several authors have evaluated genetic divergence in cowpea (Santos *et al*., 2014; Sousa *et al*., 2017; Santana *et al*., 2019). For example, Santana *et al*. (2019) evaluated the genetic divergence between cowpea genotypes by morpho-agronomic traits in the state of Pernambuco, and they found that the combinations Pitiúba x Cabeçudo and Pitiúba x Manteiga could result in hybrids with greater heterotic effect. Santos *et al*. (2014) evaluated the agronomic performance and genetic divergence between cowpea genotypes grown in the Cerrado/Pantanal ecotone region. They found that the crossings of MNC03 737F 5 4 with MNC03 725F 3 and MNC03737F 5 11 obtained promising populations in terms of grain productivity.

Hence, this research aimed to evaluate and quantify the genetic divergence between 76 cowpea accessions to identify the characters that most contribute to an efficient selection of superior parents.

Materials and methods

Plant material and experiment site

Seventy-six cowpea accessions from the Germplasm Active Bank of the Federal University of Ceará (BAG/UFC) were evaluated (Tab. 1). The group of genotypes used in this study was chosen due to the great genetic variability and potential for use in breeding programs, observed during activities routinely developed at the BAG related to cowpea, such as seed multiplication and regeneration. The test was carried out in an experimental field belonging to the BAG/UFC, located in Fortaleza-CE (03°44' S, 38°34' W, and

TABLE 1. Genotypes of cowpea from the Active Germplasm Bank of the Federal University of Ceará.

N°	Register	Accession name	N°	Register	Accession name	N°	Register	Accession name
1	CE-07	Das Almas	27	CE-117	Iron K-892-C15-63	53	CE-229	Chiapas-277
2	CE-14	Potomac	28	CE-119	Selected from CE-31	54	CE-230	Progresso-66 (grená)
3	CE-27	Quem-Quem	29	CE-124	Selected from CE-92	55	CE-243	Malhado Preto
4	CE-28	Cacheado	30	CE-131	TVu 106	56	CE-252	TVu 179-P1
5	CE-30	Galanjão	31	CE-133	TVu 756	57	CE-262	TVu 381
6	CE-32	Pitombeira	32	CE-135	TVu 793	58	CE-465	TVu 2934
7	CE-33	José do Santo	33	CE-136	TVu 1015	59	CE-578	CE-578
8	CE-44	Novato	34	CE-137	TVu 1204	60	CE-599	Selected from CE-394
9	CE-46	Milagroso	35	CE-139	TVu 1233	61	CE-646	BR3-Tracateua
10	CE-48	Selected from CE-42	36	CE-140	TVu 1240	62	CE-656	CNCx 188-13-1E/P
11	CE-51	Selected from CE-13	37	CE-141	Selected from CE-134	63	CE-726	CNCx 658-26E
12	CE-52	CE-52	38	CE-143	Selected from CE-48	64	CE-895	CNCx 333-33E
13	CE-53	Selected from CE-13	39	CE-148	TVu 1248	65	CE-899	CNCx 333-38 E
14	CE-54	Selected from CE-13	40	CE-156	TVu 1593	66	CE-924	Epace 11-Jaguaribe
15	CE-58	IR-58.71	41	CE-160	TVu 1976	67	CE-931	Selected from CE-10
16	CE-62	Selected from CE-1	42	CE-161	TVu 1977	68	CE-935	BRS Gurgueia
17	CE-69	Selected from CE-1	43	CE-162	TVu 1981	69	CE-936	Canapuzinho
18	CE-74	Praiano	44	CE-171	TVu 4279	70	CE-938	BRS Paraguaçu
19	CE-83	Azulão-2	45	CE-175	TVu 4369	71	CE-959	BRS Potiguar
20	CE-90	Ferrugem	46	CE-181	Caupi Branco	72	CE-960	TVu 379
21	CE-91	TVu 966	47	CE-190	Costa Rica V-11	73	CE-962	-
22	CE-101	Hagreen-66	48	CE-191	Costa Rica V-12	74	CE-967	TVu 382
23	CE-102	7907-Purple Hull	49	CE-192	Costa Rica V-15	75	CE-970	-
24	CE-105	Clay-23	50	CE-194	Costa Rica V-39	76	CE-971	-
25	CE-106	Australia-67	51	CE-196	Costa Rica V-43			
26	CE-108	Mississipi Silver-24	52	CE-226	Guerrero-109			

altitude of 12 m a.s.l.). According to the Köppen and Geiger methodology, the region's climate is of the tropical Aw type, with an average annual temperature and precipitation of 26.3 ± 1.7°C and 1,448 mm, respectively.

Experiment conditions

Each genotype was distributed in a 5.0 m long row, with 0.5 m spacing between plants. Sowing was carried out using three seeds per hole. Fifteen days after planting, thinning was performed, leaving the most vigorous plant in each hole, with 10 plants per genotype. To avoid possible crossings between the different accessions, the spacing adopted between rows was 2.0 m. At the time of sowing, fertilization was carried out with the commercial fertilizer NPK (04-14-08), following the recommendations for the crop. The fertilizer was applied in furrows located next to the planting pits. The control of weeds, pests, and diseases was carried out according to the needs observed in the field.

Variables analyzed

The qualitative descriptors evaluated throughout the crop cycle were seedling pigmentation, flower color, inflorescence type, growth habit, central leaflet shape, plant size, pod position, pod color, pod shape, grain color, grain shape, seed brightness, seed texture, and resistance to diseases caused by viruses. For this last descriptor, a visual analysis of the plants was performed to detect the characteristic symptoms of Cowpea severe mosaic virus (CPSMV), the main pathogen of the crop (Lima *et al*., 2012). All plants were classified for possible resistance/susceptibility based on a scale ranging from 0 to 4, where a score of 0 was assigned for absence of symptoms and scores of 1, 2, 3 and 4 for 25, 50, 75 and 100% of plants with typical symptoms of these viruses, respectively.

The following quantitative characters were evaluated: initial flowering (IF), pod length (PL), number of grains per pod (NGP), the mass of 100 grains (M100G), and cycle. The statistical analysis was carried out with all the characters (14 qualitative and 5 quantitative), which were converted into multi-categorical variables.

The following variables were also collected: average number of days from emergence to full flowering (NDEFF), average number of days from emergence to pod formation (NDEPF), average number of days from emergence to physiological maturation (NDEPM), average number of days from emergence to harvest point (NDEHP), number of pods per plant (NPP), and production per plant (P/Pl).

Statistical analysis

Based on the frequency distribution of the genotypes in the different phenotypic classes of each descriptor, the genetic diversity was estimated using the Shannon-Weaver index (Shannon & Weaver, 1964), according to the following equation:

$$H' = \frac{[N \ ln(N) - \sum_{i=1}^{S} ni \ ln(ni)]}{N} \quad (1)$$

where H' is the Shannon-Weaver index; ni is the number of individuals sampled from the i-th species; N is the total number of individuals sampled; S is the total number of species sampled, and ln is the Neperian-based logarithm.

The Shannon-Weaver index (H') measures the degree of uncertainty in predicting to which species or group an individual chosen randomly, from a sample with S species and N individuals, will belong. Thus, the lower the value of H', the lower the degree of uncertainty, and, therefore, the lower the diversity of the sample (Shannon & Weaver, 1964). This index can also be used to quantify the level of entropy of the characters. The entropy of a given descriptor will be greater based on the number of phenotypic classes and the balanced frequency distribution of the accessions in the different phenotypic classes (Oliveira *et al*., 2017).

To establish a single pattern of analysis for the different variables (qualitative and quantitative), these were converted into multi-categorical variables (Cruz *et al*., 2011; Silva *et al*., 2013; Paiva *et al*., 2014) and later standardized according to the following equation:

$$Zij = \frac{Xij - \overline{Xj}}{S(Xi)} \quad (2)$$

where Z is the standardized value, with zero average and variance within the range from -1 to +1; Xij is the actual observation value within each class; $\overline{X}$j is the average of the class observations; S is the variance of observations within each class; i is the number of observations, and j is the class of each observation.

After standardization of all multi-categorical variables, the genetic distances between the 76 cowpea accessions were estimated based on the square of the Euclidean distance, with the grouping of the genotypes carried out using Ward's hierarchical method.

Two dendrograms were generated to graphically represent the genetic diversity between accessions. The first included all 19 characters evaluated, and the second was constructed only with the characters that exhibited frequency above 50% in a certain class and an entropy value greater than 75% (H'>0.75), aiming to reduce expenses with labor and time dedicated to data collection.

The data obtained from the phenological and production characters were analyzed using descriptive statistics and subjected to Pearson's correlation test, with all statistical analyses performed using the Minitab® software version 18.1.

Results and discussion

Table 2 shows the multi-categorical descriptors, the number of phenotypic classes, and the Shannon-Weaver index. The highest entropy values were observed for the characters flower color (0.97), pod color (0.93), and seed brightness (0.92). High values for entropy are normally associated with a greater amount of the phenotypic category for the applied descriptor and also with a greater balance in the proportion between the frequency of genotypes in the different phenotypic categories (Vieira *et al*., 2008). Thus, this result demonstrates the existence of variability between the referred characters, making them relevant in the differentiation between genotypes.

TABLE 2. Number of phenotypic classes (NPC) and entropy levels (H') for the multi-categorical descriptors applied to the 76 accessions of cowpea.

Descriptors	NPC	H'
Seedling pigmentation	2	0.18
Flower color	3	0.97
Inflorescence type	2	0.10
Growth habit	2	0.36
Central leaflet shape	5	0.76
Plant size	4	0.73
Pod position	3	0.81
Pod color	4	0.93
Pod shape	4	0.60
Grain color	15	0.78
Grain shape	4	0.63
Seed brightness	3	0.92
Seed texture	2	0.74
Initial flowering	5	0.29
Pod length	5	0.70
Number of grains per pod	3	0.72
Mass of 100 grains	5	0.83
Cycle	5	0.57
Resistance to diseases caused by viruses	5	0.90

Low entropy values (H'<0.75) associated with a frequency above 50% were found for eleven descriptors, namely inflorescence type, seedling pigmentation, initial flowering, growth habit, cycle, pod shape, grain shape, pod length, number of grains per pod, plant size and seed texture. Therefore, these descriptors were suggested for disposal (Tab. 2). Although the descriptors pod length and number of grains per pod were provided for discard, they are relevant for cowpea breeding programs as they directly influence grain yield. Therefore, only the other nine descriptors were eliminated.

The disposal of nine of the 19 characters evaluated (Tab. 2) reduced by 47.4% the number of descriptors. A result superior to this was found by Silva *et al*. (2019) in a study to select morpho-agronomic characters in *Physalis angulata* L. germplasm using univariate and multivariate techniques that recorded disposal of 55.9% of the total number of the descriptors evaluated, without a significant loss of information.

Among all the characters evaluated in the present study, those with the lowest entropy coefficients were inflorescence type and seedling pigmentation, with H' values of 0.10 and 0.18, respectively. This result implies a low variability of these characters, which can be considered as those that least influenced the divergence between the cowpea accessions.

Ward's hierarchical method, based on the dissimilarity matrix estimated using the quadratic Euclidean distance, made it possible to group the 76 genotypes into five distinct groups from a cut at approximately 60% of the maximum distance (Fig. 1).

Group I had the highest concentration of genotypes, consisting of 55 of the 76 accessions evaluated, which expressed the most frequent characters. Group II was composed of 14 accessions (16, 17, 22, 28, 35, 36, 38, 44, 46, 59, 61, 70, 74, and 76). The genotypes belonging to this group have a small/medium pod length, mass of 100 grains that classifies them as small and medium, and a cycle ranging from early to medium. Group III consisted of 5.3% of the tested genotypes (29, 56, 65, and 68), which showed a determinate growth habit and erect plant size, important characters for breeding programs that aim to obtain cultivars adapted to mechanization.

Group IV was composed only of accession number 4 (Cacheado), which differs from the others since it is the only one that showed a type of compound inflorescence.

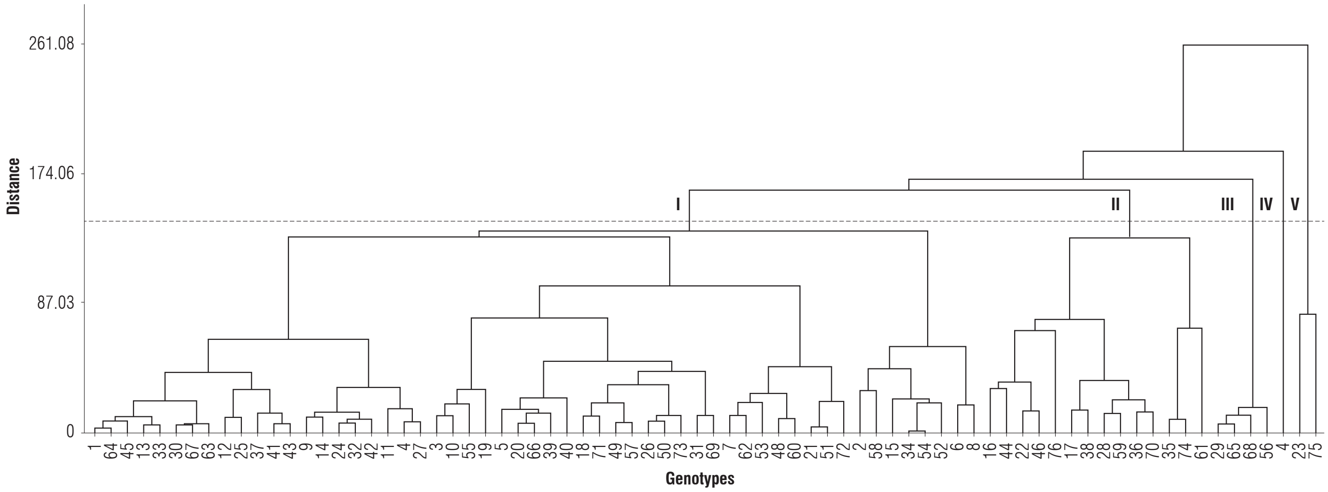


FIGURE 1. Dendrogram generated from the genetic dissimilarity matrix between 76 accessions of cowpea.

According to Barros *et al*. (2011), compound inflorescence has a direct influence on production by morphologically altering the reproductive part of the plant, which causes bifurcations at a certain height of the peduncle and new segments that will produce flowers and fruits. Group V consisted of two components (23 and 75), the only ones that had anthocyanin pigmentation in the seedling phase.

The most divergent combination, with a distance of 261.08, was established between genotypes 1 (Das Almas) and 75 (CE-970), showing greater genetic divergence between these accessions, which is desirable in breeding programs aiming to produce superior hybrids. On the other hand, the most similar combination was obtained between accessions 34 (TVu 1204) and 54 (Progresso-66), with a distance of 1.47, which should be avoided in order to explore heterosis in the progeny.

The first dendrogram was created considering all the descriptors evaluated (Fig. 1). For the construction of the second dendrogram, nine characters were discarded. The elimination of these characters resulted in a new distribution of accessions, represented graphically in Figure 2.

In this new dendrogram, seven groups were generated. Group I consisted of 24 accessions (1, 21, 22, 26, 29, 30, 31, 33, 34, 36, 37, 41, 43, 44, 45, 46, 50, 51, 54, 59, 64, 65, 67, and 70). These genotypes have white-colored seeds ranging from white with brown halo, white with mottled halo, and white-brown. Also, they showed mass of 100 grains that characterize them as extra small, small, and medium. In group II, ten accessions (13.2%) were grouped, which showed pods distributed above the foliage level, with a matte or intermediate opaque seed shine and with extra small to small size based on the seed weight. Group III

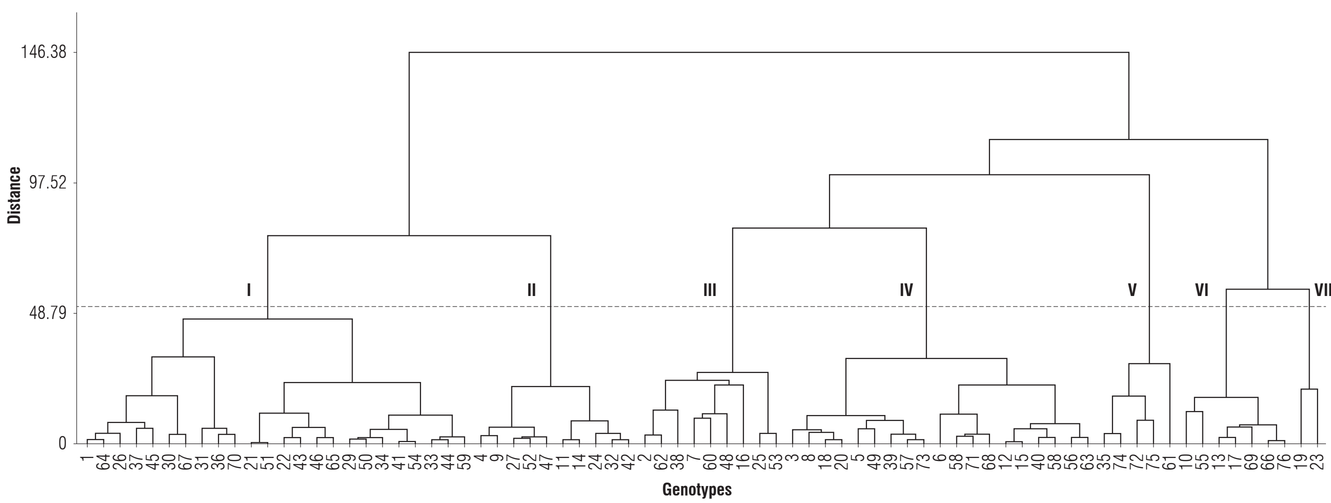


FIGURE 2. Dendrogram generated from the characters that showed higher levels of entropy (H'>0.75) associated with a frequency below 50% in a given class.

was constituted by nine genotypes (2, 7, 16, 25, 38, 48, 53, 60, and 62), whose plants showed brilliant seeds of cream color, white-purple, and black-white.

The genotypes that formed group IV produced pods positioned above the foliage, shiny seeds, and weight of 100 seeds that classify them as medium to large. Group V consisted of accessions 35, 61, 72, 74, and 75. These accessions have violet flowers and produce seeds of great or extra-large weight. In group VI, there were seven genotypes that exhibited greater pod length and less frequent seed color (purple mottled, brown-white, brown mottled white, cream, and black). The two accessions that were part of group VII (19 and 23) have an oval central leaflet shape and produce large pods with black colored or vinegar seeds.

Table 3 shows the descriptive statistics of the phenological and production variables used in the characterization of the 76 cowpea genotypes. The amplitude of the coefficient of variation (CV) varied from 9.19 to 59.44% for NDEHP and P/Pl, respectively. Santana *et al.* (2019), in a study of genetic divergence between cowpea accessions, found a smaller amplitude for CV values, which varied from 4.39 to 23.65% for the characters grain index and grain yield, respectively. In studies with cowpea, it is common to find high CV values for grain production (Bertini *et al.*, 2009; Públio Júnior *et al.*, 2017; Camara *et al.*, 2018; Souza *et al.*, 2019).

The evaluated genotypes showed high variability regarding the cycle. Regarding the character NDEFF, some accessions started flowering at 33 d after emergence, and others only at 58 d. The general average for this descriptor was 45 d. The character NDEPF ranged from 35 to 70 d, with an average of 48 d. When considering NDEPM, there was a variation from 40 to 76 d, with a general average of 56 d. The accessions also expressed great variation concerning NDEHP, with the earliest reaching the harvest point at 51 d and the latest at 82 d. On average, the harvest point was reached at 62 d.

According to Freire Filho *et al.* (2005), precocity is a relevant character as it represents the possibility of up to three crops per year, including rainfed and irrigated crops. As a result, it should be possible to increase and/or stabilize cowpea production in regions with long periods of drought. Thus, the mentioned descriptors were relevant and should be considered when selecting parents since they are directly related to the cultivation cycle.

Approximately 53% of accessions started to flower fully between 42 and 48 d after emergence, classified as of early and medium cycle, respectively (Fig. 3A). The majority of accessions began to form pods between 40 and 50 d (Fig. 3B). Approximately 91% of accessions started the maturation process between 48 and 64 d (Fig. 3C), and 67% reached the harvest point between 58 to 64 d after emergence (Fig. 3D). Accession 23 (7907-Purple Hull) was the only one considered extra early, starting its flowering and harvest at 33 and 51 d, respectively. On the other hand, accessions 2 (Potomac), 4 (Cacheado), and 8 (Novato) only started flowering between 54 and 58 d and harvest from 74 to 82 d.

Regarding the descriptor NPP, there was considerable variation between genotypes, with most of them producing 15 to 30 pods (Fig. 3E). Accessions 1 (Das Almas), 5 (Galanjão), and 9 (Milagroso) are noteworthy for having shown an NPP greater than 50. This character is essential for breeding programs aimed at the identification of parents since the selection for increasing the number of pods per plant results, indirectly, in increased grain yield (Santos *et al.*, 2012).

A more uniform distribution was observed for P/Pl, with about 93% of accessions producing less than 100 g and only five accessions with higher production, with emphasis on genotypes 1 (Das Almas) and 5 (Galanjão), which produced more than 130 g/plant (Fig. 3F). The low production values recorded for most genotypes are due to the low weight of 100 grains and the low number of grains per pod.

TABLE 3. Descriptive statistics of the quantitative characters of the 76 accessions of cowpea.

Characters	Minimum	Maximum	Average	Standard deviation	Variance	CV (%)
NDEFF	33.00	58.00	44.83	4.68	21.95	10.45
NDEPF	35.00	70.00	47.96	5.81	33.77	12.12
NDEPM	40.00	76.00	55.68	6.64	44.19	11.94
NDEHP	51.00	82.00	61.91	5.69	32.37	9.19
NPP	2.57	53.13	22.90	11.97	143.27	52.27
P/Pl (g)	5.50	139.38	54.52	32.41	1050.29	59.44

NDEFF - average number of days from emergence to full flowering; NDEPF - average number of days from emergence to pod formation; NDEPM - average number of days from emergence to physiological maturation; NDEHP - average number of days from emergence to harvest point; NPP - number of pods per plant; P/Pl - production per plant; CV - coefficient of variation.

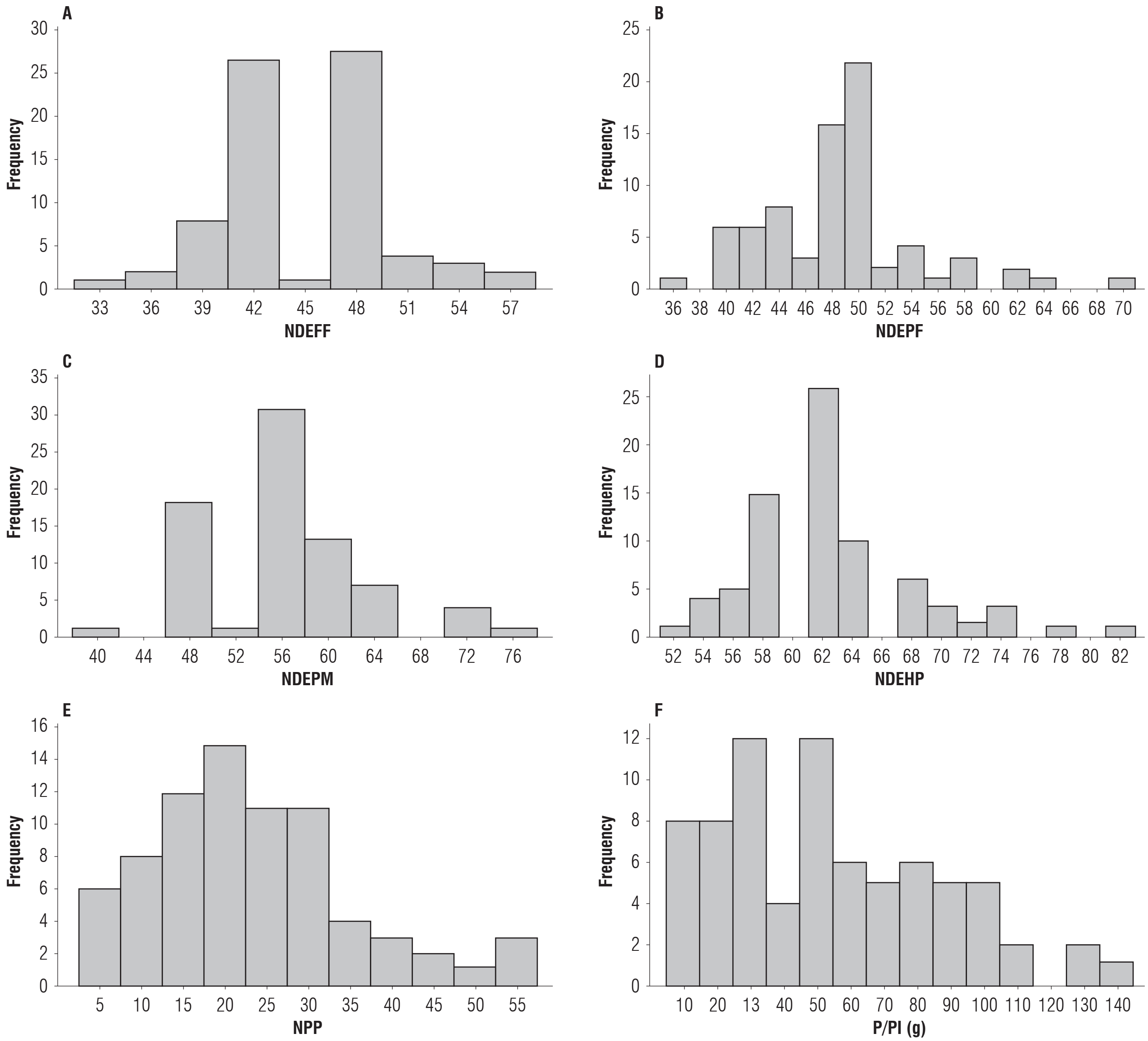


FIGURE 3. Frequency distribution of the 76 cowpea genotypes evaluated for six characters. A) NDEFF - average number of days from emergence to full flowering; B) NDEPF - number of days from emergence to pod formation; C) NDEPM - number of days from emergence to physiological maturation; D) NDEHP - number of days from emergence to harvest point; E) NPP - number of pods per plant, and F) P/Pl - production per plant.

Regarding virus attack, the accessions were classified as resistant/susceptible according to a grade scale. Among the evaluated genotypes, 29 showed no symptoms (1, 2, 3, 5, 8, 10, 13, 17, 19, 21, 28, 29, 31, 35, 36, 40, 51, 55, 62, 66, 67, 68, 69, 70, 71, 72, 73, 75 and 76). The other accessions showed symptoms ranging from mild to severe. Since only visual analysis of symptoms was performed, further studies will be needed to confirm the resistance of the genotypes to the CPSMV.

Regarding the estimated linear correlation between quantitative characters, an amplitude from -0.016 to 0.933 was found (Tab. 4). Pearson's correlation coefficient (r) measures the strength of the relationship between the characters studied, assuming values between -1 and 1, which are interpreted as perfect negative and positive correlations, respectively. According to the values shown in Table 4, the variables NPP and P/Pl were highly correlated ($r = 0.886$; $P = 0.000$). These results corroborate the findings of Santos *et* *al*. (2012) and show that the accessions that expressed the highest number of pods also showed higher production.

TABLE 4. Pearson's correlation coefficients and their respective probabilities obtained for the six quantitative characters evaluated in 76 cowpea genotypes.

	NDEFF	NDEPF	NDEPM	NDEHP	NPP
NDEPF	0.768				
	0.000				
NDEPM	0.754	0.933			
	0.000	0.000			
NDEHP	0.749	0.925	0.890		
	0.000	0.000	0.000		
NPP	-0.016	0.004	0.061	0.038	
	0.894	0.974	0.603	0.745	
P/Pl (g)	0.035	0.015	0.093	0.101	0.886
	0.767	0.900	0.422	0.384	0.000

NDEFF - average number of days from emergence to full flowering; NDEPF - average number of days from emergence to pod formation; NDEPM - average number of days from emergence to physiological maturation; NDEHP - average number of days from emergence to harvest point; NPP - number of pods per plant; P/Pl - production per plant; upper cells show the correlation coefficient and lower cells show the *P*-value.

The phenological variables NDEPF, NDEPM, and NDEHP showed a high correlation with each other (Tab. 4). This result was expected since these characters are closely related to the crop cycle. Therefore, the accessions that started the formation of pods earlier showed the same behavior for physiological maturation and harvest point.

Of the 76 accessions evaluated in this study, five deserve to be mentioned and should be incorporated into cowpea breeding programs. They are: 1 (Das Almas), 5 (Galanjão), 13 (Selected from CE-13), 23 (7907-Purple Hull), and 46 (Caupi Branco). Accession 23 was considered the most precocious of all. However, it showed a low NPP and, therefore, low grain production. The other five genotypes combined medium cycle, NVP above average, and production above 100 g per plant.

Regarding the dendrogram shown in Figure 2, among the five genotypes mentioned, 1 (Das Almas) and 46 (Caupi Branco) belong to group I, while 5 (Galanjão) belongs to group IV. The genotypes 13 (Selected from CE-13) and 23 (7907-Purple Hull) belong to group VI and VII, respectively. Based on the assumption that classified accessions are homogeneous within the group but heterogeneous between groups, crossings should be performed between accessions belonging to different groups and with desirable agronomic characters. Following these criteria, crosses involving genotype 23 (7907-Purple Hull), which showed an extra early cycle, with 1 (Das Almas) or 5 (Galanjão), which expressed the highest NPP and P/Pl, are promising.

Conclusions

The descriptors flower color, pod color, and seed brightness showed greater genetic variability. Among the genotypes evaluated, Das Almas, Galanjão, selected from CE-13, 7907-Purple Hull, and Caupi Branco can be incorporated as parents into cowpea breeding programs.

Crossings between the genotype 7907-Purple Hull and Das Almas or Galanjão are indicated for use in breeding programs to obtain superior genotypes in precocity and grain yield.

Acknowledgments

The authors would like to express their gratitude to the National Council for Scientific and Technological Development (CNPq) for funding the scientific initiation scholarship, the Phytotechnics Department of the Federal University of Ceará (UFC) for providing the infrastructure and technical support for conducting and evaluating the experiment, particularly the Center for Studies and Research in Improvement and Biotechnology of Plant Genetic Resources, and the Seed Analysis Laboratory.

Conflict of interest statement

The authors declare that there is no conflict of interest regarding the publication of this article.

Author's contributions

CHCMB, AKFS and FRGA designed the experiment; CHCMB obtained financial support for the project that led to this publication; CHCMB and AKFS supervised and conducted the planning and execution of the research activity; FRGA conducted the experimental research and collected the data; CLP and FRGA applied statistical and computational techniques to analyze the study data; FLST and FRGA wrote and translated the initial draft; all authors reviewed the manuscript.

Literature cited

Barros, F. R., Anunciação Filho, C. J., Rocha, M. M., Nunes, J. A. R., Silva, K. J. D., Freire Filho, F. R., & Ribeiro, V. Q. (2011). Potencial genético de progênies de feijão-caupi segregantes quanto ao tipo da inflorescência. *Pesquisa Agropecuária Brasileira*, *46*(2), 182–189. https://doi.org/10.1590/S0100-204X2011000200010

Bertini, C. H. C. M., Teófilo, E. M., & Dias, F. T. C. (2009). Divergência genética entre acessos de feijão-caupi do banco de germoplasma da UFC. *Revista Ciência Agronômica*, *40*(1), 99–105.

Camara, F. T., Mota, A. M. D., Nicolau, F. E. A., Pinto, A. A., & Silva, J. M. F. (2018). Produtividade de feijão caupi crioulo em função do espaçamento entre linhas e número de plantas por cova. *Revista de Agricultura Neotropical*, *5*(2), 19–24.

Cruz, C. D., Ferreira, F. M., & Pessoni, L. A. (2011). *Biometria aplicada ao estudo da diversidade genética*. Suprema.

Cruz, C. D., Regazzi, A. J., & Carneiro, P. C. S. (2014). *Modelos biométricos aplicados ao melhoramento genético* (3rd ed.). Editora UFV.

Freire Filho, F. R., Lima, J. A. A., & Ribeiro, V. Q. (Eds.). (2005). *Feijão-caupi: avanços tecnológicos*. Embrapa.

Freire Filho, F. R., Ribeiro, V. Q., Rocha, M. M., Silva, K. J. D., Nogueira, M. S. R., & Rodrigues, E. V. (2011). Produção e importância socioeconômica. In F. R. Freire Filho (Ed.), *Feijão-caupi no Brasil: produção, melhoramento genético, avanços e desafios* (pp. 18–38). Embrapa.

Lima, J. A. A., Nascimento, A. K. Q., Silva, A. K. F., & Aragão, M. L. (2012). Biological stability of a strain of *Cowpea severe mosaic virus* over 20 years. *Revista Ciência Agronômica*, *43*(1), 105–111. https://doi.org/10.1590/S1806-66902012000100013

Oliveira, J. S., Faleiro, F. G., Junqueira, N. T. V., Vieira, E. A., & Viana, M. L. (2017). Caracterização fenotípica e diversidade genética de *Passiflora* spp. baseada em descritores multicategóricos. *Revista de Ciências Agrárias*, *60*(3), 223–234. https://doi.org/10.4322/rca.2427

Paiva, J. B., Freire Filho, F. R., Teófilo, E. M., & Ribeiro, V. Q. (2014). *Feijão-caupi: melhoramento genético no Centro de Ciências Agrárias*. UFC.

Públio Júnior, E., Morais, O. M., Rocha, M. M., Públio, A. P. P. B., & Bandeira, A. S. (2017). Características agronômicas de genótipos de feijão-caupi cultivados no sudoeste da Bahia. *Científica*, *45*(3), 223–230. https://doi.org/10.15361/1984-5529.2017v45n3p223-230

Rotili, E. A., Cancellier, L. L., Dotto, M. A., Peluzio, J. M., & Carvalho, E. V. (2012). Divergência genética em genótipos de milho, no Estado do Tocantins. *Revista Ciência Agronômica*, *43*(3), 516–521. https://doi.org/10.1590/S1806-66902012000300014

Santana, S. R. A., Medeiros, J. E., Anunciação Filho, C. J., Silva, J. W., Costa, A. F., & Bastos, G. Q. (2019). Genetic divergence among cowpea genotypes by morphoagronomic traits. *Revista Caatinga*, *32*(3), 841–850. https://doi.org/10.1590/1983-21252019v32n329rc

Santos, A., Ceccon, G., Correa, A. M., Durante, L. G. Y., & Regis, J. A. V. B. (2012). Análise genética e de desempenho de genótipos de feijão-caupi cultivados na transição do cerrado-pantanal. *Cultivando o Saber*, *5*(4), 87–102.

Santos, J. A. S., Teodoro, P. E., Correa, A. M., Soares, C. M. G., Ribeiro, L. P., & Abreu, H. K. A. (2014). Desempenho agronômico e divergência genética entre genótipos de feijão-caupi cultivados no ecótono Cerrado/Pantanal. *Bragantia*, *73*(4), 377–382. https://doi.org/10.1590/1678-4499.0250

Shannon, C. E., & Weaver, W. (1964). *The mathematical theory of communication*. The University of Illinois Press.

Silva, F. L., Baffa, D. C. F., Oliveira, A. C. B., Pereira, A. A., & Bonomo, V. S. (2013). Integração de dados quantitativos e multicategóricos na determinação da divergência genética entre acessos de cafeeiro. *Bragantia*, *72*(3), 224–229. https://doi.org/10.1590/brag.2013.039

Silva, H. K., Passos, A. R., Schnadelbach, A. S., Moreira, R. F. C., Conceição, A. L. S., & Lima, A. P. (2019). Selection of morphoagronomic descriptors in *Physalis angulata* L. using multivariate techniques. *Journal of Agricultural Science*, *11*(1), 289–302. https://doi.org/10.5539/jas.v11n1p289

Sousa, S., Tavares, T., Barros, H., Nascimento, I., Santos, V., & Fidelis, R. (2017). Divergência genética de feijão-caupi (*Vigna unguiculata*) no sul do Tocantins. *Revista de Ciências Agrárias*, *40*(2), 419–429. https://doi.org/10.19084/RCA16069

Souza, K. N., Torres Filho, J., Barbosa, L. S., & Silveira, L. M. (2019). Avaliação de genótipos de feijão-caupi para produção de grãos verdes em Mossoró-RN. *Colloquium Agrariae*, *15*(1), 9–14. https://doi.org/10.5747/ca.2019.v15.n1.a265

Vieira, E. A., Fialho, J. F., Silva, M. S., Fukuda, W. M. G., & Faleiro, F. G. (2008). Variabilidade genética do banco de germoplasma de mandioca da Embrapa cerrados acessada por meio de descritores morfológicos. *Científica*, *36*(1), 56–67.

Growth parameters and organoleptic characteristics of plantain (*Musa* AAB Simmonds cv. Farta Velhaco) at different planting densities

Parámetros de crecimiento y características organolépticas del plátano (*Musa* AAB Simmonds cv. Farta Velhaco) en diferentes densidades de siembra

Glaucio da Cruz Genuncio[1], Jhonathann Willian Furquin da Silva[2], Davidson Henrique Cordeiro[3], Tainah de Oliveira Mayer[3], and Elisamara Caldeira do Nascimento[2*]

ABSTRACT

In view of the role of density and spacing in the growth parameters and organoleptic characteristics of plantains, this study sought to identify the best planting density for the cultivar Farta Velhaco (Terra group) cultivated in Mato Grosso (Brazil), evaluating three spacings. For this, we carried out an experiment in a randomized block design according to the following spaces (between plants x between rows): 2.0 m x 3.0 m (density of 1,600 plants ha^{-1}); 2.5 m x 3.0 m (density of 1,320 plants ha^{-1}) and 3.0 m x 3.0 m (density of 1,110 plants ha^{-1}), with 25 replicates per treatment. The following attributes were evaluated: plant height (cm); diameter of the pseudostem (cm); number of leaves; number of hands per bunch; number of fruits per hand; fresh fruit weight (g); fruit height (cm); external and internal fruit lengths (cm); fruit diameter (cm); bunch weight (kg), and productivity (t ha^{-1}), in addition to the percentage of total soluble solids, titratable acidity, pH and flavor of the fruits. The density of 2.0 m x 3.0 m in the cultivation of the plantain group Terra cv. Farta Velhaco improves fruit classification attributes as well as the fruit organoleptic characteristics such as the percentage of total soluble solids and flavor.

Key words: adaptation of cultivars, tropical agriculture, family farms.

RESUMEN

En función del papel que cumplen la densidad y el espaciamiento en los parámetros de crecimiento y las características organolépticas de los plátanos, este estudio buscó identificar la mejor densidad de siembra para el cultivar Farta Velhaco (grupo Terra) cultivado en Mato Grosso (Brasil), evaluando tres espaciamientos. Para ello, realizamos un experimento en un diseño de bloques al azar según los siguientes espacios (entre plantas x entre hileras): 2.0 m x 3.0 m (densidad de 1600 plantas ha^{-1}); 2.5 m x 3.0 m (densidad de 1320 plantas ha^{-1}) y 3.0 m x 3.0 m (densidad de 1110 plantas ha^{-1}), con 25 repeticiones por tratamiento. Se evaluaron las siguientes características: altura de la planta (cm); diámetro del pseudotallo (cm); número de hojas; número de manos por racimo; número de frutos por mano; peso fresco de la fruta (g); altura del fruto (cm); longitudes externas e internas del fruto (cm); diámetro del fruto (cm); peso del racimo (kg) y productividad (t ha^{-1}), además del porcentaje de sólidos solubles totales, la acidez titulable, el pH y el sabor de frutos. La densidad de siembra 2.0 x 3.0 m en el cultivo del plátano grupo Terra cv. Farta Velhaco mejora los atributos de clasificación de la fruta, así como sus características organolépticas como el porcentaje de sólidos solubles totales y el sabor.

Palabras clave: adaptación de cultivares, agricultura tropical, granjas familiares.

Introduction

The banana (*Musa* spp.) is one of the most important tropical fruits, and its productivity depends on many production factors. Planting density and the type of seed used are important factors that influence productivity. Additionally, the number of hands per bunch, number of fruits per bunch, and weight and length of fruits contribute to the production of quality fruits (Camolesi *et al*., 2012).

The different banana cultivars are classified according to the genomic group and its subgroups, mainly differing in the size of the plant. This last factor directly interferes with the spacing adopted for the breeding stock and the productivity obtained at the end of the cycle, and it also influences the resistance to pathogens (Perrier *et al*., 2011). On the other hand, the low genetic variability of this crop represents an imminent risk due to the lack of new cultivars or their decimation by pests, as occurred with the export of Latin American bananas based exclusively on the cultivar Gros Michel that is susceptible to the disease of Panama (Silva *et al*., 2016). The evaluation of cultivars in experiments conducted in the field can select expressive

Received for publication: 22 April, 2021. Accepted for publication: 1 December, 2021.

Doi: 10.15446/agron.colomb.v39n3.95232

[1] Departamento de Fitotecnia e Fitossanidade, Faculdade de Agronomía e Zootecnia, Universidade Federal de Mato Grosso, Cuiabá (Brazil).
[2] Programa de Pós Graduação em Agricultura Tropical, Universidade Federal de Mato Grosso, Cuiabá (Brazil).
[3] Curso de Agronomía, Universidade Federal de Mato Grosso, Cuiabá (Brazil).
[*] Corresponding author: elisamara.caldeira@gmail.com

phenotypic characters from environmental influence and, consequently, responses to production and productivity. The measurement of productivity in banana plants is complex and results from the association of different factors influenced by the environment. Thus, for selection to be carried out efficiently, it is important to know the associations between agronomic characters of great importance for its cultivation (Amorim *et al*., 2008). As a result, several banana breeding programs have emerged (Lédo *et al*., 2018) and different cultivars have been made available to farmers (Castricini *et al*., 2017; Irish *et al*., 2019).

Of the numerous triploid subgroups, some stand out for being widely cultivated far from their region of origin (Asia), as in the case of the AAB group "African Plantain" (Ploetz *et al*., 2007), to which the cultivar Farta Velhaco or Pacova belongs. This cultivar stands out for its starch content and its possibility of use in the cuisine of Mato Grosso, a state in the interior of Brazil. Banana is the main tropical fruit produced in Mato Grosso, the main crop of family farming in the state, and is typically used for local or regional commercialization (IBGE, 2020).

In this sense, identifying the best density of plants that improves agronomic characters is important since denser crops tend to increase the cost of planting and the need for more specific nutrition and irrigation management. Additionally, denser crops influence the obtaining of maximum crop production according to its cycle.

In view of the importance of choosing the appropriate spacing for increasing productivity and quality of plantain fruits, this study was carried out to identify the best planting density for the cultivar Farta Velhaco (Terra group) cultivated in the region of Baixada Cuiabana, Mato Grosso, Brazil.

Materials and methods

Experimental area

The experiment was conducted in the Fruit Sector of the Experimental Farm of the Federal University of Mato Grosso - UFMT, located in Santo Antônio do Leverger - MT, Brazil. The experimental farm was 30 km from Cuiabá, at 15°47'11" S, 56°04'17" W and 140 m a.s.l. The regional climate is classified as Aw according to Köppen and Geiger (1928) with distinct periods of droughts and rains. The mean annual temperature is around 26°C, with a mean precipitation of 1360 mm, and relative air humidity of 66%.

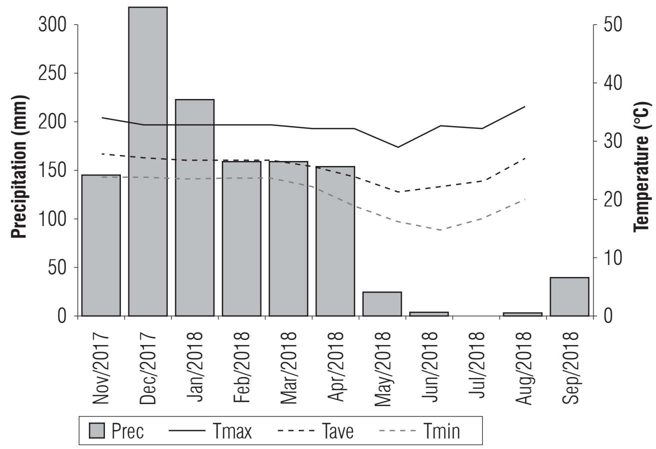


FIGURE 1. Precipitation (Prec, mm), maximum (Tmax), average (Tave) and minimum (Tmin) temperatures (°C) in the experimental area (November 2017 to September 2018).

The results of the soil chemical analysis (Teixeira *et al*., 2017) at 20 cm depth were as follows: pH ($CaCl_2$) = 5.8; P = 37.5 mg dm^{-3}; K = 99.0 mg dm^{-3}; Ca = 2.5 $cmol_c$ dm^{-3}; Mg = 0.4 $cmol_c$ dm^{-3}; Al = 0.5 $cmol_c$ dm^{-3}; H + Al = 0.8 $cmol_c$ dm^{-3}; base saturation of 55%, and soil organic matter of 1.75 g dm^{-3}. The soil was classified as an Alfisols (74% sand, 8% silt, 19% clay) (Soil Survey Staff, 2014).

The soil was corrected according to the results of the soil chemical analysis with an application of 2.0 t ha^{-1} of dolomitic limestone, relative power of total neutralization 88%, aimed to raise the base saturation to 70%; 100 kg ha^{-1} of N in the form of urea, 120 kg ha^{-1} of P_2O_5 (simple superphosphate), and 300 kg ha^{-1} of K_2O (potassium chloride) were applied at 30, 60, 90, 120, and 150 d after transplanting the seedlings to the field following the recommendations of Borges and Souza (2009) and Oliveira *et al*. (2014).

We used the cultivar Farta Velhaco (Terra Group), with an average height of 3.5 to 4.5 m, pseudostem diameter of around 25 cm at the base of the plant, 3 to 7 bunches per plant, a vegetative cycle of 390 to 420 d, and production of around 690 to 720 d.

Seedling preparation and planting

Micropropagated seedlings of the cultivar Farta Velhaco were used, which, when removed from the laboratory, were transplanted into tubes of 280 cm³ (63 mm in diameter and 190 mm in height) using Carolina Soil® (Pardinho, São Paulo, Brazil) substrate and acclimatized in a greenhouse at a temperature between 27°C and 32°C, containing greenhouse screen shading at 50% and constant nebulization for 60 d. At 61 d, the seedlings were transferred to plastic containers of 25 cm x 35 cm x 0.015 cm (with a volume of 3.6 L) and placed under a 35% shading screen for a period of 60 d. In both phases, a modified nutrient solution (Hoagland & Arnon, 1950) was applied at a volume of 250 ml per plant twice a week. At 120 d after transplanting, the seedlings were taken to an open area for hardening for 7 d and then planted according to the different treatments (spacing).

Experimental design

The experiment was conducted in a completely randomized design with three treatments and one cultivar (Farta Velhaco). The treatments consisted of spacings of 2.0 m x 3.0 m (between plants x between lines) with a density of 1,600 plants ha^{-1}; 2.5 m x 3.0 m, with a density of 1,320 plants ha^{-1}, and 3.0 m x 3.0 m, with a density of 1,110 plants ha^{-1}, with 25 replicates per treatment, and each experimental unit was composed of three plants.

Phenological and organoleptic analyses

The phenological variables of plant height (m), diameter of the pseudostem at 30 cm from the ground (cm), and number of leaves were measured at 26, 27, 57, 87, 126, 157 and 186 d after rhizome emission. The fruit bunch was harvested at the agronomic harvest point (bunch formation). The fruit height (cm), external and internal fruit lengths (cm), fruit diameter (mm), number of fruits per hand, number of hands per bunch, fresh fruit weight (g), bunch weight (kg), and productivity (t ha^{-1}) were evaluated. For fruit evaluations, the first bunch produced was harvested due to the greater homogeneity of the fruits in this period of the experiment.

Measurements were taken using a measuring tape, a scale, and a digital caliper. To obtain the weight of the bunch, the number of fruits per hands was multiplied by the number of hands per bunch. For the calculation of productivity, the values of the weight of the bunch were multiplied by the estimated density of plants for each treatment.

The organoleptic variables measured in the fruits were total soluble solids content (°Brix), using a field refractometer (model RT-10 ATC, Salvi Casagrande, Mettler Toledo, Brazil); total titratable acidity (% citric acid) by acid/base titration was performed in the laboratory. Fruit pH was determined using a pH benchtop meter (Orion Dual Star, Thermo Fisher, Brazil). The flavor was obtained by the ratio between the percentage of total soluble solids and total titratable acidity.

The phenological and organoleptic variables were checked for homogeneity of variances according to O'Neill and Matthews (2000) and for normality of errors according to Shapiro and Wilk (1965). A one-way analysis of variance was then applied. When significant, the Tukey's post hoc test was used with a *P*-value set to 0.05 using the ExpDes.pt package (Ferreira *et al*., 2014) in R version 3.6.3 (R Core Team, 2019). For variables whose data did not meet the normality of errors and/or homogeneity of variances, the Kruskal and Wallis (1952) non-parametric test was used through the R version 3.6.3 (R Core Team, 2019).

Results and discussion

Growth characters

Planting densities had significant effects on the phenology of the plantain Farta Velhaco cultivated under the edaphoclimatic conditions of Baixada Cuiabana. Wind, light, and temperature are the main edaphoclimatic characteristics that affect this cultivar. Plants grown at a density of 1,110 plants ha^{-1} (spacing 3.0 m x 3.0 m) were taller and had higher pseudostem diameter when compared to the other treatments (Fig. 2).

Evaluating both the height and the diameter of the pseudostem is an important factor because it determines the growth vigor of the plant in addition to its carrying capacity, both in terms of resistance to wind and to the weight of the bunch in the reproductive stage (Arantes *et al*., 2010).

According to Aguiar *et al*. (2016), this group of bananas is characterized by height between 3.0 to 5.0 m. These data agree with those obtained for Farta Velhaco when conducted under local edaphoclimatic conditions. However, very tall plants tend to have thinner pseudostems, which facilitates tipping by winds (Mahmoud *et al*., 2018). In this sense, a positive correlation can be observed between the height and the diameter of the banana plants at a density of 1,110 plants ha^{-1} (3.0 m x 3.0 m spacing) (Fig. 1), which may confer greater resistance to wind, a major factor for maintaining the banana plantation. It should also be noted that there was no incidence of strong winds (75 km/h) during the experiment.

The number of leaves was higher in plants at a density of 1,600 plants ha^{-1} (spacing 2.0 m x 3.0 m) (Fig. 1). The evaluation of the number of photosynthetically active leaves is a fundamental condition for estimating the time for bunch emission and serves as a parameter to assess the balance between photosynthesis and banana production (Arantes *et al*., 2010). The number of functional leaves is an important phenological descriptor as it directly influences planting density and crop management, with consequences for production.

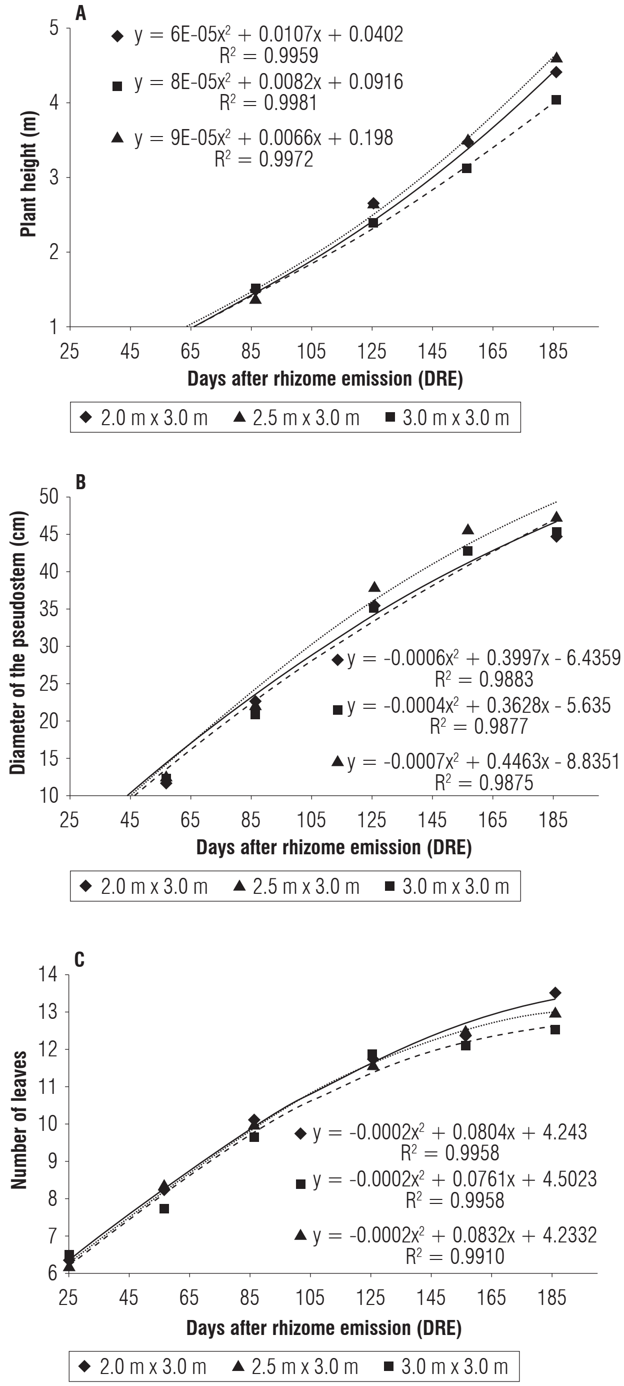


FIGURE 2. A) Plant height, B) diameter of the pseudostem, and C) number of leaves of Farta Velhaco plantains planted at different spacings, in Mato Grosso (Brazil) during 185 d after transplanting.

Lessa *et al.* (2012), when conducting a banana plantation for three productive cycles, observed a positive correlation between the diameter of the pseudostem and the number of leaves until flowering. Pereira *et al.* (2000) found that to produce the first bunch of banana cv. Dwarf Silver it is necessary to emit approximately nine functional leaves.

In this study, there was an average of thirteen functional leaves prior to the inflorescence emission to produce Farta Velhaco. For Chaudhuri and Baruah (2010), the greater number of functional leaves of plants grown in areas with lower planting density indicates less competition for nutrients, soil moisture and light intensity in the vegetative and reproductive stages, when compared to plants grown at higher densities.

Thus, the possible adaptation of Farta Velhaco to photosynthetic and physiological activities is an important feature, as it promotes more balanced vegetative growth and, consequently, better reproductive development when grown at different spacings.

Income and income-attributing characters

The planting density influenced yield and income-attributing characters in the plantain crop. As a result, and when evaluating the productive attributes of number of hands per bunch, number of fruits per hand, and fresh fruit weight (Fig. 3), it was possible to observe reductions both in the number of hands per bunch and in the fresh weight of the fruit in plants with a planting density of 1,320 plants ha^{-1} (spacing 2.5 m x 3.0 m) with mean values of 8.55 kg per bunch, whereas the plants grown at a density of 1,600 plants ha^{-1} (2.0 mx 3.0 m) produced 10.77 kg per bunch and a density of 1,110 plants ha^{-1} (3.0 m x 3.0 m) resulted in the production of 10.13 kg per bunch.

For productivity (obtained by multiplying the mass of the bunch by the number of plants ha^{-1} for each treatment), values of 17.95 t ha^{-1} could be observed for the plants at the highest planting density (2.0 x 3.0 m) in comparison to those obtained at other densities: 11.28 t ha^{-1} in spacing of 2.5 m x 3.0 m and 11.24 t ha^{-1} in spacing of 3.0 m x 3.0 m.

According to the State Secretariat for Family Agriculture of Mato Grosso, productivity results for Farta Velhaco are, on average, 15.0 t ha^{-1} when conducted at a density of 1,111 plants ha^{-1} for the state of Mato Grosso (Salmazo *et al.*, 2021). Thus, the data obtained by this research expressed that when using the density of 1,600 plants ha^{-1}, productivity can be increased by 2.95 t ha^{-1} for this cultivar, which is an important agronomic indicator for gains in production and productivity. On the other hand, they point out that the average yield of the banana group is 20.0 t ha^{-1}.

The evaluation of banana genotypes, mainly from the Cavendish group, is typically done for at least two cycles. However, studies carried out aiming at the evaluation of banana cultivars type Terra are rare, mainly due to the scarcity of varieties of this group. As a result, it might be acceptable to evaluate the productive attributes of the land group in a single cycle since many genotypes are currently cultivated as an annual crop (Arantes *et al*., 2010).

Silva *et al*. (2003) highlight some productive parameters for the Terra group, such as bunch weight of 25 kg, number of fruits per bunch of 160 fruits, and number of hands per bunch of 10. Thus, cv. Farta Velhaco, under the experimental conditions of this study, obtained productive values below those recommended by those authors when planted at higher densities, where six hands per bunch and 48 fruits per bunch were observed with a total of 13 kg per bunch. However, in order to obtain gains in productivity for Farta Velhaco, planting density becomes an important factor for the economic viability of the crop.

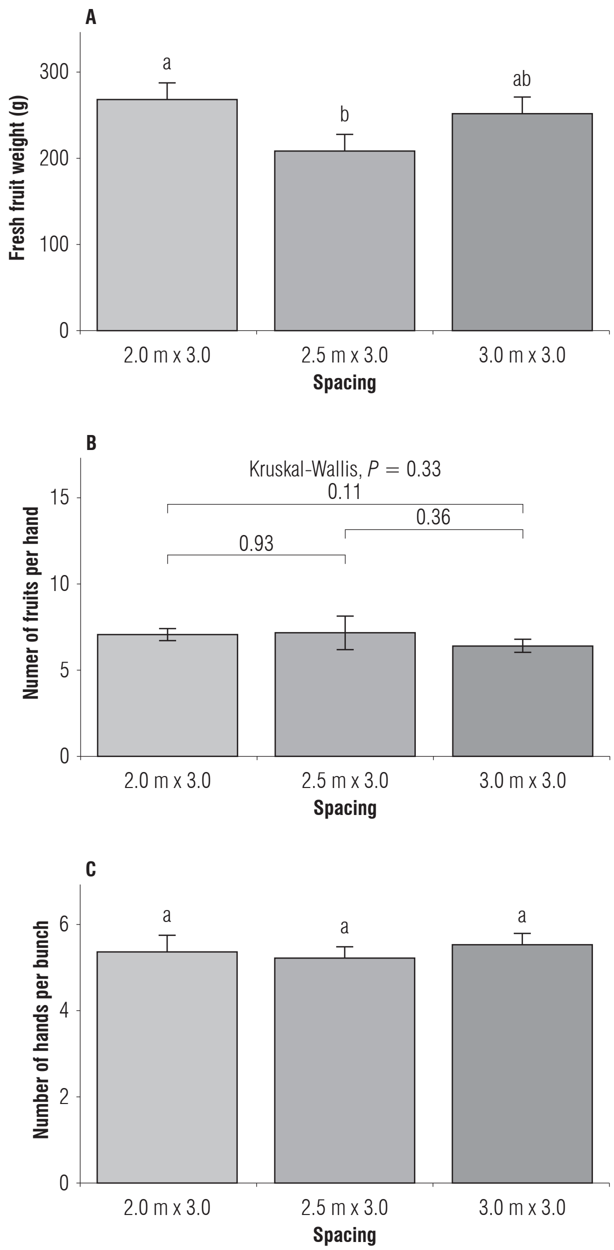


FIGURE 3. A) Fresh fruit weight, B) number of fruits per hand, and C) number of hands per bunch of Farta Velhaco plantain conducted at different spacings in Mato Grosso (Brazil).

When evaluating the qualitative phenological attributes of fruits used for the commercial classification of the Terra group banana (Silva *et al*., 2003), there was a significant influence on the height, the external and internal lengths, and the fruit diameter, with significant reduction of these attributes for the fruits obtained from plants conducted at the 2.5 m x 3.0 m spacing (Fig. 4).

In general, the cultivar Farta Velhaco, when grown under a high planting density, maintained an adequate balance in the source-sink ratio at all its physiological stages. Ganapathi and Dharmatti (2018), when evaluating different spacing for two cultivars marketed in India, found that shading was the main factor affecting plantations with high populations and that the plants compensated for this factor, investing in leaf development with a consequent imbalance in the source-sink ratio at the reproductive stage.

Qualitative parameters of the fruit quality

When assessing planting density for the effect on fruit quality, a significant influence can be observed on the percentage of total soluble solids and flavor. Flavor is a measure obtained by the ratio between the concentration of total soluble solids (°Brix) and titratable acidity (% of citric acid), and the higher the value, the greater the perception of improvement in the flavor of the fruit.

In this research, an improvement in the organoleptic characteristics of the fruits was observed when growing Farta Velhaco plantains in more dense systems. Despite the higher concentration of total soluble solids in the fruits of the plants arranged at a lower density (spacing 3.0 m x 3.0 m), increased flavor can be obtained in fruits from plants conducted at a higher density (Fig. 5).

As this is a cultivar recently introduced to the market, comparative data is not yet available in the literature. However, the analysis of physical-chemical and biochemical parameters (fruit quality), such as soluble solids, pH, titratable acidity and bioactive compounds, will be useful for the characterization and selection of genotypes with superior characteristics for genetic improvement as well as for the introduction of new varieties in the existing agricultural systems (Borges & Souza, 2009).

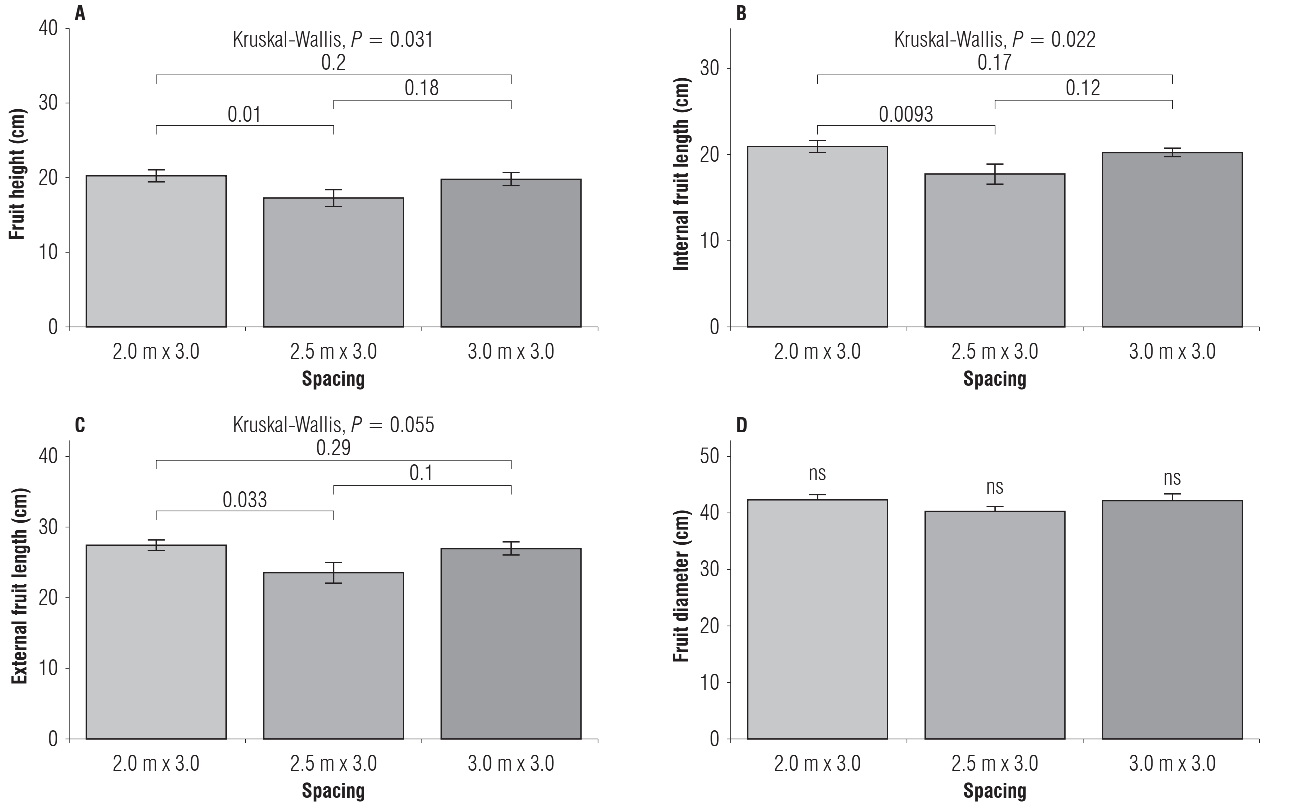


FIGURE 4. A) Fruit height, B) internal and C) external fruit lengths, and D) fruit diameter of the Farta Velhaco plantain conducted at different spacings in Mato Grosso (Brazil). ns - not significant.

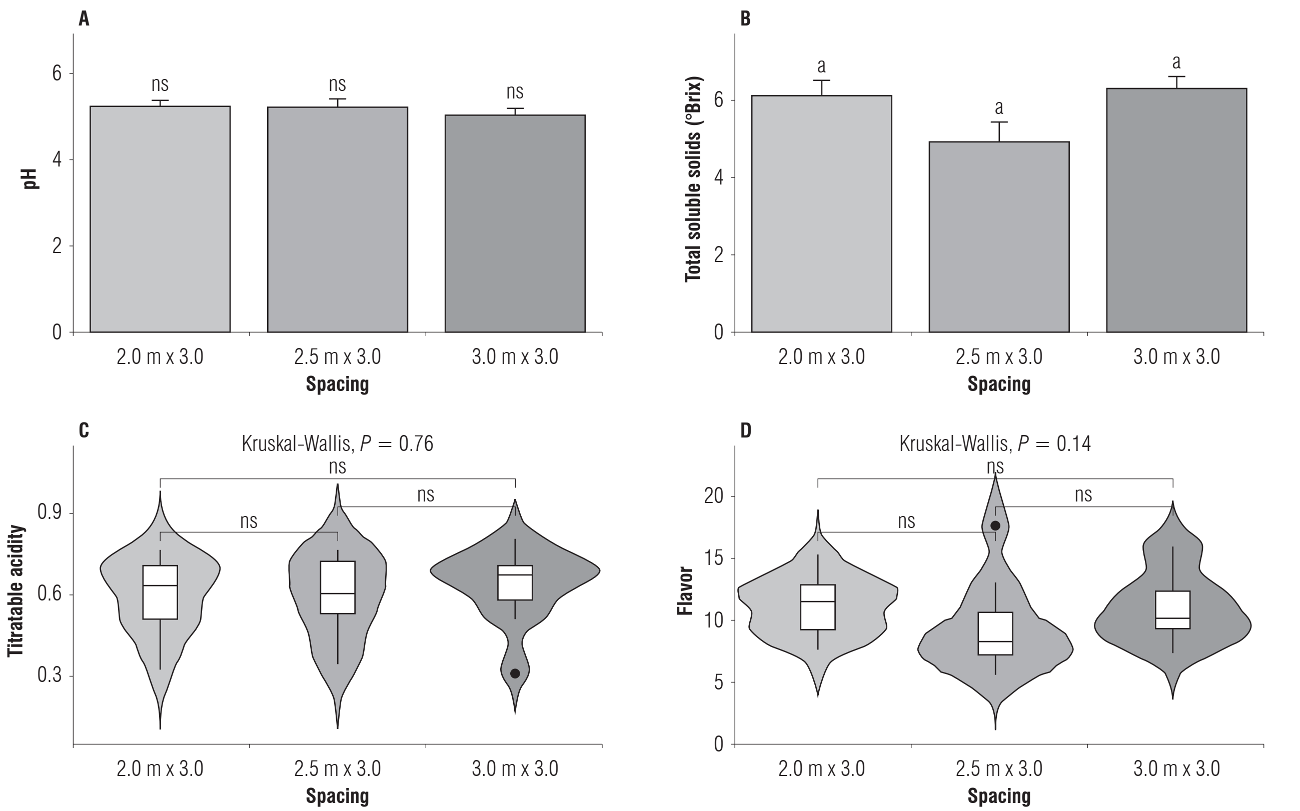


FIGURE 5. A) pH, B) total soluble solids (°Brix), C) titratable acidity (% citric acid), and D) flavor of Farta Velhaco plantain conducted in different spacing in Mato Grosso (Brazil). ns - not significant.

According to previous studies, fruits with high levels of total soluble solids are the most likely to be accepted by consumers (Gibert *et al*., 2009). Values range from 7.18 to 25.37 °Brix for plantain genotypes used for cooking. Cooked plantains are consumed at many ripening stages after being cooked; they are generally not appreciated in their fresh form due to the absence of sweetness and unpleasant firmness (De Jesus *et al*., 2013). The values below 6.26 °Brix found in this study indicate that the cultivar Farta Velhaco can be used for different forms of processing.

The genotypes with firmer fruit pulp are suitable for industrial use, mainly for the preparation of fried products, such as plantain chips. In addition, more firm fruits are more resistant to transport and durable after harvest (Youryon & Supapvanich, 2017). During the ripening process, the percentage of dry weight of the pulp decreases. This characteristic is important for the selection of genotypes for industry or even for domestic consumption, for the preparation of cooked and/or fried dishes, preferable in many countries.

Conclusions

The high planting density (2.0 x 3.0 m spacing) in the cultivation of the plantain group Terra, cv. Farta Velhaco improved the fruit classification attributes, productivity and organoleptic characteristics such as the percentage of total soluble solids and flavor, characteristics appreciated for choices related to fruit consumption.

Acknowledgments
The authors would like to express their gratitude to the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for the scientific initiation scholarships made available.

Conflict of interest statement
The authors declare that there is no conflict of interest regarding the publication of this article.

Author's contributions
GCG and ECN designed the experiment. DHC and TOM performed the field experiment and laboratory analysis. JWFS contributed to the data analysis. GCG and ECN wrote the article. All authors have reviewed the manuscript.

Literature cited

Aguiar, R. S., Zaccheo, P. V. C., Neves, C. S. V. J., Aguiar, M. S., & Oliveira, F. T. (2016). Performance of 'nanicão jangada' banana plants intercropped with winter cover crops. *Revista Brasileira de Fruticultura*, *38*(4), Article e-729. https://doi.org/10.1590/0100-29452016729

Amorim, E. P., Ramos, N. P., Ungaro, M. R. G., & Kiihl, T. A. M. (2008). Correlações e análise de trilha em girassol. *Bragantia*, *67*(2), 307–316. https://doi.org/10.1590/s0006-87052008000200006

Arantes, A. M., Donato, S. L. R., & Silva, S. O. (2010). Relação entre características morfológicas e componentes de produção em plátanos. *Pesquisa Agropecuária Brasileira*, *45*(2), 224–227. https://doi.org/10.1590/S0100-204X2010000200015

Borges, A. L., & Souza, L. S. (Eds.). (2009). *Recomendações de calagem e adubação para abacaxi, acerola, banana, laranja, tangerina, lima ácida, mamão, mandioca, manga e maracujá*. Embrapa.

Camolesi, M. R., Neves, C. S. V. J., Martins, A. N., & Suguino, E. (2012). Fenologia e produtividade de cultivares de bananeiras em Assis, São Paulo. *Revista Brasileira de Ciências Agrárias*, *7*(4), 580–585. https://doi.org/10.5039/agraria.v7i4a1747

Castricini, A., Dias, M. S. C., Rodrigues, M. G. V., & Oliveira, P. M. (2017). Quality of organic banana produced in the semiarid region of Minas Gerais, Brazil. *Revista Brasileira de Fruticultura*, *39*(2), Article e-813. https://doi.org/10.1590/0100-29452017813

Chaudhuri, P., & Baruah, K. (2010). Studies on planting density in banana cv. 'Jahaji' (AAA). *Indian Journal of Hill Farming*, *23*(2), 31–38.

De Jesus, O. N., Silva, S. O., Amorim, E. P., Ferreira, C. F., Campos, J. M. S., Silva, G. G., & Figueira, A. (2013). Genetic diversity and population structure of *Musa* accessions in *ex situ* conservation. *BMC Plant Biology*, *13*, Article 41. https://doi.org/10.1186/1471-2229-13-41

Ferreira, E. B., Cavalcanti, P. P., & Nogueira, D. A. (2014). ExpDes: an R package for ANOVA and experimental designs. *Applied Mathematics*, *5*(19), 2952–2958. https://doi.org/10.4236/am.2014.519280

Ganapathi, T., & Dharmatti, P. R. (2018). Effect of integrated nutrient modules on growth, yield and quality parameters of banana cv. Grand Naine. *International Journal of Current Microbiology and Applied Sciences*, *7*(1), 1974–1984. https://doi.org/10.20546/ijcmas.2018.701.239

Gibert, O., Dufour, D., Giraldo, A., Sánchez, T., Reynes, M., Pain, J. P., González, A., Fernández, A., & Díaz, A. (2009). Differentiation between cooking bananas and dessert bananas. 1. Morphological and compositional characterization of cultivated Colombian *Musa*ceae (*Musa* sp.) in relation to consumer preferences. *Journal of Agricultural and Food Chemistry*, *57*(17), 7857–7869. https://doi.org/10.1021/jf901788x

Hoagland, D. R., & Arnon, D. I. (1950). *The water-culture method for growing plants without soil*. The College of Agriculture, University of California - Berkeley.

IBGE. (2020). *Indicadores IBGE - levantamento sistemático da produção agrícola, estatística da produção agrícola, setembro 2020*. Instituto Brasileiro de Geografia e Estatística.

Irish, B. M., Goenaga, R., Montalvo-Katz, S., Chaves-Cordoba, B., & Van den Bergh, I. (2019). Host response to black leaf streak and agronomic performance of banana genotypes in Puerto Rico. *HortScience*, *54*(10), 1808–1817. https://doi.org/10.21273/HORTSCI13876-19

Köppen, W., & Geiger, R. (1928). *Klima der Erde*. Justus Perthes.

Kruskal, W. H., & Wallis, W. A. (1952). Use of ranks in one-criterion variance analysis. *Journal of the American Statistical Association*, *47*(260), 583–621. https://doi.org/10.1080/01621459.1952.10483441

Lédo, A. S., Silva, T. N., Martins, C. R., Silva, A. V. C., Lédo, C. A. S., & Amorim, E. P. (2018). Physicochemical characterization of banana fruit by univariate and multivariate procedures. *Bioscience Journal*, *34*(1), 24–33. https://doi.org/10.14393/BJ-v34n1a2018-37232

Lessa, L. S., Oliveira, T. K., Amorim, E. P., Assis, G. M. L., & Silva, S. O. (2012). Características vegetativas e seus efeitos sobre a produção de bananeira em três ciclos. *Revista Brasileira de Fruticultura*, *34*(4), 1098–1104. https://doi.org/10.1590/S0100-29452012000400017

Mahmoud, H. I., Elkashif, M. E., & Elamin, O. M. (2018). Effect of plant spacing and number of suckers on yield components and fruit quality of main crop and first four ratoons of banana clones in central Sudan. *Acta Horticulturae*, *1216,* 27–36. https://doi.org/10.17660/ActaHortic.2018.1216.4

Oliveira, J. A. A., Pereira, M. C. T., Nietsche, S., Souza, V. N. R., & Costa, I. J. S. (2014). Aclimatização de mudas micropropagadas de bananeira em diferentes substratos e recipientes. *Revista Brasileira de Ciências Agrárias*, *9*(1), 72–78. https://doi.org/10.5039/agraria.v9i1a3682

O'Neill, M. E., & Mathews, K. (2000). Theory and methods: a weighted least squares approach to Levene's test of homogeneity of variance. *Australian and New Zealand Journal of Statistics*, *42*(1), 81–100. https://doi.org/10.1111/1467-842X.00109

Pereira, M. C. T., Salomão, L. C. C., Silva, S. O., Sediyama, C. S., Couto, F. A. A., & Silva Neto, S. P. (2000). Crescimento e produção de primeiro ciclo da bananeira 'Prata Anã' (AAB) em sete espaçamentos. *Pesquisa Agropecuária Brasileira*, *35*(7), 1377–1387. https://doi.org/10.1590/s0100-204x2000000700012

Perrier, X., De Langhe, E., Donohue, M., Lentfer, C., Vrydaghs, L., Bakry, F., Carreel, F., Hippolyte, I., Horry, J. P., Jenny, C., Lebot, V., Risterucci, A. M., Tomekpe, K., Doutrelepont, H., Ball, T., Manwaring, J., de Maret, P., & Denham, T. (2011). Multidisciplinary perspectives on banana (*Musa* spp.) domestication. *Proceedings of the National Academy of Sciences of the United States of America*, *108*(28), 11311–11318. https://doi.org/10.1073/pnas.1102001108

Ploetz, R. C., Kepler, A. K., Daniells, J., & Nelson, S. C. (2007). Banana and plantain - an overview with emphasis on Pacific island cultivars. *Musa*ceae (banana family). In C. R. Elevitch (Ed.), *Species profiles for Pacific island agroforestry*. Permanent Agriculture Resources. http://www.bananenzeug.ch/wp-content/uploads/2018/06/banana-plantain-overview.pdf

R Core Team. (2019). *R: a language and environment for statistical computing*. R Foundation for Statistical Computing. https://www.r-project.org/

Salmazo, P., Silva, P., Valério, T. N., Grzebieluckas, C., & Krause, W. (2021). *Cartilha do fruticultor: cultivo adensado e viabilidade econômica da banana da terra*. UNEMAT.

Shapiro, S. S., & Wilk, M. B. (1965). An analysis of variance test for normality (complete samples). *Biometrika*, *52*(3/4), 591–611. https://doi.org/10.2307/2333709

Silva, M. J. R., Jesus, P. R. R., Anjos, J. M. C., Machado, M., & Ribeiro, V. G. (2016). Caracterização agronômica e pós-colheita das bananeiras 'Maravilha' e 'Preciosa' no submédio do Vale São Francisco. *Revista Ceres*, *63*(1), 46–53. https://doi.org/10.1590/0034-737X201663010007

Silva, S. O., Passos, A. R., Donato, S. L. R., Salomão, L. C. C., Pereira, L. V., Rodrigues, M. G. V., Lima Neto, F. P., & Lima, M. B. (2003). Avaliação de genótipos de bananeira em diferentes *ambientes*. *Ciência e Agrotecnologia*, *27*(4), 737–748. https://doi.org/10.1590/s1413-70542003000400001

Soil Survey Staff. (2014). *Keys to soil taxonomy* (12th ed.). United States Department of Agriculture, Natural Resources Conservation Service.

Teixeira, P. C., Donagemma, G. K., Fontana, A., & Teixeira, W. G. (Eds.). (2017). *Manual de métodos de análise de solo* (3rd ed.). Embrapa.

Youryon, P., & Supapvanich, S. (2017). Physicochemical quality and antioxidant changes in 'Leb Mue Nang' banana fruit during ripening. *Agriculture and Natural Resources*, *51*(1), 47–52. https://doi.org/10.1016/j.anres.2015.12.004

Performance of corn hybrids for *in natura* consumption in the Pontal do Triângulo Mineiro region of Brazil

Comportamiento de híbridos de maíz para consumo *in natura* en el Pontal do Triângulo Mineiro de Brasil

Vanessa Cury Galati[1*], Emilly Ruas Alkimim[1], Layane Aparecida Mendes dos Santos[1], José Henrique Ferreira Cardoso[1], Gelsimar Faria Oliveira[1], and Flávio Hiroshi Kaneko[1]

ABSTRACT

Corn is considered one of the most economically important crops in the world. However, in addition to its importance for grain production, this crop stands out for the consumption of green corn. This study aimed to characterize different corn hybrids grown in the Pontal do Triângulo Mineiro region of Brazil, considering the agronomic characteristics of importance and the preference of local consumers. We evaluated six characteristics as follows: cob length (CL), cob diameter (CD), cob weight with husks (WWH) (g), weight without husks (WWNH) (g), soluble solids content (SS) (°Brix), and plant population per ha (PP). Data were subjected to analysis of variance and the means were compared by the Scott-Knott test at a 1% probability level. For the sensory analysis, we evaluated the flavor, color, texture, and purchase intention characteristics of corn cobs. The hybrids XB6012 Bt, R9789 vip3, SX 8555 vip3, SX 7005 vip3 and BM709 PRO2 showed higher values for CL and WWNH, characteristics of importance for the fresh corn consumer market. The hybrid BM 709 PRO2 had significant potential compared to the others, due to the higher values of CL and CD, in addition to being among the hybrids with the highest WWNH. Based on the sensory analysis, the hybrids SX 7005 vip3 and DKB 390 PRO3 stood out in terms of flavor and texture, surpassing the local commercial corn.

Key words: local acceptability, yield component, sensory analysis, *Zea mays*.

RESUMEN

El maíz se considera uno de los cultivos de mayor importancia económica a nivel mundial. Sin embargo, además de su importancia para la producción de granos, este cultivo se destaca por el consumo de mazorcas frescas. El objetivo del presente estudio fue caracterizar diferentes híbridos de maíz cultivados en la región Pontal do Triângulo Mineiro de Brasil, teniendo en cuenta las variables agronómicas de importancia y la preferencia del consumidor local. Se evaluaron seis características: longitud de la mazorca (LM), diámetro de la mazorca (DM), peso de la mazorca con hojas (PH) (g), peso de la mazorca deshojada (PD) (g), contenido de sólidos solubles (SS) (°Brix) y población de plantas por ha (PP). Los datos obtenidos se sometieron a análisis de varianza y se compararon las medias mediante la prueba de Scott-Knott al 1% de probabilidad. Para el análisis sensorial, se evaluaron el sabor, color, textura e intención de compra de las mazorcas. Los híbridos XB6012 Bt, R9789 vip3, SX 8555 vip3, SX 7005 vip3 y BM709 PRO2 presentaron valores superiores para LM y PD, características de importancia para el mercado consumidor de maíz fresco. El híbrido BM 709 PRO2 tuvo un potencial significativo comparado con los demás debido a los mayores valores de LM y DM, además de estar entre los genotipos con mayor PD. Con base en el análisis sensorial, los híbridos SX 7005 vip3 y DKB 390 PRO3 se destacaron por su sabor y textura, superando al maíz comercial local.

Palabras clave: aceptabilidad local, componente de rendimiento, análisis sensorial, *Zea mays*.

Introduction

Brazil is one of the largest producers and exporters of agricultural products in the world. Grains, mainly soybeans and corn, show rapid growth in production and productivity due to the geographic expansion in the Midwest region of the country and the adoption and diffusion of technological innovations (Borlachenco & Gonçalves, 2017). Corn (*Zea mays* L.) is classified within the group of C4 plants and shows good edaphoclimatic adaptations, allowing its cultivation in varied places. Corn production for the 2019/2020 harvest exceeded 100 million t of grain, in an area of approximately 18.2 million ha (Conab, 2019).

Low average productivity of corn is usually directly related to the low investment capacity of producers for improved seeds adapted to a particular planting location that is associated with losses caused by biotic and abiotic factors.

Received for publication: 13 April, 2021. Accepted for publication: 30 December, 2021.

Doi: 10.15446/agron.colomb.v39n3.95046

[1] Federal University of Triângulo Mineiro, Iturama, Minas Gerais (Brazil).
[*] Corresponding author: vanessa.galati@uftm.edu.br

For satisfactory growth and development, corn depends on water availability and temperature and solar radiation at ideal levels for the crop (Machado *et al*., 2020). Additionally, the selection of genetic material suitable and adapted for each region of cultivation (genotype x environment interaction) is a fundamental factor that guarantees good results. So, it is expected that the material can express its genetic potential (Cruz *et al*., 2011).

In addition to its importance for grain production, the corn crop is important for cob production for fresh consumption, popularly known as "green corn" production. The cultivation of green corn has aroused interest among farmers in Brazil, as a demand exists throughout the year providing income due to its popular acceptance and the possibility of good prices.

In this sense, there are hybrids destined, for example, to the production of grains that can be interesting options for the cultivation of green corn, so they may be considered hybrids with double aptitude (Rodrigues *et al*., 2009; Costa *et al*., 2015).

Green corn is the corn harvested and consumed while still fresh, when the grains exhibit approximately 70 to 80% humidity and before there is a conversion of sugar into starch (stage R3 of development). The time required to reach this phenological stage may vary between different genotypes (Barbieri *et al*., 2005).

For the production of green corn, it is necessary to evaluate characteristics considered desirable for its cultivation as well as for commercialization, such as the commercial percentage of cobs, weight of cobs, diameter of cobs, and yellow grains with straight alignment. This is because commercialization is based on these attributes, in addition to the analysis of sensory acceptability, an extremely important characteristic in this market (Rodrigues *et al*., 2018).

Consumer acceptability can be determined by sensory analysis that corresponds to the sensations arising from physiological reactions, through stimuli that generate interpretations for each product. The sensations produced by psychological effects and the stimuli produced by physical-chemical effects quantify the sensations of the product (Albuquerque *et al*., 2008).

In the Pontal do Triângulo Mineiro region, there is no tradition for the production of grains, especially corn. The economy is focused, in general, on extensive livestock and sugar cane production. In recent years, there has been an increase in the search for more information and, consequently, more knowledge by local producers about the corn crop and its various applications, grain production, silage, and green corn production. In this sense, research focused on evaluating the behavior of different corn materials in this region is necessary to meet possible future demands and to understand the potential of different genotypes fitting the environmental conditions in the region.

This study aimed to characterize different corn hybrids grown in the Pontal do Triângulo Mineiro region, with respect to the agronomic characteristics of importance and the preference of local consumers.

Materials and methods

Genetic material and experimental conditions

This experiment was carried out under "dryland" conditions during the 2018/19 harvest in the municipality of Iturama, Minas Gerais, Brazil (19°43'20" S, 50°11'37" W, and 474 m a.s.l.). The experimental area belongs to the Rural Producers Union of Iturama and its soil is classified as a sandy Latosol with a sandy texture having the following chemical characteristics (0-20 cm) (Van Raij *et al*., 2001): $P_{(resin)}$: 15 mg dm^{-3}; organic matter: 17 g dm^{-3}; pH $_{(CaCl2)}$ 4.3; S: 3 mg dm^{-3}; Ca, Mg, K, H+Al, and Al: 12, 4, 0.8, 34, and 4 mmolc dm^{-3}, respectively. Average precipitation is shown in Figure 1.

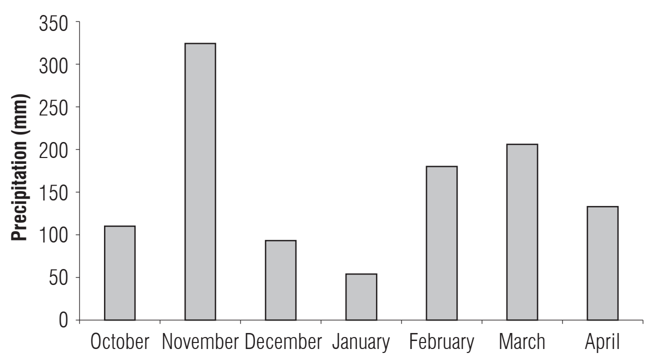


FIGURE 1. Average precipitation for the municipality of Iturama, Minas Gerais, Brazil, during the 2018/19 harvest.

In the 2017/18 harvest, the soil was corrected with 3 t ha^{-1} of dolomitic limestone incorporated with a plow harrow. Subsequently, 1 t ha^{-1} of plaster was applied to the area occupied by corn (silage) in the first harvest and sorghum (silage) in the second harvest.

For implementation of the experiment (2018/19 harvest), the area was desiccated with 2000 g of active ingredient (a.i.) ha^{-1} of glyphosate and then scarification and leveling with a leveling grid were carried out. The crop was planted with a 0.80 m interline spacing with 60,000 seeds per ha on November 28th, 2018. Fertilization at planting was carried out with 320 kg ha^{-1} of the formula 06-24-12 (sowing furrow) and 350 kg ha^{-1} of 20-00-20 in cover (vegetative development phase V_3). For weed management, spraying was carried out at V_3 with 1,500 g a.i. ha^{-1} of atrazine with manual weeding control. Management of the corn leafhopper (*Dalbulus maidis*) and fall armyworm (*Spodoptera frugiperda*) was performed with two applications of 120 g a.i. ha^{-1} of imidacloprid + 10 g a.i. ha^{-1} of lambda-cyhalothrin + 20 g a.i. ha^{-1} of chlorantraniliprole (V_2 and V_3).

Plant material evaluated consisted of 10 commercial corn hybrids: XB 6012 Bt, R 9789 vip3, Syn 555 vip3, SX 8555 vip3, SX 7005 vip3, DKB 390 PRO3, DKB 363 PRO3, BM 815 PRO2, BM 709 PRO2, AG 8740 PRO3. For the sensory analysis, a commercial hybrid was added as control of local preference for consumption as green corn, purchased in a supermarket in the municipality of Iturama.

The experimental design used was in randomized blocks with three replicates. The plots consisted of eight 10-m-long lines, and the six central lines were considered useful for the purpose of data collection and observations.

Evaluation of productive performance of corn hybrids

To evaluate the performance of the different hybrids, the following characteristics were evaluated: cob length (CL) (cm) and cob diameter (CD) (mm), measured using a digital caliper from the brand Mitutoyo with an accuracy of 0.05 mm; weight with husks (WWH) (g) and weight without husks (WWNH) (g) determined using a portable digital high-precision scale (STC02) with a capacity of up to 50 kg; soluble solids content (SS) (°Brix), obtained through a corn juice aliquot, compressed by a gauze and quantified by a field refractometer (PR-101, Atago, Tokyo, Japan). Plant population per ha (PP) was obtained by counting plants for each hybrid in an area of 3.2 m^2 (2 lines of 4 m of crop line x 0.8 m of spacing between lines) as well as a conversion into the average estimate of plants per ha.

The evaluations for the characteristics CL, CD, WWH, WWNH and SS were obtained as the average of the total from five cobs. Harvest was carried out manually, as well as the husking of the cobs in the R_3 phase of the crop. Data obtained were subjected to analysis of variance (ANOVA) and the means were compared by the Scott-Knott test at 1% probability level using the statistical program RBio (Bhering, 2017).

Analysis of the sensory profile of corn hybrids

In addition to the evaluation of agronomic characteristics, a sensory analysis was carried out at the Chemistry Laboratory at the Federal University of Triângulo Mineiro (UFTM) where flavor, color, texture and purchase intention of the corn cobs were evaluated. The sensory test was performed with 21 people (volunteers from UFTM) and characteristics were evaluated on a scale of grades. For the flavor and texture characteristics, the scale consisted of 1 (liked a lot), 2 (liked moderately), 3 (indifferent), 4 (disliked moderately) and 5 (disliked a lot). For the coloring, the scale consisted of 1 (very yellow grains), 2 (yellow), 3 (light yellow) and 4 (whitish). Purchase intention was evaluated on a scale of 1 (would certainly buy), 2 (would probably buy), 3 (indifferent), 4 (probably would not buy) and 5 (certainly would not buy).

Samples for sensory analysis consisted of pieces of approximately 5 cm in length from each of the cobs, corresponding to each of the 11 corn hybrids. These were cooked in a pressure cooker at 180°C for 20 min. Subsequently, the samples were served at room temperature (25°C) in disposable cups encoded with random numbers of three digits. Together with the sample, the evaluators received the analysis sheet with a scale of grades and a glass of water to rinse the mouth between tasting one sample to another, avoiding interference with the results.

Results and discussion

Evaluation of the productive performance of corn hybrids

The corn hybrids were harvested on February 20th, 2019, 84 d after sowing. After harvesting and evaluating the characteristics, the data were subjected to analysis of variance (ANOVA) at 1% probability level, using the statistical program RBio. Based on the results obtained, a significant difference was observed for the characteristics of cob length, cob diameter, weight with husks, and weight without husks (Tab. 1). There was no significant difference between blocks, so the experimental area was more homogeneous.

Based on the results of the ANOVA, for the characteristics that were significant, the averages were grouped by the Scott-Knott test at 1% probability using the RBio program (Tab. 2).

For the cob length and weight without husks, the hybrids XB 6012 Bt, R 9789 vip3, SX 8555 vip3, SX 7005 vip3, and BM 709 PRO2 stood out with the highest values (Tab. 2). The cob length showed values between 20.84 cm (BM 709

TABLE 1. Analysis of variance for the cob length (CL), cob diameter (CD), weight with husks (WWH), weight without husks (WWNH), soluble solids content (SS), plant population per ha (PP) of corn.

		MS					
SV	**DF**	**CL**	**CD**	**WWH**	**WWNH**	**SS**	**PP**
Block	2	0.93[NS]	2.10[NS]	3558[NS]	779[NS]	0.36[NS]	0.3[NS]
Treatment	9	11.65[**]	25.83[**]	16983[**]	5394[**]	0.83[NS]	1.72[NS]
Residue	18	0.29	2.532	2108	385	0.44	1.93
CV (%)		2.93	3.91	13.80	8.51	6.13	14.17

[**]Significant at 1% probability; NS - not significant; SV - source of variation; DF - degree of freedom; MS - medium square; CV - coefficient of variation.

TABLE 2. Average values of cob length (CL) (cm), cob diameter (CD) (mm), weight with husks (WWH) (g), and weight without husks (WWNH) (g) of corn.

Cultivars	Variables			
	CL	**CD**	**WWH**	**WWNH**
XB 6012 Bt	19.97 a	42.32 b	397.78 b	275.56 a
R 9789 vip3	19.60 a	42.39 b	488.89 a	277.78 a
Syn 555 vip3	16.37 c	41.87 b	303.33 c	225.56 b
SX 8555 vip3	19.92 a	41.33 b	351.11 b	261.55 a
SX 7005 vip3	20.38 a	39.56 c	348.89 b	241.11 a
DKB 390 PRO3	15.58 c	37.62 c	226.67 c	170.00 c
DKB 363 PRO3	16.53 c	38.42 c	302.22 c	194.44 c
BM 815 PRO2	18.22 b	41.00 b	294.45 c	221.11 b
BM 709 PRO2	20.84 a	46.54 a	360.00 b	273.33 a
AG 8740 PRO3	16.59 c	36.22 c	252.22 c	167.78 c

Means followed by the same letters do not differ significantly according to the Scott-Knott test at 5% probability level.

PRO2) and 15.58 cm (DKB 390 PRO3). The average weight of a cob without husks ranged from 277.78 g (R 9789 vip3) to 167.78 g (AG 8740 PRO3).

The hybrid BM 709 PRO2 differs significantly from the others for cob diameter. This hybrid showed an average value of 46.54 mm for this variable, which is higher than that found for the other evaluated cultivars. The hybrid BM 709 PRO2 had significant potential for commercialization as green corn, standing out in relation to the others due to the greater cob length and larger cob diameter. The same could be said for the hybrids XB 6012 Bt, R 9789 vip3 and SX 8555 vip3, although they had a smaller cob diameter than the hybrid BM 709 PRO2.

For the production of green corn, it is desirable to obtain high commercial cob weight since its commercialization is dependent on this characteristic. Cobs larger than 15 cm in length and 3 cm in diameter are considered as commercial cob patterns (Grigulo *et al*., 2011; Rodrigues *et al*., 2018). Therefore, despite the prominence of some hybrids, in general, all the evaluated materials showed cob length and cob diameter considered ideal for commercialization as green corn.

For the weight of the cob with husks, the values observed ranged from 226.67 g (DKB 390 PRO3) to 488.89 g (R 9789 vip3). The cultivar R 9789 vip3 differed significantly from the others due to its higher value (488.89 g). It is important to highlight that the weight of the cob with husks must be evaluated in the production of green corn since for processing or consumption *in natura*, the cobs are usually transported with the husk to the final destination. This is done to avoid sugar degradation and product denaturation as well as physical damage caused by transportation. Thus, the aim is to maintain the sweet taste of cobs for commercialization (Rodrigues *et al*., 2009; Grigulo *et al*., 2011).

In addition to the cluster analysis of means, we estimated the correlation between the variables evaluated (Fig. 2). The correlation measures the degree of association between two variables. High correlations between two characters indicate that the change in one character, via selection, promotes changes in the other. The correlation can be positive when it is possible to obtain gains for one variable by selecting another, or negative when the selection in one variable will negatively contribute to another (Resende, 2002). This strategy allows for rapid progress, via indirect selection, as it allows selecting variables that have complex inheritance and that are difficult to measure, through another correlated variable that is easier to measure (Oliveira *et al*., 2011).

The highest values of positive correlation were observed between the variables weight without husks and cob length (0.83), weight without husks and weight with husks (0.77), weight without husks and cob diameter (0.76), weight with husks and cob length (0.67), cob diameter and cob length (0.67), and weight with husks and cob diameter (0.54) (Fig. 2). Considering the direction and magnitude of these correlations, the selection for these variables would be advantageous since it seeks to obtain plants with greater weight without husks and with greater cob length and diameter.

The highest values of negative correlation were observed between the variables plant population per ha and weight without husks (-0.46) and plant population per ha and cob length (-0.46). This result was expected, since the larger the plant population, the greater the competition between plants (intraspecific competition) and, consequently, this will result in shorter cob lengths and lower cob weight without husks.

In this study, it was possible to verify that the average estimate of the population of plants per ha varied from 54,901 (AG 8740 and DKB 363) to 66,000 plants per ha (BM 709 PRO2) (Tab. 3).

TABLE 3. Estimated average values of plant population per ha for each evaluated hybrid of corn.

Cultivars	Plant population per ha
XB 6012 Bt	56,862
R 9789 vip3	60,784
Syn 555 vip3	62,745
SX 8555 vip3	58,823
SX 7005 vip3	62,745
DKB 390 PRO3	56,863
DKB 363 PRO3	54,901
BM 815 PRO2	58,823
BM 709 PRO2	66,666
AG 8740 PRO3	54,901

Considering that the production focused on green corn, it is important to evaluate the plant population per ha since for this type of market the ideal is to work with an adequate plant population to obtain cobs with the appropriate weight and sizes for consumption as green corn. Corn is the crop of the grass group that is most sensitive to variation in plant density, and some researchers consider the genotype itself as the main determinant of density (Pricinotto *et al*., 2019).

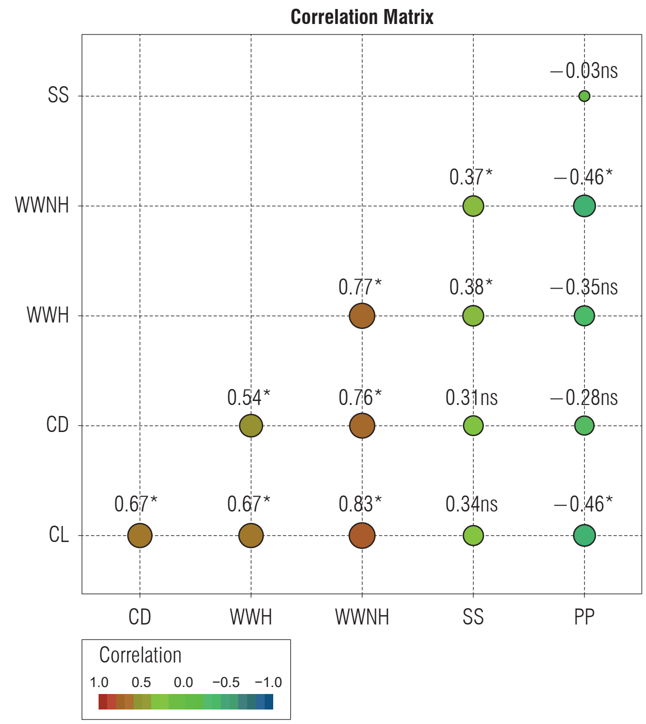


FIGURE 2. Correlation between the variables cob length (CL), cob diameter (CD), weight with husks (WWH), weight without husks (WWNH), soluble solids content (SS), plant population per ha (PP) of corn. * and ns - significant and non-significant correlations, respectively, at the 5% probability level ($P<0.05$) according to the T test.

Analysis of the sensory profile of corn hybrids

Based on the sensory analysis, we observed for the flavor that the hybrids SX7005 vip3, DKB 390 PRO3, and XB 6012 Bt showed greater acceptance by the evaluators, with the first two surpassing the "local commercial control" (Fig. 3).

For the color of the corn cobs, most of the evaluators preferred the cobs with yellow and light-yellow coloring, such as XB6012 Bt (77%), SYN 555 vip3 (71%), DKB 363 PRO3 (52%), DM 709 PRO2 (75%), AG 8740 PRO3 (78%), and SX 8555 vip3 (80%) (Fig. 3). Pereira Filho *et al*. (2002), studying different corn hybrids, found that cobs with lighter colored grains are preferred, when the product is intended for consumption of green corn *in natura*. The color of the corn endosperm is controlled by a gene that gives the yellow color with a dominant homozygote and the white color with a recessive homozygote that directly interferes with acceptance by the final consumer (Barbieri *et al*., 2005).

The corn hybrids that showed a soft texture and were more pleasant to the evaluators were SX 7005 vip3 (78%)

and DBK 390 PRO3 (73%) that was slightly below the local commercial hybrid that showed 86% acceptance (Fig. 3). It is important to note that texture is related to the thickness of the pericarp, so the thicker the pericarp, the greater the resistance to chewing. This characteristic is also influenced by the stage of grain maturation, that is, the more mature the grain, the thicker the pericarp and the lesser the acceptance related to texture (Barbieri *et al*., 2005; Camilo *et al*., 2015).

The purchase intention parameter was evaluated at the end of the sensory test. The hybrids SYN 555 vip3 (73%), SX 7005 vip3 (85%), DKB 390 PRO3 (80%) and the local commercial hybrid (62%) were the most accepted for purchase by the evaluators (Fig. 3). The hybrids with the best acceptability were harvested approximately 50 d after flowering. According to this research, the included hybrids showed acceptance of purchase higher than the local commercial hybrid.

Based on the sensory analysis, we verified that the hybrids SX 7005 vip3 and DKB 390 PRO3 stood out in flavor and texture, surpassing the local commercial corn. This result directly affected the purchase intention analysis, in which approximately 80% and 85% of people would buy the hybrids DKB 390 PRO3 and SX 7005 vip3, respectively.

As there are still few corn hybrids on the market that produce green corn, these can be an option for small producers when harvested at the right time, with high moisture and sugar content in the grains. In this way, the producer will select the hybrid that shows the best productive and sensorial characteristics to ensure consumer satisfaction.

Conclusions

Under the edaphoclimatic conditions of the region of the municipality of Iturama, located in the Pontal do Triângulo Mineiro region, Brazil, the hybrids XB6012 Bt, R9789 vip3, SX 8555 vip3, SX 7005 vip3, and BM709 PRO2 showed outstanding characteristics for the fresh corn consumer market. It is worth mentioning that the hybrid BM 709 PRO2 has significant potential for commercialization as green corn, standing out in relation to the others, due to

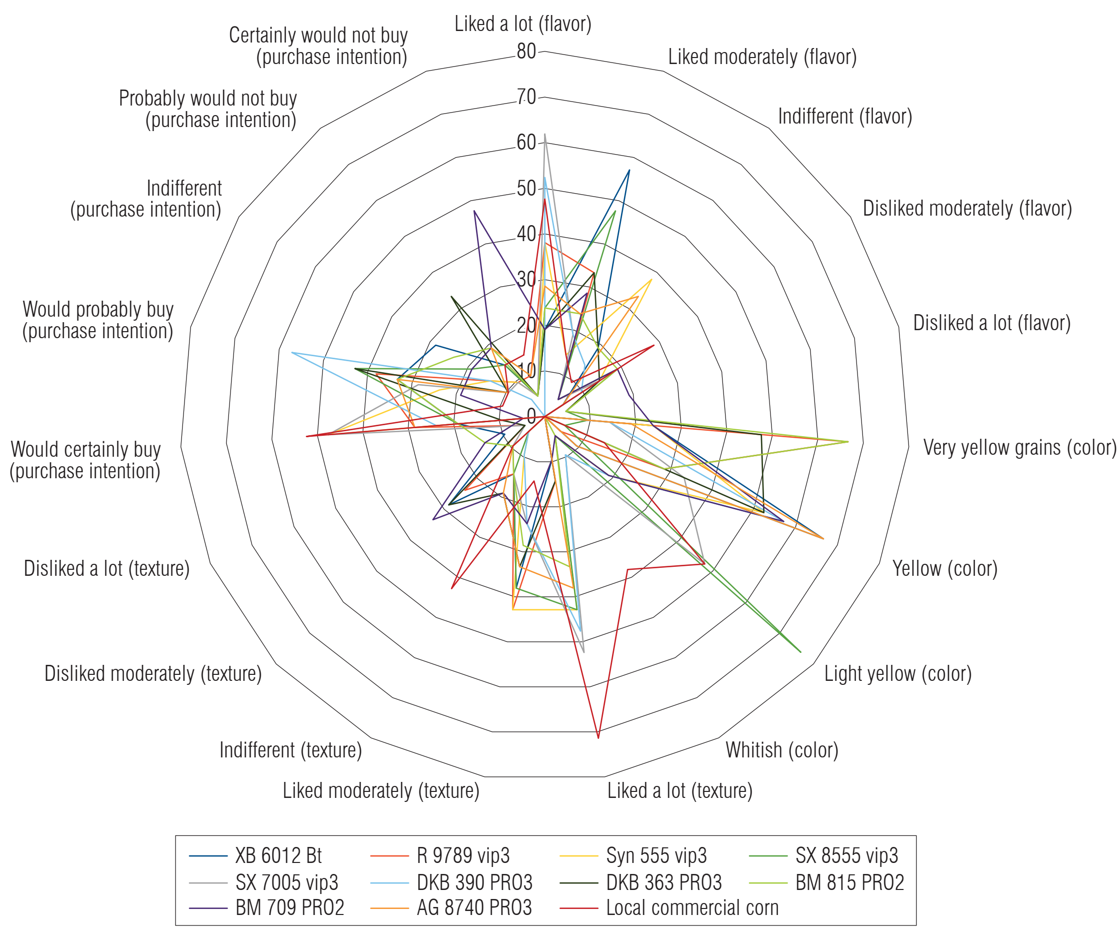


FIGURE 3. Analysis of the sensory profile of corn hybrids according to the scale of grades to assess taste, color, texture, and purchase intention.

the greater length and diameter of the cob, besides being among the hybrids with greater weight of the cob without husks. Based on the sensory analysis, the hybrids SX 7005 vip3 and DKB390 PRO3 stood out in terms of flavor and texture, surpassing the local commercial corn, which resulted in greater acceptability (purchase intention) by the evaluators.

Conflict of interest statement

The authors declare that there is no conflict of interest regarding the publication of this article.

Author's contributions

VCG, ERA, and FHK conceived and planned the trials, conducted laboratory analysis, wrote, formatted, reviewed, and edited the manuscript, statistically analyzed data, and were responsible for the design of the experiment. LAMS, JHFC and GFO conceived, planned the trials, conducted field study, and performed field and laboratory analyses.

Literature cited

Albuquerque, C. J. B., Von Pinho, R. G., & Silva, R. (2008). Produtividade de híbridos de milho verde experimentais e comerciais. *Bioscience Journal*, *24*(2), 69–76.

Barbieri, V. H. B., Luz, J. M. Q., Brito, C. H., Duarte, J. M., Gomes, L. S., & Santana, D. G. (2005). Produtividade e rendimento industrial de híbridos de milho doce em função de espaçamentos e populações de plantas. *Horticultura Brasileira*, *23*(3), 826–830. https://doi.org/10.1590/S0102-05362005000300027

Bhering, L. L. (2017). Rbio: a tool for biometric and statistical analysis using the R platform. *Crop Breeding and Applied Biotechnology*, *17*, 187–190. https://doi.org/10.1590/1984-70332017v17n2s29

Borlachenco, N. G. C., & Gonçalves, A. B. (2017). Expansão agrícola: elaboração de indicadores de sustentabilidade nas cadeias produtivas de Mato Grosso do Sul. *Interações*, *18*(1), 119–128. https://doi.org/10.20435/1984-042X-2017-v.18-n.1(09)

Camilo, J. S., Barbieri, V. H. B., Rangel, R. M., Bonnas, D. S., Luz, J. M. Q., & Oliveira, R. C. (2015). Aceitação sensorial de híbridos de milho doce e híbridos de milho verde em intervalos de colheita. *Revista Ceres*, *62*(1), 1–8. https://doi.org/10.1590/0034-737X201562010001

Conab. (2019). *Boletim da safra de grãos: nono levantamento - safra 2018/2019*. Companhia Nacional de Abastecimento. https://www.conab.gov.br/info-agro/safras/graos/boletim-da-safra-de-graos?start=30

Costa, F. R., Damaso, L. F., Mendes, R. C., Marques, D. D., & Rodrigues, F. (2015). Desempenho de híbridos de milho para consumo *in natura* em diferentes doses de nitrogênio. *Científica*, *43*(2), 109–116. https://doi.org/10.15361/1984-5529.2015v43n2p109-116

Cruz, J. C., Viana, J. H. M., Alvarenga, R. C., Pereira Filho, I. A. P., Santana, D. P., Pereira, F. T. F., & Hernani, L. C. (2011). *Cultivo do milho* (7th ed.). Embrapa Milho e Sorgo.

Grigulo, A. S. M., Azevedo, V. H., Krause, W., & Azevedo, P. H. (2011). Avaliação do desempenho de genótipos de milho para consumo *in natura* em Tangará da Serra, MT, Brasil. *Bioscience Journal*, *27*(4), 603–608.

Machado, M. L., Simão, M. L. R., Simão, F. R., & Alexandrino, R. C. S. (2020). Stress conditions in soybean areas based on measurements of soil-plant-atmosphere system and UAV images. *Pesquisa Agropecuária Tropical*, *50,* Article e61785. https://doi.org/10.1590/1983-40632020v5061785

Oliveira, E. J., Santos, V. S., Lima, D. S., Machado, M. D., Lucena, R. S., & Motta, T. B. N. (2011). Estimativas de correlações genotípicas e fenotípicas em germoplasma de maracujazeiro. *Bragantia*, *70*(2), 255–261. https://doi.org/10.1590/S0006-87052011000200002

Pereira Filho, I. A., Cruz, J. C., & Gama, E. E. G. (2002). Cultivares de milho para o consumo verde. In I. A. Pereira Filho (Ed.), *O cultivo do milho verde* (pp. 17–30). Embrapa Milho e Sorgo.

Pricinotto, L. F., Zucareli, C., Ferreira, A. S., Spolaor, L. T., & Fonseca, I. C. B. (2019). Yield and biometric characteristics of maize submitted to plant population and trinexapac-ethyl doses. *Revista Caatinga*, *32*(3), 667–678. https://doi.org/10.1590/1983-21252019v32n311rc

Resende, M. D. V. (2002). *Genética biométrica e estatística no melhoramento de plantas perenes*. Embrapa.

Rodrigues, F., Melo, P. G. S., Resende, C. L. P., Mrojinski, F., Mendes, R. C., & Silva, M. A. (2018). Aptidão de híbridos de milho para o consumo *in natura*. *Revista de Ciências Agrárias, 41*(2), 484–492. https://doi.org/10.19084/RCA17216

Rodrigues, F., Von Pinho, R. G., Albuquerque, C. J. B., Faria Filho, E. M., & Goulart, J. C. (2009). Capacidade de combinação entre linhagens de milho visando à produção de milho verde. *Bragantia*, *68*(1), 75–84. https://doi.org/10.1590/S0006-87052009000100009

Van Raij, B., Andrade, J. C., Cantarella, H., & Quaggio, J. A. (Eds.). (2001). *Análise química para avaliação da fertilidade de solos tropicais*. Instituto Agronômico.

Population dynamics of the nematodes *Heterodera glycines* and *Pratylenchus brachyurus* in a succession crop of soybean and chickpea

Dinámica poblacional de los nemátodos *Heterodera glycines* y *Pratylenchus brachyurus* en un cultivo de sucesión de soya y garbanzo

Jadir Borges Pinheiro[1], Giovani Olegario da Silva[1], Amanda Gomes Macêdo[2], Jhenef Gomes de Jesus[3], Ludmila Raulino[2], Caroline da Cruz Magalhães[4], Danielle Biscaia[1], Raphael Augusto de Castro e Melo[1], Patrícia Pereira da Silva[1], and Warley Marcos Nascimento[1]

ABSTRACT

The objective of this research was to evaluate the population dynamics of the nematodes *Heterodera glycines* race 5 and *Pratylenchus brachyurus* in a succession crop of soybeans and chickpeas. The experiment was carried out in Campos Novos dos Parecis, MT State, Brazil, from February to May 2017. Six chickpea cultivars were planted in February and evaluated in a naturally infested area of 60 ha. Five soil samples were collected at random in georeferenced and equidistant locations, forming a composite sample by replication. Five plants per georeferenced point and new soil samples were collected at regular intervals of about 30 d. The nematodes were extracted, identified, and counted. Data were subjected to variance and regression analyses. A reduction in the population levels of *H. glycines* race 5 was observed throughout the chickpea cycle, indicating that this species can be cultivated in succession with soybeans in the presence of this nematode. However, due to the maintenance in the population of *P. brachyurus* in the roots, rotation of soybean with chickpeas is not recommended in fields naturally infested with this nematode.

Key words: *Cicer arietinum* L., *Glycine max* (L.) Merrill, cyst nematode, root-lesion nematode.

RESUMEN

El objetivo de esta investigación fue evaluar la dinámica poblacional de los nematodos *Heterodera glycines* raza 5 y *Pratylenchus brachyurus* en cultivos sucesivos de soya y garbanzo. El experimento se llevó a cabo en Campos Novos dos Parecis, estado de MT, Brasil, de febrero a mayo de 2017. Se sembraron seis cultivares de garbanzo en febrero y se evaluaron en un área naturalmente infestada de 60 ha. Se recolectaron cinco muestras de suelo al azar en ubicaciones georreferenciadas y equidistantes, formando una muestra compuesta por replicación. Se recolectaron cinco plantas por punto georreferenciado y también nuevas muestras de suelo a intervalos regulares de aproximadamente 30 d. Los nematodos se extrajeron, identificaron y contaron. Los datos fueron sometidos a análisis de varianza y regresión. Se observó una reducción en los niveles poblacionales de *H. glycines* raza 5 a lo largo del ciclo del garbanzo, lo que indica que es posible cultivar esta especie en sucesión con soya en presencia de este nematodo. Sin embargo, debido al mantenimiento en la población de *P. brachyurus* en las raíces, no se recomienda la rotación de soya con garbanzos en campos naturalmente infestados con este nematodo.

Palabras clave: *Cicer arietinum* L., *Glycine max* (L.) Merrill, nematodo quístico, nematodo lesionador de raíces.

Introduction

Chickpea (*Cicer arietinum* L.) is the fourth most important legume grown in the world after soybeans, peanuts, and beans (FAOSTAT, 2021). Its grains contain fibers, proteins, vitamins, carbohydrates, mineral salts, unsaturated fatty acids, and β-carotene and are a source of protein in human and animal food supplementation (Souza, 2019). There are reports of cultivation in 55 countries, with a total production of 17.19 million t (FAOSTAT, 2021). The world average yield is approximately 1.2 t ha^{-1}; however, some countries reach higher values, such as Israel (6.04 t ha^{-1}), China (5.2 t ha^{-1}), and Moldova (3.9 t ha^{-1}) (Avelar *et al*., 2018).

In Brazil, domestic consumption and production are considered low, but in recent years, due to its nutritional characteristics and changes/trends in the patterns of food

Received for publication: 8 June, 2021. Accepted for publication: 6 December, 2021. Doi: 10.15446/agron.colomb.v39n3.96469

[1] Embrapa Hortaliças, Brasília (Brazil).
[2] Universidade de Brasília - UnB, Campus Universitário Darcy Ribeiro (Brazil).
[3] União Pioneira de Integração Social - UPIS, Campus II, Planaltina (Brazil).
[4] Centro Universitário de Desenvolvimento do Centro-Oeste - UNIDESC, Luziânia (Brazil).
[*] Corresponding author: giovani.olegario@embrapa.br

consumption, the demand for chickpea grains has grown. However, it is still necessary to import a large part of the quantity consumed in the domestic market, most of which comes from Mexico and Argentina (Artiaga *et al*., 2015), with amounts close to 8,000 t per year (Avelar *et al*., 2018). This new scenario gives chickpea an important role as an economic option for planting in the second summer harvest (known as *safrinha* in Portuguese and meaning "little harvest") in the Brazilian Cerrado biome, which takes advantage of the drought tolerance ability of this species to grow despite little rainfall (Artiaga *et al*., 2015).

The many species of plant-parasitic nematodes (PPN) are among the constraints that can cause impairments to chickpea production; some of these species include root-knot nematodes (*Meloidogyne javanica*, *M. incognita*, and *M. artiella*), cyst nematode (*Heterodera ciceri*), and the root-lesion nematode (*Pratylenchus* spp.) (Castillo *et al*., 2008; Zwart *et al*., 2019). Characterization studies in various parts of the world show that most chickpea genotypes are normally susceptible (Zwart *et al*., 2019). However, there are few reports of chickpea genotypes with nematode tolerance (Ali & Ahmad, 2000; Ansari *et al*., 2004; Hassan & Devi, 2004; Chakraborty *et al*., 2016; Sumita, 2017), making more studies in this area necessary.

PPN also damage soybeans, with *Heterodera glycines* as one of the most harmful species due to its wide geographic distribution. However, in recent years, mainly in the Midwest region of Brazil, as well as in other regions, damages were registered and also attributed to the root-lesion nematode, *Pratylenchus brachyurus*, a polyphagous species (Cruz *et al*., 2020) that colonizes even grasses such as maize and pastures.

Heterodera glycines can also colonize other plants like sunn hemp (*Crotalaria juncea*), pigeon pea (*Cajanus cajan*) (Valle *et al*., 1996), common bean (*Phaseolus vulgaris*), azuki bean (*Vigna angularis*), and mung bean (*Vigna radiata*) (Rossi & Ferraz, 2001). Poromarto and Nelson (2010) verified that chickpea was a poor host of *H. glycines* race 3; however, there are no studies on *H. glycines* race 5. Cardoso *et al*. (2019) evaluated the resistance of *Crotalaria ochroleuca* to races 1, 2, 3, 5, 6, and 14 of *H. glycines*, and verified that it was resistant to races 1, 2 and 5, but was susceptible to race 3 and showed varied reactions to races 6 and 14. Due to the increase in the cultivated areas of chickpea in succession to soybeans in the Brazilian Midwest, research on these two PPN species becomes important. Thus, the objective of this study was to evaluate the population dynamics of *Heterodera glycines* race 5 and *Pratylenchus brachyurus* in a succession crop of soybean and chickpea.

Materials and methods

The experiment was carried out in Campos Novos dos Parecis, a municipality in the state of Mato Grosso, Brazil, from February to May 2017, in a field area naturally infested with the species *Heterodera glycines* race 5 and *Pratylenchus brachyurus*. The regional climate according to the Köppen classification is tropical with a dry winter and rainy summer, CWB type. The weather of the summer season in the Cerrado biome is characterized by a semi-humid tropical climate, with average temperatures of 25°C and annual precipitation of 1,677 mm. Six chickpea cultivars, BRS Aleppo, BRS Cícero, Jamu 96, BRS Cristalino, BRS Toro, and BRS Kalifa, were evaluated. The sowing occurred on February 24, 2017.

The experiment was conducted in an area of 60 ha (10 ha per cultivar), previously cultivated with a susceptible soybean cultivar, in a completely randomized design with three replicates. Soil and plant samples were collected at regular intervals of about 30 d on March 24, April 24, and May 25. For each replicate, five soil samples of 500-600 g and roots were collected using equidistant georeferenced points. All the samples were identified, extracted, and quantified in the Nematology Laboratory of Embrapa Vegetables, Brasília - DF.

The *H. glycines* and *P. brachyurus* species were identified according to their morphologic characteristics (Gonzaga, 2006; Mekete *et al*., 2012). The identification of *H. glycines* race 5 was performed with the differentiating host test according to Riggs and Schmitt (1988).

The extraction of second-stage juveniles and cists of *H. glycines,* as well as juveniles and adults of *P. brachyurus* from the soil samples, was performed using the rapid centrifugal-flotation technique, employing a sucrose solution, in accordance with Jenkins (1964). The extraction of PPN associated with roots was performed using the method of Hussey and Barker, modified by Bonetti and Ferraz in accordance with Wilcken *et al*. (2010). After the extractions, the quantification of the nematodes was performed with a Peters chamber and the visualization was made under a trinocular stereoscopic microscope (model Eclipse 80i, Nikon, Melville, NY, USA) in accordance with Bellé *et al*. (2017).

The following response variables were evaluated: number of viable cysts of *H. glycines*/150 cm^3 of soil (NVCS), number of nonviable cists of *H. glycines*/150 cm^3 of soil (NNVCS), number of juveniles of second stage (J2) of *H. glycines*/150 cm^3 of soil (NJ2HS), number of juveniles and adults of *P. brachyurus*/150 cm^3 of soil (NJAPS), and number of juveniles and adults of *P. brachyurus*/g of fresh roots (NJAPR). The data were subjected to variance and regression analyses using the Genes statistical program (Cruz, 2016).

Results and discussion

There was no effect of the cultivars on the response variables evaluated. On the contrary, statistical differences were observed for the source of variation - date of collection or sampling time for all the evaluated variables, except for the number of juveniles and adults of *Pratylenchus* in roots (NJAPR). Table 1 indicates that the population levels of *P. brachyurus* per g of roots remained constant. There was also no interaction between the cultivars and sampling times - collection (CxCL) (Tab. 1), indicating that the population dynamics of the studied nematode species was not affected by any of these factors (cultivars and times).

Regarding the number of viable cysts of *H. glycines*/150 cm^3 of soil (NVCS), a significant reduction in the population was observed during the chickpea crop cycle (Fig. 1), regardless of the cultivar used.

An increase in the number of nonviable cysts of *H. glycines* /150 cm^3 of soil (NNVCS) was observed according to the sampling times (CL) (Fig. 2), regardless of the chickpea cultivar.

As for the number of juveniles of second stage (J2) of *H. glycines*/150 cm^3 of soil (NJ2HS), among all the evaluated cultivars, there was a reduction of population levels from 57.64 to 16.67 (Fig. 3).

The increase in the number of nonviable cysts, the reduction of viable cysts, and the reduction in the number of these PPN in the soil may be due to the hatching of

TABLE 1. Summary of the joint analysis of variance of the population dynamics of the soybean cyst nematode (*Heterodera glycines* race 5) and the root-lesion nematode (*Pratylenchus brachyurus*) in a chickpea crop.

Source of variation	Mean square				
	NVCS	NNVCS	NJ2HS	NJAPS	NJAPR
Cultivar (C)	79.13^{ns}	220.71^{ns}	912.37^{ns}	446.82^{ns}	31411.15^{ns}
Collection (CL)	211.19*	1772.54*	5245.22*	2779.94*	272338.9^{ns}
CxCL	46.95^{ns}	195.15^{ns}	920.01^{ns}	890.57^{ns}	43086.66^{ns}
Residue	62.21	160.17	1629.16	955.03	94252.54
General mean	10.24	15.01	34.20	28.64	339.56
CV (%)	36.42	29.95	59.56	41.35	23.20

*Significant at 5% of probability by F test. ns - Not significant at 5% probability by F test.NVCS - Number of viable cysts of *H. glycines*/150 cm^3 of soil; NNVCS - number of nonviable cysts of *H. glycines*/150 cm^3 of soil; NJ2HS - number of second-stage juveniles (J2) of *H. glycines*/150 cm^3 of soil; NJAPS - number of juveniles and adults of *Pratylenchus brachyurus*/150 cm^3 soil; NJAPR - number of juveniles and adults of *P. brachyurus*/g of roots (fresh weight). CV - coefficient of variation.

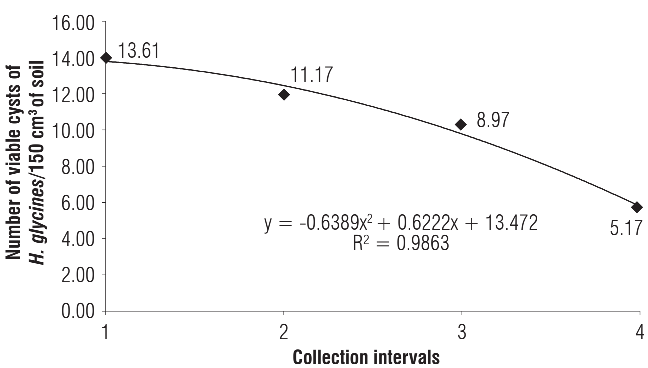


FIGURE 1. Number of viable cysts of *Heterodera glycines*/150 cm^3 of soil according to the collection intervals (30, 60, and 90 d after sowing).

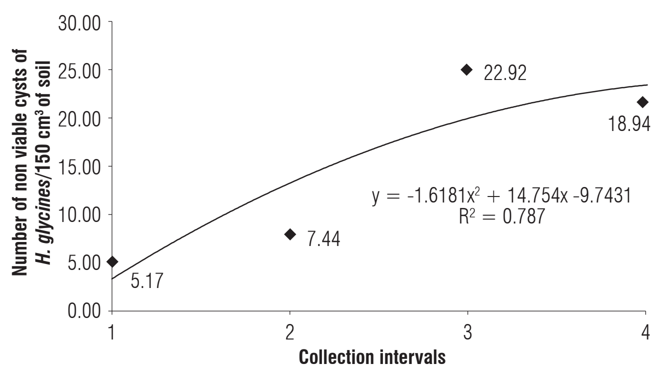


FIGURE 2. Number of nonviable cysts of *Heterodera glycines*/150 cm^3 of soil according to the collection intervals (30, 60, and 90 d after sowing).

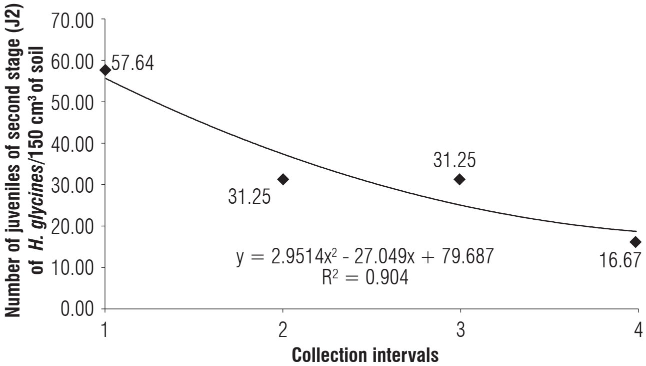


FIGURE 3. Number of juveniles of second stage (J2) of *Heterodera glycines*/150 cm^3 of soil according to the collection intervals (30, 60, and 90 d after sowing).

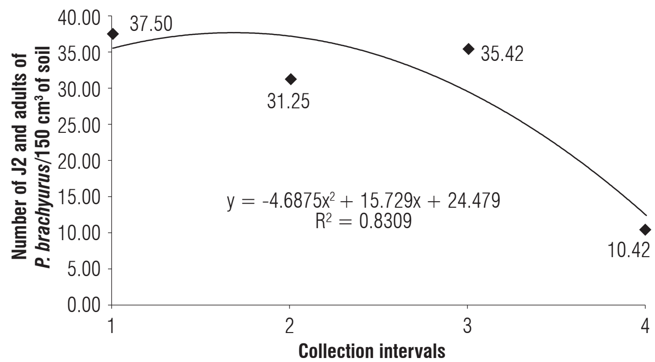


FIGURE 4. Number of juveniles of second stage (J2) and adults of *Pratylenchus brachyurus*/150 cm^3 of soil according to the collection intervals (30, 60, and 90 d after sowing).

juveniles of *H. glycines* race 5 and the absence of a host plant in the area for a period that prevented the reinfection and continuity of its life cycle (Cardoso *et al.,* 2019). This result shows that chickpea is not a host of *H. glycines* race 5 and that it can be indicated for crop rotation or succession with soybeans in the areas infested with this race. This is a significant finding, as there are no similar studies in the literature.

The number of juveniles and adults of *P. brachyurus*/150 cm^3 of soil (NJAPS) was maintained during the first 60 d of the cycle, with a subsequent sharp reduction observed from 35.42 to 10.42 between the samplings collected at 60 and 90 d of the cycle (Fig. 4). However, as previously verified, the population per g of roots remained high in all the samples. This reduction in the population of *P. brachyurus* in the soil, considering that the chickpea vegetative cycle is close to 110 d, was probably because this nematode is a migrating endoparasite that prefers roots to soil. When plants are expanding their canopy in an area with a large initial population of nematodes in the soil, it is usual for migration to occur with more intensity as the roots are also developing. At the end of the crop cycle, there will be a new migration to the soil, with a subsequent search for host plants (Puerari *et al.,* 2020).

Santos (2019) evaluated these same six cultivars in a naturally infested field with the presence of *M. javanica* for their resistance to *P. brachyurus*, finding a marked differentiation in terms of their susceptibility. Nevertheless, this author estimated only the reproduction factor (RF) based on the rate between the initial population at the time of the sowing and the final nematode population in the soil. That study verified that the cultivar Jamu 96 maintained the same population of *P. brachyurus* in both periods, *i.e.*, showing a reproduction factor equal to 1; BRS Cicero showed a reproduction factor of 0.95 and all the others had RFs ranging from 0.43 to 0.65. The initial population level of *P. brachyurus* in the soil may be a determinant factor to the degree of resistance of the cultivars; since the population in this study was considered very high, it may have resulted in a greater nematode pressure, making it impossible to verify differences in the resistance levels of the cultivars.

Santos (2019) demonstrated that these chickpea cultivars showed resistance to *M. javanica* in a naturally infested field with a mixed population of *M. javanica* and *P. brachyurus*. However, Bernardes Neto *et al.* (2019) evaluated the same cultivars in a greenhouse trial and considered them susceptible to *M. incognita* and *M. enterolobii*, which are also important for both chickpeas and soybeans.

Among the most widespread control methods for PPN are the use of nematicides, crop rotation, antagonistic plants, organic matter, fallowing, soil fumigation, solarization, and use of genetic resistance (Ravichandra, 2014; Cruz *et al.,* 2020). Crop rotation with non-host plant species for a sufficient period to allow the decomposition of infested residues reduces the viability and association of the pathogen, especially in the no-tillage system (Cruz *et al.,* 2020). It also increases beneficial soil organisms and minimizes the impact of root diseases (Cruz *et al.,* 2020).

To evaluate the response of different plant species to *H. glycines* and to better understand what crops could be recommended in rotation or succession or as green manure for soybeans, Sanches (2001) evaluated two species of velvet bean (*Mucuna pruriens* and *Mucuna utilis*) for reaction to races 2, 3, 4, 5 and 14 of *H. glycines* and verified that both are not hosts of this nematode. Evaluating the response of several species used as green manure or cover crops to race 3 of this nematode, Valle *et al*. (1996) found that sunn hemp (*Crotalaria juncea)*, pigeon pea (*Cajanus cajan*), azuki beans (*Vigna angularis*), and mungo beans (*Vigna radiata*) are hosts that can multiply these PPN. The susceptibility of azuki and mungo beans was also verified by Rossi and Ferraz (2001), who also found susceptibility in common beans (*Phaseolus vulgaris*) and white lupine (*Lupinus albus* L.) to race 3 of this nematode.

In addition to cultivated plants, *H. glycines* can also be found in weeds. Venkatesh *et al*. (2000) evaluated 22 weed species from 13 families of dicotyledonous as alternative hosts of *H. glycines* in a greenhouse in Ohio, USA, and found that the red dead-nettle (*Lamium purpureum*), common henbit (*Lamium amplexicaule*), field pennycress (*Thlaspi arvense*) and shepherd purse (*Capsella bursa-pastoris*) were hosts. A greater reproduction of race 1 and a low reproduction of race 6 were found in red dead-nettle. For race 3, the highest level of reproduction occurred in red dead-nettle, field pennycress, and common henbit (Venkatesh *et al*., 2000).

Poromarto and Nelson (2010) found that the chickpea was not an efficient host of *H. glycines* race 3, although it allowed the multiplication of a small number of this nematode. However, Cardoso *et al*. (2019), who evaluated the resistance of rattlepod (*Crotalaria ochroleuca)* to races 1, 2, 3, 5, 6, and 14 of *H. glycines*, found that this plant species was resistant to races 1, 2, and 5 but was susceptible to race 3 and showed varied reactions to races 6 and 14.

The identification of these *H. glycines* races is important since prior to this research, no study had identified resistance of this plant to race 5 of this nematode.

Conclusions

There was a reduction in the population levels of *Heterodera glycines* race 5 throughout the cycle of chickpeas, indicating that it is possible to grow this species in a succession crop with soybeans in the presence of this nematode.

Due to the maintenance of the population of *Pratylenchus brachyurus* in the roots throughout the chickpea cycle, crop rotation of soybean with chickpeas would not be recommended for fields infested with this nematode.

Conflict of interest statement

The authors declare that there is no conflict of interest regarding the publication of this article.

Author's contributions

JBP, AGM, and DB designed the experiments. JBP, AGM, JGJ, LR, CCM, and DB carried out the field and laboratory experiments. GOS and RACM contributed to the data analysis. JBP, GOS, AGM, RACM, PPS, and WMN wrote the article. All authors reviewed the manuscript.

Literature cited

Ali, S. S., & Ahmad, R. (2000). Screening of chickpea germplasm against nematode. *International Chickpea and Pigeonpea Newsletter*, *7*, 8–9.

Ansari, M. A., Patel, B. A., Mhase, N. L., Patel, D. J., Douaik, A., & Sharma, S. B. (2004). Tolerance of chickpea (*Cicer arietinum* L.) lines to root-knot nematode, *Meloidogyne javanica* (Treub) Chitwood. *Genetic Resources and Crop Evolution*, *51*, 449–453. https://doi.org/10.1023/B:GRES.0000023460.26690.23

Artiaga, O. P., Spehar, C. R., Boiteux, L. S., & Nascimento, W. M. (2015). Avaliação de genótipos de grão de bico em cultivo de sequeiro nas condições de Cerrado. *Revista Brasileira de Ciências Agrárias*, *10*(1), 102–109.

Avelar, R. I. S., Costa, C. A., Rocha, F. S., Oliveira, N. L. C., & Nascimento, W. M. (2018). Yield of chickpeas sown at different times. *Revista Caatinga*, *31*(4), 900–906. https://doi.org/10.1590/1983-21252018v31n412rc

Bellé, C., Kaspary, T. E., Kuhn, P. R., Schmitt, J., & Lima-Medina, I. (2017). Reproduction of *Pratylenchus zeae* on weeds. *Planta Daninha*, *35*, Article e017158528. https://doi.org/10.1590/S0100-83582017350100006

Bernardes Neto, J. F., Pinheiro, J. B., Silva, G. O., Biscaia, D., Macedo, A. G., Silva, P. P., & Nascimento, W. M. (2019). Reação de genótipos de grão-de-bico aos nematoides-das-galhas *Meloidogyne incognita* raça 1 e *Meloidogyne enterolobii*. *Revista Agrária Acadêmica*, *2*(4), 63–70. https://doi.org/10.32406/v2n42019/63-70/agrariacad

Cardoso, M. R., Dias-Arieira, C. R., Ribeiro, N. R., Almeida, A. A., Miamoto, A., & Lopes, A. P. M. (2019). *Crotalaria ochroleuca* susceptibility to *Heterodera glycines* races. *Journal of Agricultural Science*, *11*(7), 205–212. https://doi.org/10.5539/jas.v11n7p205

Castillo, P., Navas-Cortés, J. A., Landa, B. B., Jiménez-Díaz, R. M., & Vovlas, N. (2008). Plant-parasitic nematodes attacking chickpea and their *in planta* interactions with rhizobia and phytopathogenic fungi. *Plant Disease*, *92*(6), 840–853. https://doi.org/10.1094/PDIS-92-6-0840

Chakraborty, G., Mondal, S., Karmakar, P., Roy, D., & Samanta, P. (2016). Screening of some pulse germplasm for their reactions to root-knot nematode, *Meloidogyne incognita* (Kofoid and White) Chitwood. *Current Nematology*, *27*(2), 137–142.

Cruz, C. D. (2016). Genes software - extended and integrated with the R, Matlab and Selegen. *Acta Scientiarum Agronomy*, *38*(4), 547–552. https://doi.org/10.4025/actasciagron.v38i3.32629

Cruz, T. T., Asmus, G. L., & Garcia, R. A. (2020). *Crotalaria* species in succession to soybean for the management of *Pratylenchus brachyurus*. *Ciência Rural*, *50*(7), Article e20190645. https://doi.org/10.1590/0103-8478cr20190645

FAOSTAT. (2021). *Chick peas production*. Food and Agriculture Organization of the United Nations. http://www.fao.org/faostat/en/#search/Chick%20peas

Gonzaga, V. (2006). *Caracterização morfológica, morfométrica e multiplicação in vitro das seis espécies mais comuns de Pratylenchus Filipjev, 1936 que ocorrem no Brasil* [Doctoral dissertation, Universidade Estadual Paulista]. Repositório Institucional UNESP. https://repositorio.unesp.br/bitstream/handle/11449/105288/gonzaga_v_dr_jabo.pdf?sequence=1

Hassan, M. G., & Devi, S. (2004). Evaluation of mungbean, urdbean, pea, lentil and chickpea germplasm for resistance to root-knot nematode (*Meloidogyne incognita*). *Indian Journal of Nematology*, *34*(2), 228–229.

Jenkins, W. R. (1964). A rapid centrifugal-flotation technique for separating nematodes from soil. *Plant Disease Reporter*, *48*(9), 692.

Mekete, T., Dababat, A., Sekora, N., Akyazi, F., & Abebe, E. (Eds.). (2012). *Identification key for agriculturally important plant-parasitic nematodes*. CIMMYT.

Poromarto, S. H., & Nelson, B. D. (2010). Evaluation of northern-grown crops as hosts of soybean cyst nematode. *Plant Health Progress*, *11*(1). https://doi.org/10.1094/PHP-2010-0315-02-RS

Puerari, H. H., Miamoto, A., Ferreira, J. C. A., Cardoso, M. R., & Dias-Arieira, C. R. (2020). Penetration and development of *Pratylenchus brachyurus* in maize roots treated with acibenzolar-S-methyl. *European Journal of Plant Pathology*, *157*, 835–844. https://doi.org/10.1007/s10658-020-02044-3

Ravichandra, N. G. (2014). *Horticultural Nematology*. Springer.

Riggs, R. D., & Schmitt, D. P. (1988). Complete characterization of the race scheme for *Heterodera glycines*. *Journal of Nematology*, *20*(3), 392–395.

Rossi, C. E., & Ferraz, L. C. C. B. (2001). Efeito de diferentes plantas hospedeiras sobre a morfometria de uma população brasileira de *Heterodera glycines*. *Arquivos do Instituto Biológico*, *68*(1), 95–102.

Sanches, J. B. (2001). *Histopatologia de raízes de Mucuna pruriens (L.) DC infectadas pelo nematóide de cistos da soja, Heterodera glycines* [Doctoral dissertation, Universidade Federal de Viçosa]. Locus repositório institucional da UFV. https://www.locus.ufv.br/handle/123456789/11304

Santos, L. P. (2019). *Resistência de cultivares de grão-de-bico a Meloidogyne javanica e Pratylenchus brachyurus no Cerrado brasileiro* [Undergraduate thesis, Instituto Federal Goiano]. Repositório Instituto Federal Goiano. https://repositorio.ifgoiano.edu.br/handle/prefix/630

Souza, C. V. A. (2019). *Características agronômicas e qualidade fisiológica de sementes de grão-de-bico em função da densidade de plantas* [Master's thesis, Universidade de Brasília]. Repositório Institucional da UnB. https://repositorio.unb.br/handle/10482/35513

Sumita, K. (2017). Reaction of chickpea lines to *Meloidogyne incognita*. *World Journal of Pharmacy and Pharmaceutical Sciences*, *6*(10), 1138–1140.

Valle, L. A. C., Dias, W. P., & Ferraz, S. (1996). Reação de algumas espécies vegetais, principalmente leguminosas, ao nematóide de cisto da soja, *Heterodera glycines* Ichinohe. *Nematologia Brasileira*, *20*(2), 30–40.

Venkatesh, R., Harrison, S. K., & Riedel, R. M. (2000). Weed hosts of soybean cyst nematode (*Heterodera glycines*) in Ohio. *Weed Technology*, *14*(1), 156–160.

Wilcken, S. R. S., Rosa, J. M. O., Higuti, A. R. O., Garcia, M. J. M., & Cardoso, A. I. I. (2010). Reprodução de *Meloidogyne* spp. em porta-enxertos e híbridos de pepino. *Horticultura Brasileira*, *28*(1), 120–123. https://doi.org/10.1590/S0102-05362010000100023

Zwart, R. S., Thudi, M., Channale, S., Manchikatla, P. K., Varshney, R. K., & Thompson, J. P. (2019). Resistance to plant-parasitic nematodes in chickpea: current status and future perspectives. *Frontiers in Plant Science*, *10*, Article 966. https://doi.org/10.3389/fpls.2019.00966

Morphological variations and abundance of populations of the leafhopper *Dalbulus maidis* (DeLong) (Hemiptera: Cicadellidae) from the corn-producing region of Huila, Colombia

Variaciones morfológicas y abundancia de poblaciones del saltahojas *Dalbulus maidis* (DeLong) (Hemiptera: Cicadellidae) provenientes de la región productora de maíz del Huila, Colombia

Isda Sánchez-Reinoso[1], Camilo Ignacio Jaramillo-Barrios[2], Buenaventura Monje-Andrade[2], Augusto Ramírez-Godoy[1], and Angela María Vargas Berdugo[2]*

ABSTRACT

Dalbulus maidis is known as the vector of the pathogens causing corn stunt complex disease that decreases crop yields to percentages higher than 90%. In the most affected areas of this disease, there is an increase in the abundance of this insect. This research evaluated the abundance and morphological characterization of populations of *D. maidis* collected from the region of Huila and determined the possible morphological variations within these populations. Leafhopper samples were collected in corn crops from 40 to 70 d after planting from eight localities in the corn-producing region of Huila. Taxonomic determination was carried out and twelve morphological characters were compared to evaluate the inter-population variations. Analyses of variance were performed to determine significant differences between individuals at three levels: abundance from specific corn-producing areas, morphotype, and sex. A total of 6,722 individuals were found, separated into four morphotypes. Total length, head length, and distance between the eyes showed significant differences. This study can serve as a basis for a better understanding of the vector for future research in the corn-producing regions of Colombia.

Key words: vector, morphometry, taxonomy, morphotypes, stunt.

RESUMEN

Dalbulus maidis es el principal vector de los agentes causales de la enfermedad del complejo del achaparramiento del maíz que disminuye el rendimiento del cultivo hasta en un 90%. En las áreas de mayor afectación por esta enfermedad se presenta un incremento en la abundancia del insecto. Esta investigación evaluó la abundancia y la caracterización morfológica de poblaciones de *D. maidis* recolectadas en el departamento del Huila y determinó posibles variaciones morfológicas dentro de estas poblaciones. Las muestras de los insectos fueron recolectadas en cultivos de maíz entre 40 y 70 d después de la siembra en ocho localidades de la región productora de maíz del Huila. Se llevó a cabo una determinación taxonómica y se compararon doce caracteres morfológicos para evaluar las variaciones entre poblaciones. Se realizaron análisis de varianza para determinar diferencias significativas entre los individuos en tres niveles: abundancia de áreas específicas de producción de maíz, morfotipo y sexo. Se encontraron 6722 individuos en total, separados en cuatro morfotipos. La longitud total, la longitud de la cabeza y la distancia entre los ojos presentaron diferencias significativas. Este estudio podría ser la base para una mejor comprensión del vector para futuras investigaciones en las regiones productoras de maíz en Colombia.

Palabras clave: vector, morfometría, taxonomía, morfotipos, achaparramiento.

Introduction

Corn is one of the most important crops in Colombia's agricultural sector. This crop represents 13% of the agricultural area in the country (CIMMYT & CIAT, 2019). More specifically, the Huila region recorded technical corn production of 23,706 t covering 5,689 ha in 2018, while traditional corn showed a production of 9,957 t with an area of 6,496 ha (Agronet, 2019).

The corn leafhopper *Dalbulus maidis* (DeLong) is a hemipteran insect of the family Cicadellidae known as the most important vector of corn pathogens such as *Candidatus* phytoplasma asteris (maize bushy stunt phytoplasma

Received for publication: 7 June, 2021. Accepted for publication: 28 December, 2021.

Doi: 10.15446/agron.colomb.v39n3.96432

[1] Departamento de Agronomía, Facultad de Ciencias Agrarias, Universidad Nacional de Colombia, Bogotá (Colombia).
[2] Corporación Colombiana de Investigación Agropecuaria (AGROSAVIA), Centro de investigación Nataima, Espinal, Tolima (Colombia).
[*] Corresponding author: avargas@agrosavia.co

- MBSP) and *Spiroplasma kunkelii* (corn stunt spiroplasm - CSS) that cause the severe corn stunt complex disease symptoms that affect corn yield (Moya-Raygoza, 2002; Varón de Agudelo & Sarria Villa, 2007). The maize rayado fino virus (MRFV) belongs to this complex. However, it has less symptomatic effects compared to CSS an MBSP. The vector *D. maidis* can individually or simultaneously transfer the pathogens CSS, MBSP, and MRFV during the early vegetative stages of corn growth. While the pathogens multiply within the plant, at the early stages of infection, the plant may not display symptoms and has apparently normal growth. However, at the late phases of the vegetative and early reproductive stages of the corn, leaf yellowing and reddening of the leaf lamina edge is observed. Simultaneously, an alteration of the cell cycle of the plant is noted and expressed as premature flowering, the proliferation of branches, and wilting. The most severe symptoms of the disease, such as dwarfism, shortening of nodes, excessive branching, and reduction in size and quantity of the grain, are evident at the reproductive stage of corn (Varón de Agudelo & Sarria Villa, 2007). Yield losses in maize crops of 50% to 90% have been reported due to maize stunting in Colombia as well as Brazil and Argentina (Druetta *et al*., 2018).

Knowledge of *D. maidis* populations makes it possible to establish the dynamics of the corn stunt complex disease. Additionally, the morphological description of insect vector populations is the first step in understanding the biology and ecology of a species, its relationship with the environment (Varón de Agudelo & Sarria Villa, 2007) and, in this case, how these factors may subsequently relate to its role as a vector. In insects, several studies show that factors such as temperature, latitude, and elevation affect the species morphology and development (Hawkins & Lawton, 1995; Krasnov *et al*., 1996; Smith *et al*., 2000; Hodkinson, 2005; Rodríguez-Zabala *et al*., 2016). Larsen and Nault (1994), in Mexican populations of *D. maidis*, observed changes in size, weight, and pigmentation according to low temperatures, as well as morphological variations in the populations according to the season of the year. In Brazil, the influence of latitude and elevation on populations of *D. maidis* has been evaluated, finding morphological differences associated with variations in these two factors in the studied sites. Greater body weight, size, and pigmentation are seen at higher elevations, as well as the separation of populations into two groups (populations in the northeastern region and populations in the central-southern region) according to the sampling site (Oliveira *et al*., 2004).

Dalbulus maidis has a wide distribution in the Americas, from the United States to Argentina, including some Caribbean islands (CABI, 2020). *Dalbulus maidis* population dynamics can directly influence the disease incidence and severity (Varón de Agudelo & Sarria Villa, 2007; Oliveira *et al*., 2013).

Studies of *D. maidis* populations have not yet been carried out in Colombia. During the last few years, the Huila region has been affected by an increasing spread of corn stunting complex and increased *D. maidis* populations (ICA, 2019). Therefore, studies of the characterization of *D. maidis* are necessary, allowing for an approach to the situation in this corn-producing region, based on the morphological evaluation and abundance of individuals. Considering the hypothesis that morphological variations exist within the populations of *D. maidis* associated with different corn-producing areas from Huila, this research was carried out as part of the project of integrated management of phytosanitary alternatives in pests and diseases for rice-corn-cotton systems in the inter-Andean valleys and the Caribbean. The objective of this research was to characterize morphologically *D. maidis* populations from the corn-producing region of Huila and determine possible morphological variations between them.

Materials and methods

Insect sampling

Insect sampling was carried out according to the permit for the collection of wild species for scientific research modified by resolution 1466 of 2014 granted by ANLA to the Corporación Colombiana de Investigación Agropecuaria (AGROSAVIA). Samples were collected in yellow and white corn crops from 40 to 70 d after planting that were conventionally established by farmers for commercial purposes. Sampling localities within the Huila region were considered based on the reported presence of *D. maidis* and the prevalence gradient of the disease provided by the Instituto Colombiano Agropecuario (ICA) - Huila region. The selection of localities was based on the following criteria: localities in Huila where the disease was reported for the first time related to increased leafhopper populations in 2015: Campoalegre and Palermo; localities selected as the escape zone for the disease: Tarqui and Garzón; localities with new disease reports and no reported presence of leafhoppers: Aipe and Villavieja; localities with no reports of the disease or presence of the genus *Dalbulus*: La Plata and Neiva. In this way, it was possible to cover areas from the south to the north of Huila (Fig. 1). After selecting the localities, samples were collected from October 2018 to May 2019. Two samples were collected from each farm visited using a Dietrick vacuum sampler or vacuum insect collector (D-VAC model 22, USA) from linear transects of 20 m from the edge of the crop towards the center since this is the pattern of arrival and dissemination of *D. maidis* in corn crops. The D-VAC vacuum sampler allowed the massive capture of specimens considering the behavior of *D. maidis* as a leafhopper and its great capacity to move from one plant to another. Additionally, the equipment allowed rapid access to the parts of the plants where *D. maidis* is most frequently found. A total of 72 samples corresponding to 36 corn farms were collected (Tab. 1). Subsequently, the collected material was stored in paper bags and transported in Styrofoam coolers to the Entomology laboratory of the Nataima research center of AGROSAVIA. Once in the laboratory, the samples were cleaned by removing the plant material and separating the individuals by taxonomic order, and depositing the specimens in vials with 70% alcohol at 4°C.

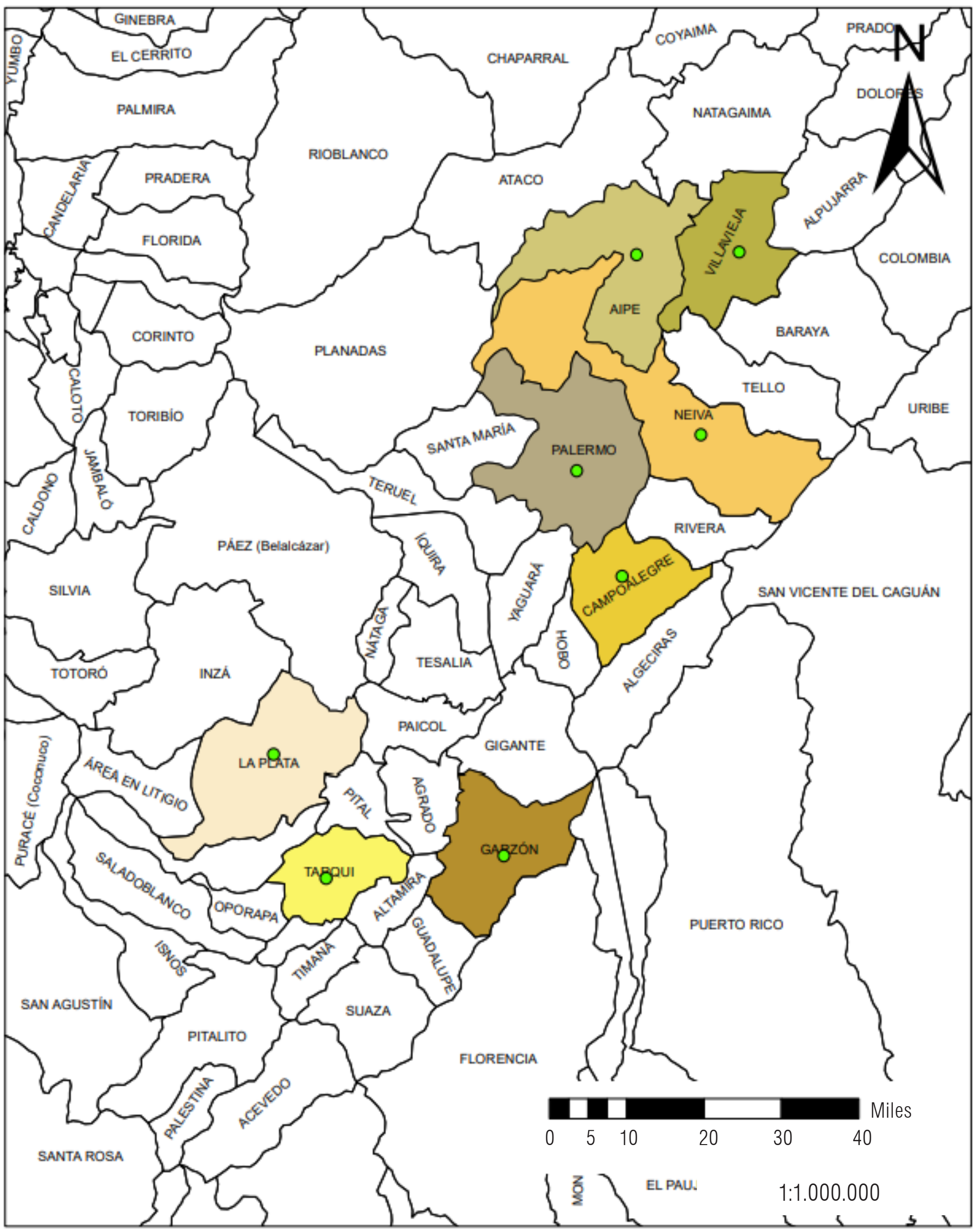


FIGURE 1. Localities in the Huila region where sampling was conducted.

TABLE 1. Weather conditions of the localities and location of the farms where samples were collected in the Huila region.

Sampling	Date	Location	Altitude (m a.s.l.)	Temperature (°C)	Relative humidity (%)	Precipitation (mm)	Coordinates
1	10/17/2018	Aipe	360	26 - 28	65 - 70	50 - 100	4°26'46.9'' N 75°11'57.0'' W
2	10/17/2018	Aipe	359	>28	65 - 70	50	3°26'42.4'' N 75°08'20.3'' W
3	04/25/2019	Aipe	363	>28	65 - 70	50	3°27'01.8'' N 75°08'32.2'' W
4	11/19/2018	Villavieja	379	>28	70 - 75	50 - 100	3°14'00.9'' N 75°13'38.9'' W
5	11/19/2018	Villavieja	373	>28	70 - 75	50 - 100	3°14'01.0'' N 75°13'37.4'' W
6	11/19/2018	Villavieja	391	>28	70 - 75	50 - 100	3°14'00.8'' N 75°13'40.6'' W
7	11/19/2018	Villavieja	390	>28	70 - 75	50 - 100	3°14'06.7'' N 75°13'40.1'' W
8	10/17/2018	Campoalegre	540	26 - 28	70 - 75	50 - 100	2°40'21.9'' N 75°21'35.0'' W
9	10/17/2018	Campoalegre	549	26 - 28	70 - 75	50 - 100	2°39'41.9 N 75°21'52.0'' W
10	10/17/2018	Campoalegre	584	24 - 26	70 - 75	50 - 100	2°37'48.8'' N 75°21'47.7'' W
11	11/20/2018	Campoalegre	535	26 - 28	70 - 75	50 - 100	2°37'46.9 N 75°21'37.5'' W
12	11/20/2018	Garzón	795	20 - 22	80 - 85	50 - 100	2°14'44.8'' N 75°37'06.9'' W
13	11/20/2018	Garzón	859	20 - 22	80 - 85	50 - 100	2°13'20.0'' N 75°36'43.5'' W
14	11/20/2018	Garzón	879	20 - 22	80 - 85	50 - 100	2°13'36-9'' N 75°36'44.5'' W
15	11/20/2018	Garzón	775	22 - 24	80 - 85	50 - 100	2°09'51.3'' N 75°41'34.8'' W
16	11/20/2018	Garzón	767	22 - 24	80 - 85	50 - 100	2°09'51.6'' N 75°41'34.9'' W
17	11/20/2018	Garzón	723	22 - 24	80 - 85	50 - 100	2°08'51.8'' N 75°40'43.2'' W
18	05/28/2019	Garzón	797	20 - 22	80 - 85	50 - 100	2°11'43.3'' N 75°37'42.2'' W
19	05/28/2019	Garzón	757	22 - 24	80 - 85	50 - 100	2°11'06.1'' N 75°39'17.1'' W
20	05/28/2019	Garzón	804	20 - 22	80 - 85	50 - 100	2°14'45.6'' N 75°37'39.0'' W
21	05/28/2019	Garzón	808	20 - 22	80 - 85	50 - 100	2°14'49.6'' N 75°37'42.9' 'W
22	05/28/2019	Garzón	817	20 - 22	80 - 85	50 - 100	2°12'41.0'' N 75°37'35.7'' W
23	10/18/2018	La Plata	1123	18 - 20	80 - 85	150 - 200	2°22'06.6'' N 75°53'44.0'' W
24	10/18/2018	La Plata	1060	18 - 20	80 - 85	150 - 200	2°22'27.8'' N 75°53'38.3'' W
25	04/24/2019	Neiva	417	26 - 28	70 - 75	50 - 100	3°00'35.1'' N 75°15'57.8'' W

continue on next page

Sampling	Date	Location	Altitude (m a.s.l.)	Temperature (°C)	Relative humidity (%)	Precipitation (mm)	Coordinates
26	11/19/2018	Palermo	508	26 - 28	75 - 80	50 - 100	2°51’32.2’’ N 75°21’18.2’’ W
27	11/19/2018	Palermo	496	26 - 28	75 - 80	50 - 100	2°50’44.6’’ N 75°20’46.3’’ W
28	05/27/2019	Tarqui	810	22 - 24	75 - 80	150 - 200	2°04’25.2’’ N 75°21’57.3’’ W
29	05/27/2019	Tarqui	799	22 - 24	75 - 80	150 - 200	2°06’41.1’’ N 75°47’19.0’’ W
30	05/27/2019	Tarqui	801	22 - 24	75 - 80	150 - 200	2°06’45.3’’ N 75°47’11.7’’ W
31	05/27/2019	Tarqui	798	22 - 24	75 - 80	150 - 200	2°06’48.0’’ N 75°47’10.8’’ W
32	05/27/2019	Tarqui	797	22 - 24	75 - 80	150 - 200	2°06’47.9’’ N 75°47’10.1’’ W
33	05/27/2019	Tarqui	775	22 - 24	75 - 80	150 - 200	2°07’00.8’’ N 75°46’50.8’’ W

Taxonomic confirmation of the *Dalbulus maidis* species

Species level confirmation was done through higher taxon order Hemiptera, family Cicadellidae to species. The morphological characters used in this study were selected following four taxonomic keys of the group that included the characters of the antennal flagellum, distal forewing cells, first posterior tarsomere, ocellar spot, and body coloration (Triplehorn & Nault, 1985; Knight & Webb, 1993; Dietrich, 2005; Triplehorn & Johnson, 2005). Female and male insects with complete morphological structures were used for determination. Morphological characters were observed with a Leica EZ4® stereoscope (Heerbrugg, Switzerland). The specimens to be characterized were entomologically mounted and deposited in the National Taxonomic Collection of Insects “Luis María Murillo” (CTNI) under custody of AGROSAVIA. The species were corroborated by digital photographs sent to an expert, Dr. Gustavo Moya-Raygoza (Department of Botany and Zoology, the University of Guadalajara, Mexico) (G. Moya-Raygoza, personal communication, November 9, 2020).

Dalbulus maidis abundance

The population frequency of *D. maidis* was evaluated by counting individuals collected from each of the corn-producing areas in the Huila region. The frequency of *D. maidis* was measured by the total number of individuals collected at each locality. To identify a possible relationship between morphology, abundance, and presence of *D. maidis*, the database of the agroclimatic network of Huila, generated by the Institute of Hydrology, Meteorology and Environmental Studies (IDEAM) was consulted. The average temperature, precipitation, and relative air humidity (RH) data generated by the network for each locality were included in the analyses.

Intraspecific morphological variations of *Dalbulus maidis*

After confirming that the insect populations collected in the eight maize-producing areas of Huila belonged to the species *D. maidis*, morphological differences within the population were determined, using a subsample of 10% of the individuals from areas with an abundance greater than 1,000 individuals. All individuals were analyzed in those areas where the abundance was less than 1,000 individuals. The separation of individuals into morphotypes was carried out in a dichotomous manner (presence/absence) by observing the following qualitative characters: head color, abdomen pigmentation, number of ocellar spots, and presence of anterior spots on the head (Triplehorn & Nault, 1985; Knight & Webb, 1993; Oliveira *et al*., 2004).

Seven morphometric characters were selected to determine differences within the populations across localities, morphotypes, and sex of individuals. The characters were selected according to their taxonomic significance to the species and for their usefulness for separating population morphotypes (Larsen & Nault, 1994; Oliveira *et al*., 2004). The characters tested were antenna length, ocellar spot diameter, head length, distance between compound eyes, right-wing length, left-wing length, and total body length. Finally, genitalia of males and females were extracted, and the aedeagus length, distance between the divergent apical processes, and ovipositor length were measured to determine any differences in the chosen specimens (Oliveira *et al*., 2004).

A photographic record of morphological characters was taken using a Leica EZ4W® stereoscope with a built-in 5.0-megapixel CMOS WiFi camera with multiple output options, capturing images of the dorsal and ventral views (20x and 35x) of the individuals. Using the Leica AirLab® application, morphometric character measurements were obtained.

Data analysis

The suitability of each morphological character for morphotype separation was evaluated employing a monothematic clustering analysis of binary variables using the MONA function (Struyf *et al*., 1997). For each morphometric character measured, an analysis of variance was performed to compare significant differences between individuals by locality (with different environmental conditions), morphotypes determined, and sex (LSD-Fisher). A factor analysis for mixed data (FAMD) was performed that combines the principal component technique with multiple correspondence analysis of qualitative and quantitative variables to observe their interactions (data not shown). A locality and morphotype cluster analysis was performed using Ward's method (Ward Jr., 1963) and the Bray-Curtis distance (Bray & Curtis, 1957). Analyses were performed using the statistical software R version 4.0.2 (R Core Team, 2020) with the packages FactoMineR, Factoextra, and Vegan (Lê *et al*., 2008; Kassambara & Mundt, 2017; Oksanen *et al*., 2019).

Results and discussion

Dalbulus maidis abundance

A total of 6,722 specimens of *Dalbulus maidis* were found in the study area. The areas with the highest abundance were Garzón and Tarqui with 3,820 and 2,650 individuals, respectively. In the Aipe-Villavieja, La Plata and Palermo areas, *D. maidis* abundance levels were null (Fig. 2). The availability of corn is an important factor affecting the abundance of *D. maidis* since they complete their biological cycle on this plant species (Meneses *et al*., 2016). The localities whose abundance was zero had the areas planted with corn decreased by the time of sampling. The migration processes of the species must be considered since *D. maidis* has good migration capacity that allows it to move long distances to a maximum of 10 km (Taylor *et al*., 1993). In our study, we must consider the displacement of corn crops in the producing areas of Huila since the localities of Tarqui and Garzón were selected by farmers for planting corn as escape zones from the problems caused by the stunting of corn in Campoalegre and Palermo. However, with the greater availability of food in these areas, there may be greater migration and an increase in the populations in Tarqui and Garzón with a greater abundance of leafhoppers in these areas.

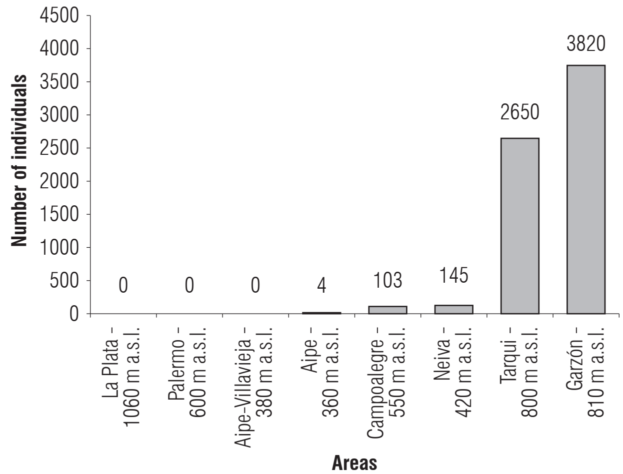


FIGURE 2. *Dalbulus maidis* abundance in eight corn-producing areas from Huila.

In combination with corn availability, climatic conditions can become important influences on the abundance of *D. maidis* in crops (Oliveira *et al*., 2013; Meneses *et al*., 2016). The eight localities were arranged into three groups according to climatic and altitude conditions (Tab. 2). The highest abundance was observed in Garzón and Tarqui, areas that had warm, semi-humid weather with an altitude range from 720 to 880 m a.s.l. and temperature from 22°C to 24°C. In contrast, the areas of Neiva and Campoalegre had warm-semiarid weather and were located from 360 to 585 m a.s.l. showing a much lower abundance compared to the Garzón and Tarqui areas (Tab. 2). The localities of Tarqui and Garzón showed temperatures optimal for *D. maidis* survival and fecundity (Meneses *et al*., 2016). Therefore, the strategy of establishing new production zones in locations with favorable conditions for the development and multiplication of *D. maidis* may increase the risk of introducing high populations of this insect to new areas and, consequently, the risk of dissemination of the maize stunt complex.

The time of sampling should be considered since the environmental conditions that may affect populations vary over time. In Brazil, variations in the abundance of Cicadellidae species were observed in 2005, 2006 and 2007 in 48 localities (Oliveira *et al*., 2013). *Dalbulus maidis* has shown changes in the abundance of populations considering the growing seasons with an increase of individuals in the months of the dry season compared to the abundance in the rainy season (Ávila & Arce, 2008; Meneses *et al*., 2016).

TABLE 2. Abundance of *Dalbulus maidis* and weather conditions of eight corn-producing areas from Huila.

Weather	Altitude (m a.s.l.)	Temperature (°C)	Area	Abundance	Gender ratio
Cold - wet	1060 - 1120	18 - 20	La Plata	0	0
Temperate - semi-wet	720 - 880	22 - 24	Garzón Tarqui	6470	1:1 1:1
Warm - semiarid	360 - 585	26 - 28	Aipe Villavieja Palermo	252	0
			Neiva Campoalegre		1:1 1:1

Morphotype evaluation

The data matrix cluster allowed identifying the separation of five groups by the morphological characteristics observed. Among the individuals evaluated, four morphotypes were described, and a group corresponding to another Cicadellidae genus, considered an external group, was discarded from the morphotype analysis of this study: morphotype II: absence of the dark abdomen character (Ab_dark), present in the other four morphotypes; morphotype III: missing opaque yellow head character (He_opye); external group: number of ocellar spots (N_ocs). Morphotypes I and IV were differentiated by the character anterior spots (Sp_ant), present in morphotype IV and absent in morphotype I (Fig. 3).

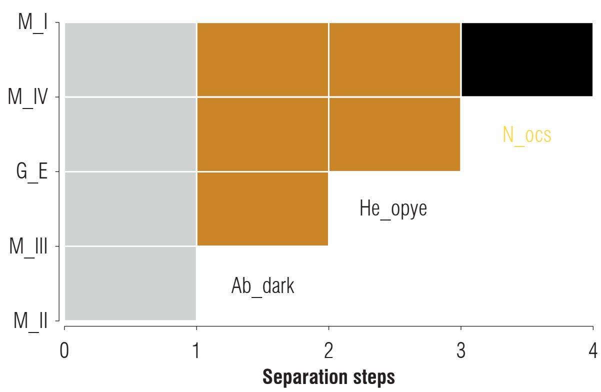


FIGURE 3. Separation banner cluster of the four *Dalbulus maidis* population morphotypes from Huila M - morphotype; G_E - external group; Ab_dark - dark abdomen character; He_opye - opaque yellow head character; N_ocs - number of ocellar spots. The black color indicates absence of the character, and the orange color indicates presence of the character.

The main characteristics of morphotype I were the opaque yellow head color and the dark brown abdomen pigmentation (Fig. 4 A, D, G). Morphotype II was characterized by the opaque yellow head and mainly by the white or pale-yellow abdomen (Fig. 4 B, E, H). Morphotype III was characterized by the abdomen and head with dark brown coloration (Fig. 4 C, F, I). Morphotype IV had similar characteristics to morphotype I; however, it was distinguished by the presence of a pair of spots on the back of the head, very close to the ocellar spots (Fig. 4 J-M).

Morphotypes I, II and IV were found in Neiva, Campoalegre, Garzón and Tarqui. Morphotype III was located only in Tarqui. The highest abundance of morphotypes was observed in Tarqui.

Morphological comparison of individuals by locality

Normality and statistical homogeneity were corroborated for the seven morphometric characters. Within the populations of *D. maidis*, antennal length, ocellar spot diameter, right and left-wing length and total body length did not show significant differences among the four corn-producing areas (Tab. 3). However, the characters of head length and distance between the eyes did show significant differences among corn-producing areas. Individuals from Garzón and Campoalegre populations differed significantly from those from Neiva; additionally, individuals from Tarqui showed significant differences with Campoalegre with respect to the character distance between eyes. Significant differences were found in the head length and distance between the eyes in the populations of *D. maidis* evaluated in the four localities of Huila; however, the average values for the characters are close, showing little variation in the characters. Thus, Neiva and Tarqui showed an average of 0.29 and 0.31 mm for head length, respectively, while Garzón and Campoalegre showed an average of 0.32 mm (Tab. 3). The characters antennal length, right-wing length, left-wing length, total length, and ocellar spot diameter did not show significant differences between them. Considering that the environmental factors reported as contributing to morphological variations (temperature, elevation, and latitude) did not show large variations in the populations of Neiva, Garzón, Campoalegre, and Tarqui (Tab. 2), these environmental factors should be considered for the evaluation of the differences between Colombian

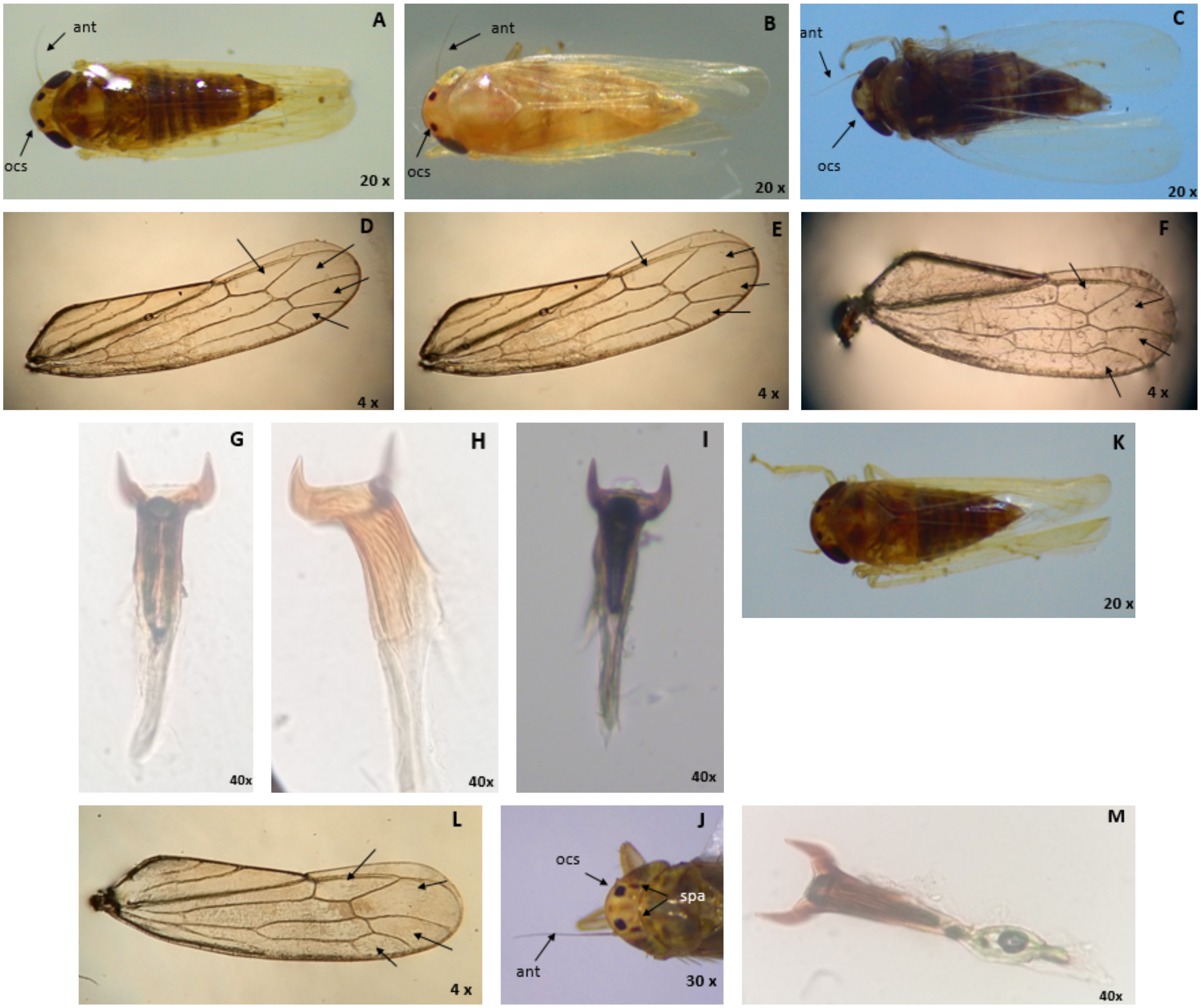


FIGURE 4. Morphological characters color of the head, ocellar spots (ocs), antenna (ant), and pigmentation of the abdomen: A) morphotype I; B) morphotype II; C) morphotype III. Anterior wing apical cells: D) morphotype I; E) morphotype II; F) morphotype III. Aedeagus with divergent apical processes: G) morphotype I; H) morphotype II; I) morphotype III. J) ocellar spots (ocs), antenna (ant), anterior spots (spa) in morphotype IV. K) Pigmentation of the abdomen and head in morphotype IV. L) apical cells anterior wings in morphotype IV. M) Aedeagus with divergent apical processes in morphotype IV.

TABLE 3. Statistical analysis with the Fisher-LSD test of seven morphometric characters of *Dalbulus maidis* populations from four corn-producing areas from Huila.

Variable	Area							
	Campoalegre		Neiva		Garzón		Tarqui	
	Mean	LSD-Fisher	Mean	LSD-Fisher	Mean	LSD-Fisher	Mean	LSD-Fisher
Antennal length	0.70	A	0.71	A	0.70	A	0.72	A
Ocellar spot diameter	0.10	A	0.10	A	0.10	A	0.10	A
Right wing length	3.37	A	3.31	A	3.38	A	3.36	A
Left wing length	3.38	A	3.34	A	3.40	A	3.39	A
Body length	3.88	A	3.80	A	3.82	A	3.83	A
Head length	0.32	B	0.29	A	0.32	B	0.31	AB
Eye distance	0.51	B	0.50	AB	0.50	AB	0.48	A

populations of *D. maidis* from different localities. Additionally, the distances between localities are relatively short, so migration events of individuals between populations are possible.

Morphological comparison of individuals by morphotypes

The morphometric character comparison among the morphotypes showed that five of them did not show significant differences (Tab. 4). As in the comparisons by area, the character distance between eyes showed a significant difference between morphotypes, separating morphotype III from the others. Total body length showed significant differences between morphotypes I and II. The antennal length was measured because the genus *Dalbulus* has the third antennal segment longer than other Cicadellidae family members (Knight & Webb, 1993; Triplehorn & Johnson, 2005). However, no records about antennal size were found to be specific for *D. maidis*, so the length range of a diagnostic character at the genus level may be somewhat broad and not show a difference at the morphotype level. The ocellar spot diameter is a relevant character in *D. maidis* since it is defined in this specimen as twice the size of the ocelli (Nielson, 1968; Knight & Webb, 1993; Pinedo-Escatel & Blanco-Rodríguez, 2016). The ocelli of *D. maidis* are very small, so the ocellar spots size is in proportion to the character of the species, but it does not allow seeing differences at the level of morphotypes in the species. The distance between the compound eyes and the length of the head are characters that have been previously evaluated for the observation of morphological variations (polymorphisms) within the species *D. maidis* (Oliveira *et al*., 2004). However, in our study, these characters did not show significant differences among the four morphotypes found. Variations in these two characters could be influenced by altitude and temperature; among the populations evaluated, these environmental conditions do not show large differences, despite their fluctuation in each locality. The length of the anterior wings has also been used to determine morphological variations among *D. maidis* populations, as in the case of the Brazilian populations, which allow separating the evaluated localities into two groups (Oliveira *et al*., 2004). However, the analysis of the morphotypes of the populations showed no significant differences for this character, although the left wing is slightly longer than the right wing (Tab. 4).

Significant differences were also found between morphotypes for the characteristic of the distance between the eyes, where morphotype III showed the lowest value for this characteristic (0.45 mm) separating it from the other morphotypes (Tab. 4). Thus, morphotype III separates a little more from the other individuals with another character of the head, since this morphotype is the only one showing a darker coloration of the head different from the opaque yellow characteristic of *D. maidis*. Additionally, considering that the morphotype III was only found in Tarqui, it is the most differentiated morphotype of those evaluated in the populations of Huila.

Statistical analysis of the ovipositor length from females of the population showed significant differences for morphotype I compared to the other three morphotypes (*P*-value 0.0016). We observed that morphotype I had a shorter ovipositor (698.10 µm) on average, compared to morphotypes II, III, and IV that had an average length of 800 µm (Tab. 4). A comparison of the aedeagus length showed significant differences between morphotype I and morphotypes II and III (Tab. 4). Morphotype I had the longest average length at 145.29 µm, while morphotype II had the shortest length among the four morphotypes with

TABLE 4. Statistical analysis with the Fisher-LSD test of ten morphometric characters of *Dalbulus maidis* populations morphotypes from Huila.

Variable	Morphotype							
	I		II		III		IV	
	Mean	LSD-Fisher	Mean	LSD-Fisher	Mean	LSD-Fisher	Mean	LSD-Fisher
Antennal length	0.71	A	0.69	A	0.73	A	0.72	A
Ocellar spot diameter	0.10	A	0.10	A	0.10	A	0.10	A
Right wing length	3.35	A	3.39	A	3.31	A	3.34	A
Left wing length	3.37	A	3.41	A	3.34	A	3.36	A
Body length	3.75	A	3.93	B	3.77	AB	3.84	AB
Head length	0.31	A	0.31	A	0.31	A	0.31	A
Eye distance	0.49	B	0.50	B	0.45	A	0.51	B
Ovipositor length	698.10	A	793.40	B	841.52	B	812.87	B
Aedeagus length	145.29	B	128.49	A	131.26	A	140.34	AB
Apical processes distance	47.17	B	50.13	C	45.03	A	43.97	A

around 128.49 µm. Morphotype III showed no significant differences from morphotypes II and IV, while morphotype IV was between the values of morphotypes II and III and I (140.34 µm). The observations of the ovipositor and aedeagus provide information for future research to clarify the reproductive processes and clarify if the differences in size of the genitalia could generate the reproductive isolation between morphotypes.

The distance between the divergent apical processes of the aedeagus was also evaluated, and significant differences were found across the four morphotypes (Tab. 4). Morphotypes IV and III did not differ among themselves; however, they differed from morphotypes I and II. Morphotype II showed the greatest distance between divergent processes, 50.13 µm on average, while morphotypes IV and III obtained the lowest values, 43.97 and 45.03 µm, respectively. Regarding the comparison between males and females, significant differences were only found in the total body length, with females being longer than males (Tab. 5). The total sized body reported for the *Dalbulus* species is between 3.5 and 4.0 mm, a range in which the four morphotypes were found (Nielson, 1968; Larsen & Nault, 1994; Oliveira *et al.*, 2004).

TABLE 5. Statistical analysis with the Fisher-LSD test of seven morphometric characters of males and females of *Dalbulus maidis* populations from four Huila areas.

Variable	Gender			
	Female		Male	
	Mean	LSD-Fisher	Mean	LSD-Fisher
Antennal length	0.70	A	0.72	A
Ocellar spot diameter	0.10	A	0.10	A
Right wing length	3.34	A	3.37	A
Left wing length	3.36	A	3.40	A
Head length	0.31	A	0.31	A
Eye distance	0.49	A	0.50	A
Body length	3.96	B	3.71	A

Differences in the variable of total body length could be seen between males and females of *D. maidis*. In total body length, the females tended to be slightly larger than males as previously reported in several studies for this species (Nielson, 1968; Triplehorn & Nault, 1985; Larsen & Nault, 1994; Oliveira *et al.*, 2004), with a higher frequency of females ranging in size from 3.70 to 4.70 mm, while the male range was mainly between 3.50 and 3.70 mm. Although most males were in the 3.50 to 3.70 mm size range, the overall spread of the data showed a range from 3.40 to 4.00 mm. These values are within the range defined by Nielson (1968), who described that males of *D. maidis* have a length from 3.50 to 4.00 mm, while females have a length from 4.00 to 4.10 mm. This also matches the results of our study, since the individuals had values close to 4.50 mm (Tab. 5). Female insects are generally larger than males (Triplehorn & Johnson, 2005). In *D. maidis,* the difference in size between males and females is related to the development rate of the individuals, since females have a slower development with more time for feeding and growth than males (Oliveira *et al.*, 2004). Additionally, an adaptive explanation is given for this characteristic since females are responsible for breeding and laying eggs and being larger increases their probability of reproduction and breeding compared to smaller females (Larsen & Nault, 1994). The diameter of the ocellar spot, antennal length, length of the head, distance between the eyes, and length of the right and left wing are not differential characters between males and females.

A separate cluster of individuals was obtained from the average of the seven morphometric characters of each morphotype by corn-producing area, allowing the identification of two main clades (Fig. 5). Thus, in the dendrogram, morphotype I from Garzón and morphotype IV from Neiva was the group most separated from the other individuals; the second clade showed three subgroups. Morphotypes II and IV from Campoalegre and morphotype II from Tarqui showed greater similarity among the characters, so they were grouped in the same clade. Morphotypes IV from Garzón, I from Tarqui and Neiva and IV from Tarqui formed the next group. Morphotypes III from Tarqui, I from Campoalegre, and II from Neiva were grouped in the third subgroup on the dendrogram.

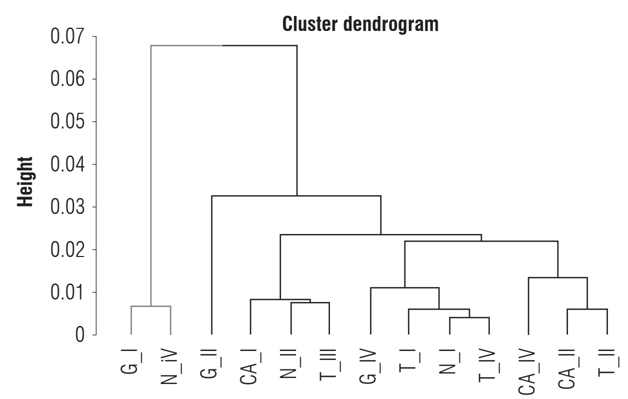


FIGURE 5. Clustering by area and morphotype of *Dalbulus maidis* populations from corn-producing areas in Huila. G_I - Garzón morphotype I; G_II - Garzón morphotype II; G_IV - Garzón morphotype IV; N_I - Neiva morphotype I; N_II - Neiva morphotype II; N_IV - Neiva morphotype IV; CA_I - Campoalegre morphotype I; CA_II - Campoalegre morphotype II; CA_IV - Campoalegre morphotype IV; T_I - Tarqui morphotype I; T_II - Tarqui morphotype II; T_III - Tarqui morphotype III; T_IV - Tarqui morphotype IV.

Conclusions

The morphological and morphometric analyses carried out in this study made it possible to separate the individuals of the *D. maidis* populations of the department of Huila into four morphotypes. Although morphological variations did not show significant interpopulation differences, variations at the intraspecific level were observed in the four localities. As such, this study could serve as a baseline for further studies of Colombian populations of *Dalbulus maidis* with a morphological description of the species in the department of Huila.

Conflict of interest statement

The authors declare that there is no conflict of interest regarding the publication of this article.

Author's contributions

AMV formulated the original idea; ISR carried out the laboratory observations and data collection; CIJ contributed to the statistical analyses; BMA and ARG advised on the morphological observations; ISR and AMV wrote the article.

Literature cited

Agronet. (2019). *Reporte: área, producción y rendimiento nacional por cultivo*. Ministerio de Agricultura y Desarrollo Rural. https://www.agronet.gov.co/estadistica/Paginas/home.aspx?cod=1#

Ávila, C. J., & Arce, C. C. M. (2008). Flutuação populacional da cigarrinha-do-milho em duas localidades do Mato Grosso do Sul. *Ciência Rural*, *38*(4), 1129–1132. https://doi.org/10.1590/s0103-84782008000400035

Bray, J. R., & Curtis, J. T. (1957). An ordination of the upland forest communities of southern Wisconsin. *Ecological Monographs*, *27*(4), 325–349. https://doi.org/10.2307/1942268

CABI. (2020). *Plantwise knowledge bank - maize leafhopper Dalbulus maidis distribution*. https://www.plantwise.org/KnowledgeBank/datasheet/17847

CIMMYT, & CIAT. (2019). *Maíz para Colombia visión 2030*. Centro Internacional de Mejoramiento de Maíz y Trigo, Centro de Investigación de Agricultura Tropical.

Dietrich, C. H. (2005). Keys to the families of Cicadomorpha and subfamilies and tribes of Cicadellidae (Hemiptera: Auchenorrhyncha). *Florida Entomologist*, *88*(4), 502–517.

Druetta, M., Luna, I., & Gimenez, M. P. (2018). *El achaparramiento del maíz por corn stunt spiroplasma (CSS)*. INTA, Ministerio de Agroindustria. https://inta.gob.ar/sites/default/files/inta_eea-quimili_corn_stunt_spiroplasma_en_maiz.pdf

Hawkins, B. A., & Lawton, J. H. (1995). Latitudinal gradients in butterfly body sizes: is there a general pattern? *Oecologia*, *102*, 31–36. https://doi.org/10.1007/BF00333307

Hodkinson, I. D. (2005). Terrestrial insects along elevation gradients: species and community responses to altitude. *Biological Reviews*, *80*(3), 489–513. https://doi.org/10.1017/S1464793105006767

ICA. (2019, August 15). *El ICA y AGROSAVIA hacen equipo para fortalecer la sanidad de los cultivos de maíz en el Huila*. Instituto Colombiano Agropecuario. https://www.ica.gov.co/noticias/ica-agrosavia-fortalece-sanidad-cultivo-maiz-huila

Kassambara, A., & Mundt, F. (2017). *Factoextra: extract and visualize the results of multivariate data analyses* (*R package version 1.0.5.*). https://cran.r-project.org/web/packages/factoextra/index.html

Knight, W. J., & Webb, M. D. (1993). The phylogenetic relationships between virus vector and other genera of macrosteline leafhoppers, including descriptions of new taxa (Homoptera: Cicadellidae: Deltocephalinae). *Systematic Entomology*, *18*(1), 11–55. https://doi.org/10.1111/j.1365-3113.1993.tb00653.x

Krasnov, B., Ward, D., & Shenbrot, G. (1996). Body size and leg length variation in several species of darkling beetles (Coleoptera: Tenebrionidae) along a rainfall and altitudinal gradient in the Negev Desert (Israel). *Journal of Arid Environments*, *34*(4), 477–489. https://doi.org/10.1006/jare.1996.0126

Larsen, K. J., & Nault, L. R. (1994). Seasonal polyphenism of adult *Dalbulus* leafhoppers (Homoptera: Cicadellidae). *Annals of the Entomological Society of America*, *87*(3), 355–362. https://doi.org/10.1093/aesa/87.3.355

Lê, S., Josse, J., & Husson, F. (2008). FactoMineR: an R package for multivariate analysis. *Journal of Statistical Software*, *25*(1), 1–18.

Meneses, A. R., Querino, R. B., Oliveira, C. M., Maia, A. H. N., & Silva, P. R. R. (2016). Seasonal and vertical distribution of *Dalbulus maidis* (Hemiptera: Cicadellidae) in Brazilian corn fields. *Florida Entomologist*, *99*(4), 750–754. https://doi.org/10.1653/024.099.0428

Moya-Raygoza, G. (2002). Distribución y hábitats de *Dalbulus* spp. (Homoptera: Cicadellidae) durante la estación seca en México. *Acta Zoologica Mexicana, 85*, 119–128.

Nielson, M. W. (1968). *The leafhopper vectors of phytopathogenic viruses (Homoptera, Cicadellidae) taxonomy, biology, and virus transmission. Technical Bulletin no. 1382.* Agricultural Research Service, United States Department of Agriculture. https://naldc.nal.usda.gov/download/CAT87201370/PDF

Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P. R., O'Hara, R. B., Simpson, G. L., Solymos, P., Stevens, M. H. H., Szoecs, E., & Wagner, H. (2019). *Vegan: community ecology package (R package version 2.5-6.).* https://cran.r-project.org/package=vegan

Oliveira, C. M., Lopes, J. R. S., Dias, C. T. S., & Nault, L. R. (2004). Influence of latitude and elevation on polymorphism among populations of the corn leafhopper, *Dalbulus maidis* (DeLong and Wolcott) (Hemiptera: Cicadellidae), in Brazil. *Environmental Entomology*, *33*(5), 1192–1199. https://doi.org/10.1603/0046-225X-33.5.1192

Oliveira, C. M., Oliveira, E., Souza, I. R. P., Alves, E., Dolezal, W., Paradell, S., Lenicov, A. M. M. R., & Frizzas, M. R. (2013). Abundance and species richness of leafhoppers and planthoppers (Hemiptera: Cicadellidae and Delphacidae) in Brazilian maize crops. *Florida Entomologist*, *96*(4), 1470–1481. https://doi.org/10.1653/024.096.0427

Pinedo-Escatel, J. A., & Blanco-Rodríguez, E. (2016). Notas del género *Dalbulus* DeLong, 1950 (Hemiptera: Cicadellidae) en México. *Folia Entomológica Mexicana*, *2*(1), 16–19.

R Core Team. (2020). *R: a language and environment for statistical computing.* R Foundation for Statistical Computing. https://www.r-project.org/

Rodríguez-Zabala, J., González, R., Correa, M. M., & Gómez, G. F. (2016). Análisis morfométrico de dos poblaciones de *Anopheles (Anopheles) calderoni* (Diptera: Culicidae) del suroccidente colombiano. *Revista Mexicana de Biodiversidad*, *87*(3), 966–971. https://doi.org/10.1016/j.rmb.2016.06.005

Smith, R. J., Hines, A., Richmond, S., Merrick, M., Drew, A., & Fargo, R. (2000). Altitudinal variation in body size and population density of *Nicrophorus investigator* (Coleoptera: Silphidae). *Environmental Entomology*, *29*(2), 290–298.

Struyf, A., Hubert, M., & Rousseeuw, P. J. (1997). Clustering in an object-oriented environment. *Journal of Statistical Software*, *1*(4), 1–30. https://doi.org/10.18637/jss.v001.i04

Taylor, R. A. J., Nault, L. R., & Styer, W. E. (1993). Experimental analysis of flight activity of three *Dalbulus* leafhoppers (Homoptera: Auchenorrhyncha) in relation to migration. *Annals of the Entomological Society of America, 86*(5), 655–667. https://doi.org/10.1093/aesa/86.5.655

Triplehorn, B. W., & Nault, L. R. (1985). Phylogenetic classification of the genus *Dalbulus* (Homoptera: Cicadellidae), and notes on the phylogeny of the Macrostelini. *Annals of the Entomological Society of America*, *78*(3), 291–315. https://doi.org/10.1093/aesa/78.3.291

Triplehorn, C. A., & Johnson, N. F. (2005). *Borror and DeLong's introduction to the study of insects* (7th ed.). Thompson Brooks/Cole.

Varón de Agudelo, F., & Sarria Villa, G. A. (2007). *Enfermedades del maíz y su manejo.* Instituto Colombiano Agropecuario, Federación Nacional de Cultivadores de Cereales y Leguminosas. Produmedios.

Ward Jr., J. H. (1963). Hierarchical grouping to optimize an objective function. *Journal of the American Statistical Association*, *58*(301), 236–244.

In vitro inhibition attempts and bio-elicitation of *Solanum lycopersicum* L. by chitin and chitosan against *Ralstonia solanacearum*, the causal agent of bacterial wilt

Intentos de inhibición *in vitro* y bioelicitación de *Solanum lycopersicum* L. mediante quitina y quitosano contra *Ralstonia solanacearum*, el agente causal de la marchitez bacteriana

Amine Rkhaila[1*], Mouhatti Saga[1], Sofia Ghizlan Grohs[1], and Khadija Ounine[1]

ABSTRACT

Ralstonia solanacearum is one of the most destructive pathogens of vegetables causing very important economic losses. In 2019, 20 strains of *R. solanacearum* were collected from potato tubers in Morocco. After their identification, the inhibitory effect of colloidal solutions of chitin and/or chitosan was evaluated *in vitro* on a solid medium (Mueller-Hinton) supplemented with these biopolymers. The concentrations (25, 50, or 100 mg L^{-1}) contributed to significant inhibition of the growth of the isolated strains that led to an inhibition of 45.04% with the combination of chitin-chitosan (100 mg L^{-1}), 58.92% with the addition of 100 mg L^{-1} of chitin, and 68.74% in the presence of chitosan at 25 mg L^{-1}. Likewise, in experiments with *Solanum lycopersicum* L. seedlings, chitin derivatives significantly promoted stem and root growth. Stem length increased by 54.95% when chitin was added at 25 mg L^{-1}, while soil amendment with 100 mg L^{-1} of chitosan increased root length by 82.55% compared to the control. The severity of bacterial wilt due to *R. solanacearum* was reduced by 117.02% when we added 100 mg L^{-1} of chitosan to the soil. However, the severity of this disease decreased by 142.86% when the soil was amended with chitin at 50 mg L^{-1}. These findings are consistent with prior research that suggests using this technique to manage bacterial wilt caused by *R. solanacearum*.

Key words: biopolymers, defense stimulation, tomato, growth inhibition.

RESUMEN

Ralstonia solanacearum es uno de los patógenos más destructivos en plantas hortícolas, ocasionando pérdidas económicas muy importantes. En 2019, 20 aislamientos de *R. solanacearum* fueron recolectados de tubérculos de papa en Marruecos. Posterior a su identificación, se evaluó *in vitro* el efecto inhibitorio de soluciones coloidales de quitina y/o quitosano en medio sólido (Mueller-Hinton) suplementado con estos biopolímeros. Las concentraciones (25, 50, o 100 mg L^{-1}) contribuyeron a una inhibición significativa del crecimiento de los aislamientos, generando una inhibición del 45.04% con la combinación de quitina-quitosano (100 mg L^{-1}), del 58.92% con la adición de 100 mg L^{-1} de quitina, y del 68.74% en presencia de quitosano a 25 mg L^{-1}. Así mismo, en los experimentos con plántulas de *Solanum lycopersicum* L., los derivados de la quitina demostraron promover el crecimiento del tallo y de las raíces de forma significativa. La longitud del tallo aumentó en un 54.95% cuando se añadió quitina a 25 mg L^{-1}, mientras que la enmienda del suelo con 100 mg L^{-1} de quitosano aumentó la longitud de la raíz en un 82.55% en comparación con el control. La severidad de la marchitez bacteriana causada por *R. solanacearum* se redujo en un 117.02% cuando se adicionaron 100 mg L^{-1} de quitosano al suelo. Sin embargo, la severidad de la enfermedad se redujo en un 142.86% cuando se enmendó el suelo con quitina a 50 mg L^{-1}. Estos resultados son coherentes con investigaciones anteriores que sugieren el uso de esta técnica para el manejo de la marchitez bacteriana causada por *R. solanacearum*.

Palabras clave: biopolímeros, estimulación de la defensa, tomate, inhibición del crecimiento.

Introduction

Market gardening is a vital agricultural sector in Africa because of its nutritional value and the significant economic income it generates. This sector has the capacity to serve as an impetus for agricultural and economic diversification by directing production towards local or export markets (Sadi *et al*., 2020).

The tomato sector in Morocco plays an important socioeconomic role. In fact, at the economic level, tomato exports have a major place since they generate about 1.1 billion USD in foreign currency. Socially, the sector is a generator of jobs creating an average of 9 million working days per year at the production level but also in packaging and processing (Elame, 2019).

Received for publication: August 10, 2021. Accepted for publication: December 30, 2021. Doi: 10.15446/agron.colomb.v39n3.97746

[1] Plant, Animal and Agro-Industry Production Laboratory, Department of Biology, Faculty of Sciences, Ibn Tofail University, Kenitra (Morocco).
[*] Corresponding author: amine.rkhaila@uit.ac.ma

Bacterial wilt caused by *Ralstonia solanacearum* is one of the major land-based vascular diseases that produces considerable damage to many crops of worldwide economic importance (Mansfield *et al*., 2012). The disease was discovered in Morocco for the first time on potatoes (*Solanum tuberosum*) in 1999 as the so-called biovar 2 (Poussier *et al*., 2000). Over the last 20 years, this taxonomy has undergone several revisions. The classifications were consolidated into four separate phylotypes based on the strain's geographic origin (Phylotype I, Phylotype II, Phylotype III, and Phylotype IV) (Cellier, 2010). According to Ravelomanantsoa *et al*. (2018), the Moroccan strain is grouped into Phylotype IIB-1 that belongs to cluster A. Penetration of this bacterium into plant tissues is ensured by wounds produced during secondary root development, insect injuries, and cultural practices. After the death of the host plant and until contact with a new host, the bacterium develops various survival strategies, such as viable non-cultivable forms and biofilm formation (EPPO, 2018). Due to the ability of *R. solanacearum* to develop endophytically, migrate through water, interact with weeds, and persist in soil, particularly in the deeper layers, this pathogen causes direct yield losses that vary significantly depending on the host, soil type, cultivar, cropping plan, climate, and type of strain (Elphinstone, 2005). According to some studies, bacterial wilt can result in significant yield losses in bananas (80% to 100%), potatoes (33% to 90%), tomatoes (0% to 91%), groundnuts (0% to 20%), and tobacco (10% to 30%) (Yuliar *et al*., 2015).

Considering the serious consequences that pesticides may have on human health (male infertility, cancers, negative effects on fetuses, etc.), chitin and chitosan seem to be well-known and eco-friendly biocontrol agents due to their biodegradable, nontoxic, and biocompatible properties (Hassan & Chang, 2017). They act by increasing the synthesis of secondary metabolites that enhance plant immunological defenses (phenylalanine ammonia lyase (PAL) and tyrosine ammonia lyase (TAL)) (Khan *et al*., 2003). Recently, some studies have found that chitin and its derivatives have strong antibacterial activities *in vitro* and *in vivo* against a variety of plant pathogenic bacteria like *Ralstonia solanacearum* (Farag *et al*., 2017) and *Xanthomonas* spp. (Ramkissoon *et al*., 2016). These reactions are the consequence of the induction of multiple defensive responses in host plants, including the accumulation of phytoalexins, proteinase inhibitors, and pathogen-related (PR) proteins, callose formation and lignin production (El Hadrami *et al*., 2010).

This biotechnological approach to agriculture promises to increase the efficacy of agrochemicals while reducing their negative impact on the environment. Multiple evidence suggests that chitin and chitosan might be an innovative solution for the regulated release of different agrochemicals such as pesticides, micronutrients, fertilizers, and plant hormones, and they can play a dual function in plant growth promotion and biocontrol of phytopathogens (Malerba & Cerana, 2018). Foliar treatment of a commercial chitosan formulation reduced the prevalence of *Xanthomonas vesicatoria* in tomato plants *in vivo* (Li *et al*., 2010). Furthermore, the use of the same chitosan solution has made it possible to limit the incidence of *Pseudomonas fluorescens* infections on broccoli plants (Ramkissoon *et al*., 2016).

The aim of this study was to evaluate the efficacy of multiple treatments based on chitin and/or chitosan to protect *Solanum lycopersicum* L. against *R. solanacearum* attack.

Materials and methods

R. solanacearum isolation and biochemical identification

Sample collection

Potato tubers (variety Désirée (Red Skin)) were collected from a local market of the M'Nasra's region in the province of Kenitra, Morocco (Fig. 1). The collected samples show typical symptoms of *R. solancearum* infection (creamy exudates from the vascular rings and eyes of tubers).

Isolation medium

Five potato tubers were carefully cleaned with water and surface sterilized with 70% ethanol; a sterile scalpel was used to cut a transverse section. After crushing the tuber, vascular exudates were sampled with a sterile loop and streaked immediately onto SMSA medium (Siri *et al*., 2011), and incubated in Petri dishes at 28°C for 3 to 5 d. Colonies with the characteristic *R. solanacearum* phenotype (irregular shaped, fluidal, and completely white or with a pink core) were subcultured onto tetrazolium chloride agar (TZC) medium and purified for further study (Sikirou *et al*., 2017).

Biochemical tests for *R. solanacearum* identification

For validation of *R. solanacearum* isolates, four biochemical assays were conducted, including gram staining reaction, potassium hydroxide solubility test, the Levan test, and the sugar fermentation test (Rahman *et al*., 2010).

Identification of virulent and avirulent isolates

The virulent (colonies with pink or light red color or characteristic red center and whitish margin) and avirulent (smaller, off-white and non-fluidal colonies) strains of *R.*

FIGURE 1. Potato tubers (variety Désirée (Red Skin)) infected by the causal agent of the bacterial wilt, *Ralstonia solanacearum.*

solanacearum were identified in a triphenyl tetrazolium chloride (TTC) medium containing 0.005% TTC (Kelman, 1954).

Virulence of the *R. solanacearum* strain

The virulence of *R. solanacearum* was assessed by inoculating tobacco (*Nicotiana tabacum*) plants at the six-leaf stage by infiltration, using a hypodermic syringe on the underside of the leaves. The bacterial concentration used for this purpose was of the order of 10^8 CFU ml^{-1} (DO600 = 0.28) (Adebayo & Ekpo, 2005).

The inoculated plants were then repotted in pots containing Maâmora forest soil (previously sterilized by tyndallization) (Bank *et al*., 2008) plus commercial peat at a rate of 1/3. According to the USDA, the soil of the Maâmora forest is made up of fine loamy sands with a clay component (De Mahieu *et al*., 2020). These pots were then transferred to a greenhouse at a temperature of 23 ± 2°C for 7 d.

The appearance of necrotic spots on the surface of the areas of inoculation by *R. solanacearum* demonstrated virulence. However, when no reaction was observed, virulence was considered negative (Schaad *et al*., 2001).

Source of chitin and chitosan

Chitin and chitosan were extracted from shrimp shells of *Prapenaeus longirostris* following the protocol described in previous studies (Rkhaila & Ounine, 2018). Table 1 summarizes the characteristics of these products.

Colloidal chitin preparation

Colloidal chitin was made from extracted chitin using a modified process developed by Hsu and Lockwood (1975). Forty g of chitin powder were added to 600 ml of concentrated HCl. The chitin was precipitated by the addition of 2 L of distilled water at 10°C after 2 h 30 min of stirring. The filtrate was rinsed with water until neutrality.

Chitosan solubilization

The modified technique of El Ghaouth *et al*. (1991) was adopted to solubilize extracted chitosan. Eighty ml of distilled water and 2.5 ml of HCl 10 N were added to 1 g of chitosan. After that, the volume was completed with distilled water up to 100 ml. Consequently, the pH of the solution was adjusted to 5.6 using 0.1 ml NaOH.

Solanum lycopersicum seed origin

The seeds used in the experiments belonged to species of the *Solanaceae* family from a standard variety (Rio Grande) and were homogeneous and of the same generation. The seeds are listed in the French varieties in the official catalog of the ONSSA - Morocco (Office National de Sécurité Sanitaire des Produits Alimentaires, Morocco) and authorized for sale on the Moroccan markets.

Evaluation of the effect of biopolymers on the growth of *R. solanacearum in vitro*

After adjusting the bacterial concentration of a 24 h culture of *R. solanacearum* to 10^8 CFU ml^{-1} (Rodrigues *et al*.,

TABLE 1. Summary of quantities obtained after extraction of chitin and chitosan from shrimp (*Prapenaeus longirostris*) shells.

Product	Quantity (g)	Ash (%)	Moisture (%)	Degree of acetylation (%)
Shrimp shell powder	100.0	-	55.08	-
Chitin	50.00	51.2	-	90
Chitosan	23.93	-	-	1.076

2011), 0.1 ml of this suspension was inoculated into 10 ml of Mueller Hinton broth. An aliquot of 1 ml of this well homogenized suspension was taken. Ten µl of this suspension was inoculated by streaking on Mueller Hinton agar. The plates were incubated at 26 ± 1°C for 24 h.

The liquid dilution method coupled with agar spreading was used as described by Hoekou *et al*. (2012) with some modifications. The assay was made by introducing into a sterile hemolysis tube 0.5 ml of the colloidal solution of chitin, chitosan, or chitin-chitosan mixture at 25, 50, or 100 mg L^{-1} with 10 µl of the microbial suspension. In the control tube, the extract was substituted with 0.5 ml of sterile nutrient broth. The tubes were incubated at 26 ± 1°C for 48 h; then, the tests and controls were distributed on Mueller Hinton agar medium at a rate of 10 plates per tube.

After incubation at 37°C for 48 h, colonies were counted on each plate and the percentages of survival and inhibition were calculated in relation to the negative control according to Equation 1 (Hoekou *et al*., 2012):

$$\%\ \text{Inhibition} = \left(1 - \frac{\text{Number of bacterial colonies counted on the test Petri dish}}{\text{Number of bacterial colonies counted on the control Petri dish}}\right) \times 100 \qquad (1)$$

In vivo test on *R. solanacearum*

Inoculation of *S. lycopersicum* seedlings

Solanum lycopersicum seedlings were grown in peat-based germination plates. At the three-leaf stage, the seedlings were collected from their substrate, and the roots cleaned with running water and lightly rubbed with cotton soaked in soapy water. Subsequently, the roots were washed until the soap was completely removed. Then, these were soaked for 30 min in various concentrations of chitin, chitosan, or chitin-chitosan mixture at 25, 50 or 100 mg L^{-1} with Tween 80 (0.01%) added. For control, the root system was soaked in sterile distilled water containing Tween 80 (0.01%, v/v) (Benhamou & Thériault, 1992). Finally, these seedlings were transferred to plastic pots containing Maâmora soil and commercial peat at a ratio of 1/3.

One d later, the seedlings were removed for a second time from their pots and washed with tap water. Once dried, the roots were wounded with a small diameter sterile needle to facilitate the entry of the bacteria (Chandrashekara *et al*., 2012). The tomato seedlings were then inoculated by soaking for 10 min in *R. solanacearum* bacterial suspension (10^8 CFU ml^{-1}) before being replanted in their starting pots. During the greenhouse-growing period (7 weeks), the plants were amended every week (once a week) with a 100 ml solution of chitin, chitosan or the chitin-chitosan mixture at 25, 50, or 100 mg L^{-1}. Then, the length of the shoot/root part and the foliar alteration index were calculated.

Assessment of the degree of infection

Leaf alteration

The expression of leaf symptoms was estimated by a leaf index using the following rating scale (Douira *et al*., 1994):

0: leaves of healthy appearance;

1: cotyledonary leaf: wilting or yellowing of the cotyledonary leaf;

2: falling of the cotyledonary leaf;

3: wilting or yellowing of the true leaf;

4: necrosis of the true leaf;

5: fall of the true leaf.

The sum of the scores in relation to the number of leaves constitutes the leaf alteration index. An average index was then calculated for each plant (Douira & Lahlou, 1989):

$$\text{LAI} = \frac{[\Sigma\,(i\,Xi)]}{6\,NtF} \qquad (2)$$

where LAI is the leaf alteration index; i is the leaf appearance scores from 0 to 5; Xi is the number of leaves with i score, and NtF is the total number of leaves.

Statistical analysis

A completely randomized design was used, and data were analyzed by analysis of variance (ANOVA) using the Tukey's test at α = 0.05 and the IBM SPSS Statistics for Windows, Version 21.0. (IBM Corp. Armonk, NY, USA). Three replicates were made for the *in vitro* and *in vivo* tests.

Results and discussion

Biochemical tests for *R. solanacearum* identification

The crystal violate reaction showed that the 20 isolated strains were gram negative because they did not retain the violet color. Microscopic examination revealed that the cells of virulent isolates are gram-negative, straight, or curved rod shaped. This observation was confirmed by the appearance of a viscous mass when potassium hydroxide was applied to bacterial cultures since KOH easily dissolves the thin layer of peptidoglycan of the cell

TABLE 2. Biochemical tests for *Ralstonia solanacearum* identification.

Strain	Gram staining reaction		Potassium hydroxide	Levan test	Sugar fermentation			
	Gram +	Gram –			Dextrose	Sucrose	Lactose	Mannitol
R. solanacearum	–	+	+	+	+	+	+	+

wall of gram-negative bacteria. This fact contributes to the release of its contents that generate the viscous mass (Razia *et al*., 2021). Furthermore, the positive result of the Levan test suggested that the *R. solanacearum* strains are Levan producers (Tab. 2).

For sugar fermentation, *R. solanacearum* isolates had the ability to oxidize carbohydrates, as shown by color changes (reddish to yellow). The sugar fermentation test revealed that all *R. solanacearum* strains could oxidize the four basic sugars (dextrose, sucrose, mannitol, and lactose) generating acid and gas (Tab. 2).

Identification of virulent and avirulent isolates

A Kelman tetrazolium chloride (CTC) agar test distinguished virulent from avirulent isolates of *R. solanacearum*. Additionally, in the TZC medium, these isolates generated pink or light red colonies or colonies with a distinctive red center and a white border (11 isolated strains), whereas avirulent isolates (9 isolated strains) produced tiny, off-white, and non-fluid colonies after 24 h of incubation. The results of this test showed that all *R. solanacearum* isolates (11 virulent strains) from the five potato tubers had gathered the typical pink or light-red colonies or colonies with a distinctive red center and a white border on the TZC medium (Ahmed *et al.,* 2013). This proved that all isolates of *R. solanacearum* (11 virulent strains) were virulent (Chamedjeu *et al.,* 2018) (Fig. 2).

FIGURE 2. Characteristic aspect of *Ralstonia solanacearum.*

Virulence of *R. solanacearum*

To ensure that the isolated strains were virulent, the underside of tobacco leaves was inoculated with a bacterial suspension of *R. solanacearum* at a concentration of 10^8 CFU ml^{-1}. After incubation for 7 d, the development of necrotic spots was observed around the infiltration zones (Fig. 3). These findings are consistent with those published by Heikrujam *et al.* (2020), who discovered that *R. solanacearum* could produce a hypersensitive reaction in tobacco leaves after 7 d.

FIGURE 3. Appearance of necrotic spots on the underside of tobacco leaves produced by *Ralstonia solanacearum.*

In vitro test on *R. solanacearum*

The results of the inhibition effects of treatments based on chitin, chitosan, or their mixtures on *R. solanacearum* are reported in Table 3.

From these results, all treatments resulted in the inhibition of *R. solanacearum* growth on Mueller Hinton agar. Furthermore, we observed a dependence between the treatment concentration and the *in vitro* inhibitory effect of *R. solanacearum*.

Based on the results of the *in vitro* test, we noted that all chitosan concentrations produced the highest suppression of bacterial growth, with values of 68.74%, 63.65%, and

67.29% for doses of 25, 50, and 100 mg L^{-1}, respectively. However, the concentrations of chitin and the chitin-chitosan mixture showed a lower inhibitory effect than chitosan alone, as evidenced by inhibition percentages that did not exceed 58.92% for chitin and 45.04% for the mixture of the two biopolymers.

TABLE 3. *Ralstonia solanacearum* growth inhibition percentage (Mueller Hinton solid media).

Treatment (mg L^{-1})		% Inhibition
Chitin	25	20.87 ± 0.5 a
	50	37.15 ± 1.6 b
	100	58.92 ± 3.8 d
Chitosan	25	68.74 ± 4.3 de
	50	63.65 ± 3.2 d
	100	67.29 ± 4.0 de
Chitin + Chitosan	25	37.48 ± 2.4 b
	50	40.24 ± 1.1 bc
	100	45.04 ± 3.2 c

Means in the same column with the same letter do not differ significantly from each other at the 5% level of significance according to the Tukey's test.

The study performed by Borines *et al.* (2015) demonstrates that chitosan caused growth inhibition of *R. solanacearum in vitro*, resulting in the appearance of 7 mm inhibition zones around discs imbibed with 300 mg L^{-1} of chitosan solubilized in acetic acid. The same authors related this effect to the high substitution of amino groups on chitosan chains, resulting in the enhancement of the positive cationic nature of chitosan in acidic solutions. This could lead to a greater chance of interaction between chitosan and the negatively charged cell walls of microorganisms (Mohy Eldin *et al.*, 2008; Borines *et al.*, 2015).

Whereas the results obtained by Ting *et al.* (2013) confirmed that an increasing chitosan concentration (from 0 to 0.15 mg ml^{-1}) improved the inhibition of *R. solanacearum* with a higher rate of 74.3% obtained by applying 0.15 mg ml^{-1} of chitosan, analysis by transmission electron microscopy showed that this effect is the result of the destruction of the bacterial cell membrane under the action of chitosan. Thus, chitosan inhibits the formation of biofilm in many bacteria, such as *Pseudomonas aeruginosa, Listeria monocytogenes,* and *R. solanacearum* (Kim *et al.*, 2018).

In vivo inoculation of seedlings of *S. lycopersicum* with *R. solanacearum*

Table 4 reports the results of the growth parameters and the leaf alteration index of *S. lycopersicum* plants inoculated with *R. solanacearum.*

The inoculation of *S. lycopersicum* seedlings strongly influenced the development of both parts (shoots and roots) in the control plants that was expressed by mean lengths varying from 36.4 ± 7.1 cm to 29.8 ± 8.0 cm, respectively. However, plants treated with the biopolymers showed an ability to overcome infection and the expression of the characteristic symptoms of bacterial wilt at both stem and root levels.

An improvement of 17.58% in the growth of the shoot was recorded in plants amended with chitin at 100 mg L^{-1}. Moreover, the treatment with 50 mg L^{-1} of this polymer resulted in the development of this parameter by more than 31%, while the lowest concentration (25 mg L^{-1}) allowed for a significant increase in the growth of this part of the plants, by the expression of a difference of 54.95%.

For treatments based on chitosan, the effect of this biopolymer was strictly related to its concentration. Consequently, the increase of the concentration generated a longer shoot. Improvements of 18.96, 15.66, and 40.11% compared to the inoculated control were observed in the plants amended with the chitosan at concentrations 25, 50, or 100 mg L^{-1}, respectively (Fig. 4).

Plants whose soil was amended with the chitin-chitosan mixture at 25 mg L^{-1} expressed an average length of 41.6 ± 6.5 cm; in other words, an improvement of 14.87% in stem length was observed compared to the control.

Regarding the mean root length, we observed a highly significant improvement of this parameter in plants treated with chitosan at different concentrations. The strongest growth promotion was recorded by 100 mg L^{-1} of chitosan. This translates into a difference of 82.55% compared to the control.

Similarly, chitin at 100 mg L^{-1} and the chitin-chitosan mixture at 25 mg L^{-1} showed a remarkable increase in root length compared to the inoculated control, resulting in a difference of 34.90% and 51.34%, respectively (Fig. 4).

Seedlings amended with chitin and/or chitosan and inoculated with *R. solanacearum* showed the lowest LAI compared to the control. This allowed a high level of protection for the plants against bacterial wilt. Consequently, differences ranging from 48.69% to 65.05% were recorded in the presence of the chitin-chitosan mixture, while significant differences were observed when the soil was amended with chitosan (72.30% to 117.02%) or in the presence of chitin (80.24% to 142.86%). These results

TABLE 4. Means of stem and root length (cm) and leaf alteration index (LAI) of plants treated by chitin, chitosan or chitin-chitosan mixture (25, 50, or 100 mg L^{-1}) and control plants.

Treatment (mg L^{-1})		Stem length (cm)	Amelioration %	Root length (cm)	Amelioration %	LAI
Control	0	36.4 ± 7.1 ab	-	29.8 ± 8.0 a	-	0.510 ± 0.51 a
Chitin	25	56.4 ± 4.7 d	54.95	35.2 ± 5.2 b	18.12	0.254 ± 0.30 b
	50	48.0 ± 5.0 c	31.69	37.1 ± 6.1 b	24.50	0.210 ± 0.21 b
	100	42.8 ± 4.6 b	17.58	40.2 ± 7.4 b	34.90	0.283 ± 0.12 b
Chitosan	25	43.3 ± 6.3 bc	18.96	30.0 ± 4.6 a	00.67	0.264 ± 0.18 b
	50	42.1 ± 4.1 b	15.66	26.6 ± 7.2 a	10.78	0.296 ± 0.22 b
	100	51.0 ± 5.3 cd	40.11	54.4 ± 6.1 c	82.55	0.235 ± 0.29 b
Chitin + Chitosan	25	41.6 ± 6.5 b	14.87	45.1 ± 4.8 bc	51.34	0.343 ± 0.36 b
	50	38.0 ± 5.4 a	04.40	34.2 ± 6.7 a	14.76	0.309 ± 0.20 b
	100	37.5 ± 5.0 ab	03.02	32.4 ± 7.0 a	08.72	0.335 ± 0.36 b

Means in the same column with the same letter do not differ significantly from each other at the 5% significance level according to the Tukey's test.

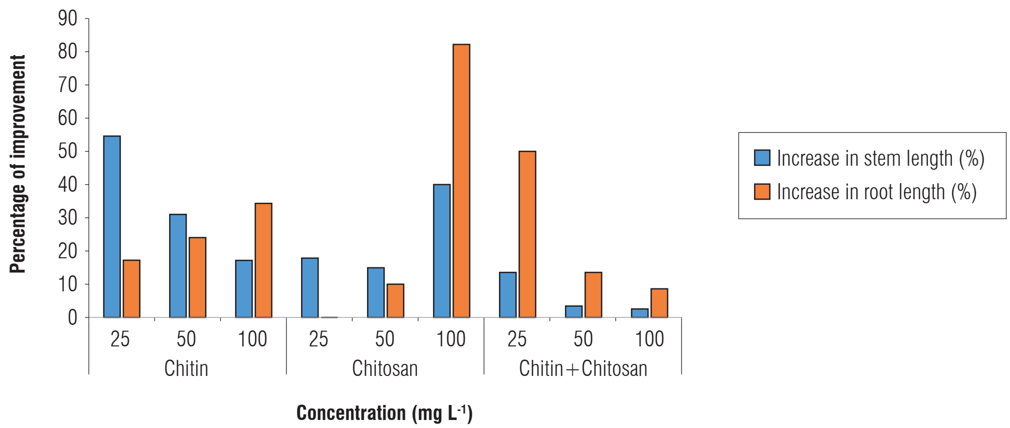


FIGURE 4. Stem and root lengths in control plants and plants inoculated with *Ralstonia solanacearum,* the bacterial wilt agent.

agree with those of Borines *et al.* (2015), who found that the soaking of roots in the 200 mg L^{-1} chitosan treatment (previously solubilized in acetic acid) before transplanting was effective in protecting the plants against the disease and, consequently, controlling bacterial wilt.

Algam *et al.* (2010) showed that the soil amendment with chitosan at 10 mg ml^{-1} significantly reduced wilt due to *R. solanacearum* by 72% in tomato plants grown in a greenhouse. Seed treatment with the same concentration induced a highly significant reduction of *R. solanacearum* incidence by 48%.

We can deduce from this that previous research employed high concentrations of biopolymers (chitin and chitosan) on the same plant; however, in our case the quantities used were lower but the results were more significant in terms of increased plant growth and bacterial biocontrol. These beneficial effects are explained by the fact that chitosan induces callose deposition in plants (Luna *et al.*, 2011). Furthermore, chitin oligomers increase the synthesis of the pathogenesis related (PR) proteins (Van Loon *et al.*, 2006) (for example, PR proteins (NPR1) in roots and leaves (PR1 and PR5) (Beatrice *et al.*, 2017)). Chandra *et al.* (2015) found that chitosan treatment enhances the plant immune response by inducing defense enzyme activity, increasing phenolic contents and maximizing defense-related gene expression.

Conclusions

In this study, four different concentrations (25, 50, and 100 mg L^{-1}) of chitin, chitosan, and a chitin-chitosan mixture were employed *in vitro* on *R. solanacearum* and, subsequently, they were used preventively and curatively as soil amendments on tomato plants. The results revealed that chitosan concentrations (25, 50, and 100 mg L^{-1}) inhibited the development of *R. solanacearum* more than other treatments. Furthermore, *in vivo* experiments revealed that the lowest concentration of chitin (25 mg L^{-1}) allowed for excellent stem growth, whereas the same concentration of chitosan contributed significantly to root length increase. The leaf alteration index showed a decrease during the application of all treatments compared to the control. These findings led us to suggest the use of these biopolymers to manage this ubiquitous disease in matrix crops over the world.

Conflict of interest statement

The authors declare that there is no conflict of interest regarding the publication of this article.

Author's contributions

AR and KO developed the methodology and created the models. AR conceptualized and wrote the original draft. KO oversaw the writing and editing of the article's final text and supervised the work. SGG and MS contributed to the data analysis. AR, KO, SGG, and MS wrote, reviewed, and edited the manuscript.

Literature cited

Adebayo, O. S., & Ekpo, E. J. A. (2005). Biovar of *Ralstonia solanacearum* causing bacterial wilt of tomato in Nigeria. *Plant Disease*, *89*(10), 1129. https://doi.org/10.1094/PD-89-1129C

Ahmed, N. N., Islam, M. R., Hossain, M. A., Meah, M. B., & Hossain, M. M. (2013). Determination of races and biovars of *Ralstonia solanacearum* causing bacterial wilt disease of potato. *Journal of Agricultural Science*, *5*(6), 86–93. https://doi.org/10.5539/jas.v5n6p86

Algam, S. A. E., Xie, G., Li, B., Yu, S., Su, T., & Larsen, J. (2010). Effects of *Paenibacillus* strains and chitosan on plant growth promotion and control of *Ralstonia* wilt in tomato. *Journal of Plant Pathology*, *92*(3), 593–600.

Bank, T. L., Kukkadapu, R. K., Madden, A. S., Ginder-Vogel, M. A., Baldwin, M. E., & Jardine, P. M. (2008). Effects of gamma-sterilization on the physico-chemical properties of natural sediments. *Chemical Geology*, *251*(1–4), 1–7. https://doi.org/10.1016/j.chemgeo.2008.01.003

Beatrice, C., Linthorst, J. M. H., Cinzia, F., & Luca, R. (2017). Enhancement of *PR1* and *PR5* gene expressions by chitosan treatment in kiwifruit plants inoculated with *Pseudomonas syringae* pv. *actinidiae*. *European Journal of Plant Pathology*, *148*, 163–179. https://doi.org/10.1007/s10658-016-1080-x

Benhamou, N., & Thériault, G. (1992). Treatment with chitosan enhances resistance of tomato plants to the crown and root rot pathogen *Fusarium oxysporum* f. sp. *radicis-lycopersici*. *Physiological and Molecular Plant Pathology*, *41*(1), 33–52. https://doi.org/10.1016/0885-5765(92)90047-Y

Borines, L. M., Sagarino, R. M., Calamba, R. B., Contioso, M. A. A., Jansalin, J. G. F., & Calibo, C. L. (2015). Potential of chitosan for the control of tomato bacterial wilt caused by *Ralstonia solanacearum* (Smith) Yabuuchi *et al*. *Annals of Tropical Research*, *37*(2), 57–69. https://doi.org/10.32945/atr3725.2015

Cellier, G. (2010). *Description des écotypes du phylotype II dans le complexe d'espèces Ralstonia solanacearum: diversité et évolution* [Doctoral dissertation, Université de la Réunion]. HAL Theses. https://tel.archives-ouvertes.fr/tel-00716870

Chamedjeu, R. R., Masanga, J., Matiru, V., & Runo, S. (2018). Isolation and characterization of *Ralstonia solanacearum* strains causing bacterial wilt of potato in Nakuru County of Kenya. *African Journal of Biotechnology*, *17*(52), 1455–1465. https://doi.org/10.5897/AJB2018.16659

Chandra, S., Chakraborty, N., Dasgupta, A., Sarkar, J., Panda, K., & Acharya, K. (2015). Chitosan nanoparticles: a positive modulator of innate immune responses in plants. *Scientific Reports*, *5*, Article 15195. https://doi.org/10.1038/srep15195

Chandrashekara, K. N., Prasanna Kumar, M. K., & Saroja, S. (2012). Aggressiveness of *Ralstonia solanacearum* isolates on tomato. *Journal of Experimental Sciences*, *3*(9), 5–9.

De Mahieu, A., Ponette, Q., Mounir, F., & Lambot, S. (2020). Using GPR to analyze regeneration success of cork oaks in the Maâmora forest (Morocco). *NDT & E International*, *115*, Article 102297. https://doi.org/10.1016/j.ndteint.2020.102297

Douira, A., & Lahlou, H. (1989). Variabilité de la spécificité parasitaire chez *Verticillium albo-atrum* Reinke et Berthold, forme à microsclérotes. *Cryptogamie Mycologie*, *10*, 19–32.

Douira, A., Lahlou, H., Elhaloui, N. E., & Bompeix, G. (1994). Mise en évidence de la variabilité du pouvoir pathogène dans la descendance d'une souche de *Verticillium albo-atrum*, forme à microsclérotes, après son adaptation à une nouvelle plante hôte et retour sur la plante d'origine. *Revue de la Faculté des Sciences de Marrakech*, (8), 107–118.

Elame, F., Lionboui, H., Wifaya, A., Mokrini, F., Mimouni, A., & Azim, K. (2019). Analyse économique de la compétitivité de la filière tomate dans la région du Souss-Massa (Maroc). *Revue Marocaine des Sciences Agronomiques et Vétérinaires*, *7*(4), 595–599.

El Ghaouth, A., Arul, J., Ponnampalam, R., & Boulet, M. (1991). Chitosan coating effect on storability and quality of fresh strawberries. *Journal of Food Science*, *56*(6), 1618–1620. https://doi.org/10.1111/j.1365-2621.1991.tb08655.x

El Hadrami, A., Adam, L. R., El Hadrami, I., & Daayf, F. (2010). Chitosan in plant protection. *Marine Drugs*, *8*(4), 968–987. https://doi.org/10.3390/md8040968

Elphinstone, J. G. (2005). The current bacterial wilt situation: a global overview. In C. Allen, P. Prior, & A. C. Hayward (Eds.), *Bacterial wilt disease and the Ralstonia solanacearum species complex* (pp. 9–28). American Phytopathological Society Press.

EPPO. (2018). PM 7/21 (2) *Ralstonia solanacearum*, *R. pseudosolanacearum* and *R. syzygii* (*Ralstonia solanacearum* species complex). *EPPO Bulletin*, *48*(1), 32–63. https://doi.org/10.1111/epp.12454

Farag, S. M. A., Elhalag, K. M. A., Hagag, M. H., Khairy, A. S. M., Ibrahim, H. M., Saker, M. T., & Messiha, N. A. S. (2017). Potato bacterial wilt suppression and plant health improvement after application of different antioxidants. *Journal of Phytopathology*, *165*(7–8), 522–537. https://doi.org/10.1111/jph.12589

Hassan, O., & Chang, T. (2017). Chitosan for eco-friendly control of plant disease. *Asian Journal of Plant Pathology*, *11*(2), 53–70. https://doi.org/10.3923/ajppaj.2017.53.70

Heikrujam, S. C., Singh, R. I., Laiphrakpam, P. C., & Laishram, S. (2020). Isolation and characterization of *Ralstonia solanacearum* from infected tomato plants of Bishnupur district of Manipur. *The Pharma Innovation Journal*, *9*(2), 138–141. https://doi.org/10.22271/tpi.2020.v9.i2c.4363

Hoekou, Y. P., Batawila, K., Gbogbo, K. A., Karou, D. S., Ameyapoh, Y., & Souza, C. (2012). Evaluation des propriétés antimicrobiennes de quatre plantes de la flore togolaise utilisées en médecine traditionnelle dans le traitement des diarrhées infantiles. *International Journal of Biological and Chemical Sciences*, *6*(6), 3089–3097. https://doi.org/10.4314/ijbcs.v6i6.10

Hsu, S. C., & Lockwood, J. L. (1975). Powdered chitin agar as a selective medium for enumeration of actinomycetes in water and soil. *Applied Microbiology*, *29*(3), 422–426. https://doi.org/10.1128/am.29.3.422-426.1975

Kelman, A. (1954). The relationship of pathogenicity in *Pseudomonas solanacearum* to colony appearance on a tetrazolium medium. *Phytopathology*, *44*, 693–695.

Khan, W., Prithiviraj, B., & Smith, D. L. (2003). Chitosan and chitin oligomers increase phenylalanine ammonia-lyase and tyrosine ammonia-lyase activities in soybean leaves. *Journal of Plant Physiology*, *160*(8), 859–863. https://doi.org/10.1078/0176-1617-00905

Kim, G., Dasagrandhi, C., Kang, E. H., Eom, S. H., & Kim, Y. M. (2018). *In vitro* antibacterial and early stage biofilm inhibitory potential of an edible chitosan and its phenolic conjugates against *Pseudomonas aeruginosa* and *Listeria monocytogenes*. *3 Biotech*, *8*, Article 439. https://doi.org/10.1007/s13205-018-1451-4

Li, B., Liu, B., Su, T., Fang, Y., Xie, G., Wang, G., Wang, Y., & Sun, G. (2010). Effect of chitosan solution on the inhibition of *Pseudomonas fluorescens* causing bacterial head rot of broccoli. *The Plant Pathology Journal*, *26*(2), 189–193. https://doi.org/10.5423/PPJ.2010.26.2.189

Luna, E., Pastor, V., Robert, J., Flors, V., Mauch-Mani, B., & Ton, J. (2011). Callose deposition: a multifaceted plant defense response. *Molecular Plant-Microbe Interactions*, *24*(2), 183–193. https://doi.org/10.1094/MPMI-07-10-0149

Malerba, M., & Cerana, R. (2018). Recent advances of chitosan application in plants. *Polymers*, *10*(2), Article 118. https://doi.org/10.3390/polym10020118

Mansfield, J., Genin, S., Magori, S., Citovsky, V., Sriariyanum, M., Ronald, P., Dow, M., Verdier, V., Beer, S. V., Machado, M. A., Toth, I., Salmond, G., & Foster, G. D. (2012). Top 10 plant pathogenic bacteria in molecular plant pathology. *Molecular Plant Pathology*, *13*(6), 614–629. https://doi.org/10.1111/j.1364-3703.2012.00804.x

Mohy Eldin, M. S., Soliman, E. A., Hashem, A. I., & Tamer, T. M. (2008). Chitosan modified membranes for wound dressing applications: preparations, characterization and bio-evaluation. *Trends in Biomaterials and Artificial Organs*, *22*(3), 158–168.

Poussier, S., Trigalet-Demery, D., Vandewalle, P., Goffinet, B., Luisetti, J., & Trigalet, A. (2000). Genetic diversity of *Ralstonia solanacearum* as assessed by PCR-RFLP of the *hrp* gene region, AFLP and 16S rRNA sequence analysis, and identification of an African subdivision. *Microbiology*, *146*(7), 1679–1692. https://doi.org/10.1099/00221287-146-7-1679

Rahman, M. F., Islam, M. R., Rahman, T., & Meah, M. B. (2010). Biochemical characterization of *Ralstonia solanacerum* causing bacterial wilt of brinjal in Bangladesh. *Progressive Agriculture*, *21*(1–2), 9–19. https://doi.org/10.3329/pa.v21i1-2.16744

Ramkissoon, A., Francis, J., Bowrin, V., Ramjegathesh, R., Ramsubhag, A., & Jayaraman, J. (2016). Bio-efficacy of a chitosan based elicitor on *Alternaria solani* and *Xanthomonas vesicatoria* infections in tomato under tropical conditions. *Annals of Applied Biology*, *169*(2), 274–283. https://doi.org/10.1111/aab.12299

Ravelomanantsoa, S., Vernière, C., Rieux, A., Costet, L., Chiroleu, F., Arribat, S., Cellier, G., Pruvost, O., Poussier, S., Robène, I., Guérin, F., & Prior, P. (2018). Molecular epidemiology of bacterial wilt in the Madagascar highlands caused by Andean (Phylotype IIB-1) and African (Phylotype III) brown rot strains of the *Ralstonia solanacearum* species complex. *Frontiers in Plant Science*, *8*, Article 2258. https://doi.org/10.3389/fpls.2017.02258

Razia, S., Chowdhury, M. S. M., Aminuzzaman, F. M., Sultana, N., & Islam, M. (2021). Morphological, pathological, biochemical and molecular characterization of *Ralstonia solanacearum* isolates in Bangladesh. *American Journal of Molecular Biology*, *11*(4), 142–164. https://doi.org/10.4236/ajmb.2021.114012

Rkhaila, A., & Ounine, K. (2018). Shrimp shells, chitin and chitosan powders effect on growth of *Lycopersicon esculentum* and their ability to induce resistance against *Fusarium oxysporum* f. sp. *radicis-lycopersici* attack. *Indian Journal of Agricultural Research*, *52*(5), 512–517. https://doi.org/10.18805/IJARe.A-305

Rodrigues, L. M. R., Destéfano, S. A. L., Diniz, M. C. T., Comparoni, R., & Neto, J. R. (2011). Pathogenicity of Brazilian strains of *Ralstonia solanacearum* in *Strelitzia reginae* seedlings. *Tropical Plant Pathology*, *36*(6), 409–413. https://doi.org/10.1590/S1982-56762011000600011

Sadi, S. M., Saidou, A. K., Boube, M., & Aune, J. B. (2020). Effets de la fertilisation à base de la biomasse du *Sida cordifolia* L. sur les performances agronomiques et la rentabilité économique de la tomate (*Lycopersicum esculentum* Mill.) en culture irriguée. *European Scientific Journal*, *16*(3), 127–150. https://doi.org/10.19044/esj.2020.v16n3p127

Schaad, N. W., Jones, J. B., & Chun, W. (Eds.). (2001). *Laboratory guide for identification of plant pathogenic bacteria* (3rd ed.). American Phytopathological Society Press.

Sikirou, R., Beed, F., Ezin, V., Hoteigni, J., & Miller, S. A. (2017). Distribution, pathological and biochemical characterization of *Ralstonia solanacearum* in Benin. *Annals of Agricultural Sciences*, *62*(1), 83–88. https://doi.org/10.1016/j.aoas.2017.05.003

Siri, M. I., Sanabria, A., & Pianzzola, M. J. (2011). Genetic diversity and aggressiveness of *Ralstonia solanacearum* strains causing bacterial wilt of potato in Uruguay. *Plant Disease*, *95*(10), 1292–1301. https://doi.org/10.1094/PDIS-09-10-0626

Ting, S., Guiyue, W., & Guanlin, X. (2013). Effects of chitosan against *Ralstonia solanacearum* and its biofilm formation. *Plant Protection*, *39*(1), 89–92.

Van Loon, L. C., Rep, M., & Pieterse, C. M. J. (2006). Significance of inducible defense-related proteins in infected plants. *Annual Review of Phytopathology*, *44*, 135–162. https://doi.org/10.1146/annurev.phyto.44.070505.143425

Yuliar, Nion, Y. A., & Toyota, K. (2015). Recent trends in control methods for bacterial wilt diseases caused by *Ralstonia solanacearum*. *Microbes and Environments*, *30*(1), 1–11. https://doi.org/10.1264/jsme2.ME14144

Physicochemical characterization of cantaloupe fertilized with various potassium sources

Caracterización fisicoquímica del melón cantalupo fertilizado con diferentes fuentes de potasio

Anouschka Leonilda de Glint[1], Morgana Baptista Gimenes[1], Andreza Lopes do Carmo[1], Vanessa Cury Galati[1*], Alexsander Seleguini[1], Aluisio dos Santos Salustiano Gradella[1], Gabriel Pimenta do Nascimento[1], and Eric Haydt Castello Branco van Cleef[1]

ABSTRACT

The northeastern region of Brazil is responsible for 95% of the country's total melon production and 97% of total exported melons. This is mainly due to the edaphoclimatic conditions and the technological packages employed in the country for fertilization strategies. The aim of this study was to evaluate the effects of topdressing potassium fertilizers associated or not with sowing fertilization with Ekosil™ on the physicochemical traits of cantaloupes. The study was conducted in a randomized complete block design, with a 4×2 factorial arrangement (topdressing potassium nitrate [PN], potassium chloride [PC], potassium sulfate [PS] and Ekosil™ [EK], with or without sowing fertilization with Ekosil™). The evaluated traits in the fruits were: fresh mass (FM), soluble solids content (SS), titratable acidity (TA), SS/TA ratio (RA) and pigment contents (chlorophyll [CL] and carotenoids [CA]). No effect of treatments was observed on pH, CL, and CA (P>0.05). Interaction of sowing and topdressing fertilization was observed for the other variables (P<0.005). The FM was greater for PC and EK with sowing fertilization with Ekosil™. Without Ekosil™, PS, PN and EK resulted in heavier fruits. The SS was greater for PS when using Ekosil™ and for PS and PC without sowing fertilization with Ekosil™. The PC and PN resulted in greater TA, and the RA was greater for PS. Under the edaphoclimatic conditions of the Pontal do Triângulo Mineiro region, topdressing fertilization with potassium sulfate, without sowing fertilization with Ekosil™, resulted in heavier and sweeter cantaloupes.

Key words: *Cucumis melo*, fruit quality, mineral nutrition, pigments, potassium fertilization.

RESUMEN

La región nororiental de Brasil es responsable del 95% de la producción total de melón del país y del 97% del total de melón exportado. Esto se debe principalmente a las condiciones edafoclimáticas y a los paquetes tecnológicos utilizados en el país en cuanto a estrategias de fertilización. El objetivo de este estudio fue evaluar los efectos de la fertilización de cobertura con fuentes de potasio asociadas o no con la fertilización con Ekosil™ en la siembra sobre las características fisicoquímicas del melón cantalupo. El estudio se realizó en un diseño de bloques completos al azar, con un arreglo factorial 4×2 (fertilización de cobertura de nitrato de potasio [NP], cloruro de potasio [CP], sulfato de potasio [SP] y Ekosil™ [EK], con o sin fertilización con Ekosil™ en la siembra). Los parámetros evaluados en frutos fueron: peso fresco (PF), contenido de sólidos solubles (SS), acidez titulable (AT), relación SS/AT (RA) y pigmentos (clorofila [CL] y carotenoides [CA]). No se observó efecto de los tratamientos sobre el pH, CL y CA (P>0.05). Se observó la interacción de la fertilización en la siembra y la fertilización de cobertura para las otras variables (P<0.005). El PF fue mayor para CP y EK, con la aplicación de Ekosil™ en la siembra. Sin Ekosil™, SP, NP y EK dieron como resultado frutos más pesados. Los SS fueron mayores con SP al usar Ekosil™, y para SP y CP sin fertilización con Ekosil™ en la siembra. El CP y NP resultaron en mayor AT, y la RA fue mayor con SP. Bajo las condiciones edafoclimáticas de la región de Pontal do Triângulo Mineiro, la fertilización de cobertura con sulfato de potasio, sin fertilización con Ekosil™ en la siembra, da como resultado melones cantalupos más pesados y dulces.

Palabras clave: *Cucumis melo*, calidad del fruto, nutrición mineral, pigmentos, fertilización potásica.

Introduction

Northeastern Brazil accounts for 95% of the country's total melon production and meets the growing external demand since it is responsible for 97% of total exported melons. This is mainly due to the edaphoclimatic conditions and the technological packages used in the country that provide Brazil with up to three harvests a year. These conditions make Brazil an extremely competitive country

Received for publication: 10 May, 2021. Accepted for publication: 17 November, 2021.

Doi: 10.15446/agron.colomb.v39n3.95042

[1] Federal University of Triângulo Mineiro, Iturama, Minas Gerais (Brazil).
[*] Corresponding author: vanessa.galati@uftm.edu.br

worldwide when compared to European and Asian nations (Figueirêdo *et al*., 2017).

According to the classification of Robinson and Decker-Walters (1997), the melon (*Cucumis melo* L.) belongs to the Cucurbitaceae family and is divided into two botanical groups: 1) *Inodorus*, including yellow and frog skin, that are odorless and non-climacteric fruits, and 2) *Cantaloupensis*, including cantaloupe, gaul and charentais, that are climacteric fruits with marked aroma.

The type yellow is the most cultivated melon in Brazil because of its firm and thick skin that guarantees a longer shelf life and resistance to post-harvest transportation, facilitating exportation. Cantaloupes have become important melons in the domestic and international markets because of their characteristics of orange-colored pulp and high sugar content (°Brix). This melon is more aromatic and has light green and lacy skin, characteristics that directly influence consumer preference (Salviano *et al*., 2017). However, to have high productivity and guarantee competitiveness in the market, it is necessary to adjust the management and fertilization techniques to the edaphoclimatic conditions of each production region, such as the Pontal of Triângulo Mineiro (Minas Gerais State, Brazil) that has naturally poor soil and high annual mean temperatures (Sousa & Lobato, 2004).

Among the macronutrients, potassium plays an important role in numerous physiological processes that alter growth and, consequently, crop productivity. This nutrient regulates the opening of stomata, limiting water loss, and acts in the photosynthetic processes of plants (Zörb *et al*., 2014; Cavalcante *et al*., 2018), guaranteeing reproduction and fruit quality. Deficient potassium fertilization directly interferes with melon productivity, reducing the fruit size, lowering levels of soluble solids and ascorbic acid, changing the color and, consequently, reducing shelf life (Lester *et al*., 2010). Bardiviesso *et al*. (2015) observed that the application of 136.75 kg K_2O ha^{-1} on melons increased fruit yield and significantly affected the concentration of soluble solids and pH, meeting the appropriate standards for fruit marketing.

Various sources of potassium used in agriculture have different points of deliquescence (POD) of salts that indicate the rate of absorption by plant tissues. Schönherr and Luber (2001) observed that this rate, in turn, was dependent on environmental conditions of temperature and humidity. These authors reported POD values from commonly used potassium sources: K_2CO_3, 44%; KCl, 86%; KNO_3, 95%; and KH_2PO_4, 97%. These data can help producers make decisions about which fertilizer to use, depending on the edaphoclimatic conditions of the planting region.

However, different sources of potassium, due to their characteristics and mode of action on vegetal metabolism, can affect differently the production and quality of the melons. Thus, the objective of this study was to evaluate the effects of fertilization with various potassium sources, at planting and topdressing, on the physicochemical characteristics of cantaloupes cultivated in the Pontal of Triângulo Mineiro, Minas Gerais, Brazil.

Materials and methods

The study was conducted in the experimental area of the School Farm "Alípio Soares Barbosa" and at the Chemistry Laboratory of the Federal University of Triângulo Mineiro, Iturama Campus (UFTM/ITU), Minas Gerais, Brazil (19°43'41" S, 50°11'44" W, at an altitude of 425 m a.s.l.). The climate of the city of Iturama is classified, according to Köppen-Geiger, as Aw, with a rainy season from October to March and a dry season from April to September. The average annual rainfall is 1266 mm and the average temperature is 24.5°C.

Before planting, the soil was sampled from the 0-20 cm layer and chemically characterized (Van Raij & Quaggio,1983) as follows: pH ($CaCl_2$) 4.8; organic matter: 24 g dm^{-3}; P (resin) 47 mg dm^{-3}; K: 2.1 mmol$_c$ dm^{-3}; Ca: 22 mmol$_c$ dm^{-3}; Mg: 8 mmol$_c$ dm^{-3}; S: 12 mg dm^{-3}; H+Al: 47 mmol$_c$ dm^{-3}; sum of bases: 32.1 mmol$_c$ dm^{-3}; cation exchange capacity: 79.1 mmol$_c$ dm^{-3}; base saturation: 41%; B: 0.17 mg dm^{-3}; Cu: 1.2 mg dm^{-3}; Fe: 75 mg dm^{-3}; Mn: 4.1 mg dm^{-3}, and Zn: 3.2 mg dm^{-3}.

The field was prepared under a conventional tillage system using a disk harrow for soil decompaction, and then a light harrowing to level off the ground and eliminate weeds. To correct the acidity of the soil, one month before the beginning of the experiment, the equivalent to approximately 4 t ha^{-1} of calcitic limestone (total relative neutralizing power = 85%) was applied, defined based on the soil analysis and following the recommendation of Van Raij *et al.* (1997).

For soil correction, 20 m^3 ha^{-1} organic compost, 120 kg ha^{-1} P_2O_5 (single superphosphate) phosphorus and 250 kg ha^{-1} phonolite powder (8% K_2O; 54% SiO_2; 6.74% Na_2O) were applied in two applications: the first 30 d and during the last 10 d before transplanting the seedlings. After transplanting, topdressing fertilization was performed with nitrogen (17 g m^{-2} of N [urea]) and potassium (25 g m^{-2} of K_2O), in four applications as follows: at the planting of seedlings and 15, 30, and 45 d after transplanting. A drip-tape irrigation system was used to irrigate the experimental area, using a nominal flow of 3.6 L h^{-1}, at 60 kPa of service pressure, with emitters spaced at 0.30 m. The plants were irrigated throughout the cycle, always keeping the soil moisture above field capacity, initially in two 20-min daily shifts, and then three 20-min shifts, from the beginning of fruiting until harvest.

The experiment was conducted in a randomized complete block design with a factorial arrangement (2×4) of treatments (with or without sowing fertilization with Ekosil™ [8% K_2O, 25% total silicon] and topdressing fertilization with potassium nitrate [PN; 12% N, 43% P_2O_5, 1% S and 1% Mg], potassium chloride [PC; 60% P_2O_5], potassium sulfate [PS; 51% K_2O, 18% S], or Ekosil™ [EK; 8% K_2O, 25% silicon]).

Ekosil™ (Yoorin Fertilizantes, Poços de Caldas, MG, Brazil) is a potassium fertilizer obtained by grinding silicate rocks of volcanic origin without using chemicals and has a saline index of 0.63. It has a residual effect with a gradual release of nutrients during the vegetative and productive cycle of plants. Thus, it causes fewer potassium losses due to leaching since the accompanying anion is the silicate. It is composed of 8% K_2O and 25% total silicon.

The experimental area was composed of approximately 60 m^2, divided into four blocks spaced 1.20 m×0.30 m (rows and plants, respectively), totaling 160 evaluated plants (five per treatment combination in each block). The melon hybrid used was the Torreon F1 from Top Seed (Agristar, Guimarânia, MG, Brazil) that produces oval fruits, with lacy peel, orange pulp, a small internal cavity, and an average mass of around 1,000 g. Sowing was carried out in expanded polystyrene trays in July 2019 and field planting was carried out in August 2019. The plants were managed vertically without pruning of branches or thinning of fruits and tutored on a bamboo spreader horizontal to the soil and tied to posts fixed in the ground. The harvest point was determined when the peduncle of the fruit showed the abscission layer and was easily detached from the plant.

After harvesting, the fruits were transported to the Chemistry Laboratory of the Federal University of Triângulo Mineiro for evaluation. The characteristics evaluated in the fruits were: 1) fresh fruit mass (FM), using a digital analytical scale (model S4202, Bel Equipamentos Analíticos, Piracicaba, Brazil) and expressed in grams; 2) content of soluble solids (SS), quantified with an aliquot extracted from the pulp after crushing by gauze compression and quantified using a palette refractometer (model PR-101, Atago, Tokyo, Japan), expressed in °Brix (AOAC, 1997); 3) titratable acidity (TA), using 10 g of crushed pulp, diluting it in 50 ml distilled water, titrating with 0.1 N NaOH and expressed in grams of citric acid 100 g^{-1} pulp (AOAC, 1997); 4) ratio (RA) between the levels of soluble solids and titratable acidity; 5) pH, using a digital pH meter (model PHS-3E, Shanghai Yoke Instrument, Shanghai, China); 6) carotenoid content (CA), and 7) chlorophyll content (CL), 2.0 g of each treatment were weighed directly into centrifuge tubes, then 18 ml 80% acetone was added, homogenized and centrifuged at 1,000 *g* for 5 min in a centrifuge (model 80-2B, Kubota Corporation, Daiki, Akagidai, Japan). The readings of the supernatant were performed using a spectrophotometer (model F-7100, Hitachi High-Tech Science Co, Tokyo, Japan) with absorbances of 663 nm (chlorophyll a), 646 nm (chlorophyll b), and 470 nm (carotenoids) and expressed in mg g^{-1} of pulp (Arnon, 1949).

An analysis of variance was performed using the Glimmix procedure of SAS version 9.4 (SAS Institute Inc., Cary, USA). Means were compared using the Tukey's test and the significance was declared at 5%.

Results

An interaction of sowing fertilization with Ekosil™ and topdressing potassium sources was observed for the variables FM (P = 0.005), SS (P<0.0001), TA (P = 0.0002) and RA (P<0.0001; Fig. 1) but no interactions were observed for the variables pH, CL, and CA (P>0.05; Fig. 2).

When using sowing fertilization with Ekosil™, the topdressing potassium sources PC and EK provided heavier fruits when compared to PN and PS that were similar. However, when not using Ekosil™ at sowing, the treatments with heavier fruits were PS and EK, followed by PN and PC. When evaluating data within each potassium source, differences in the use of Ekosil™ at sowing were observed for PC resulting in heavier fruits with sowing fertilization with Ekosil™ and for PS resulting in heavier fruits when not fertilizing with Ekosil™ at sowing (Fig. 1A).

The effect of topdressing potassium sources on SS was observed with or without fertilization with Ekosil™ at sowing. The topdressing PS provided greater SS content, followed by PN and EK (without any differences between

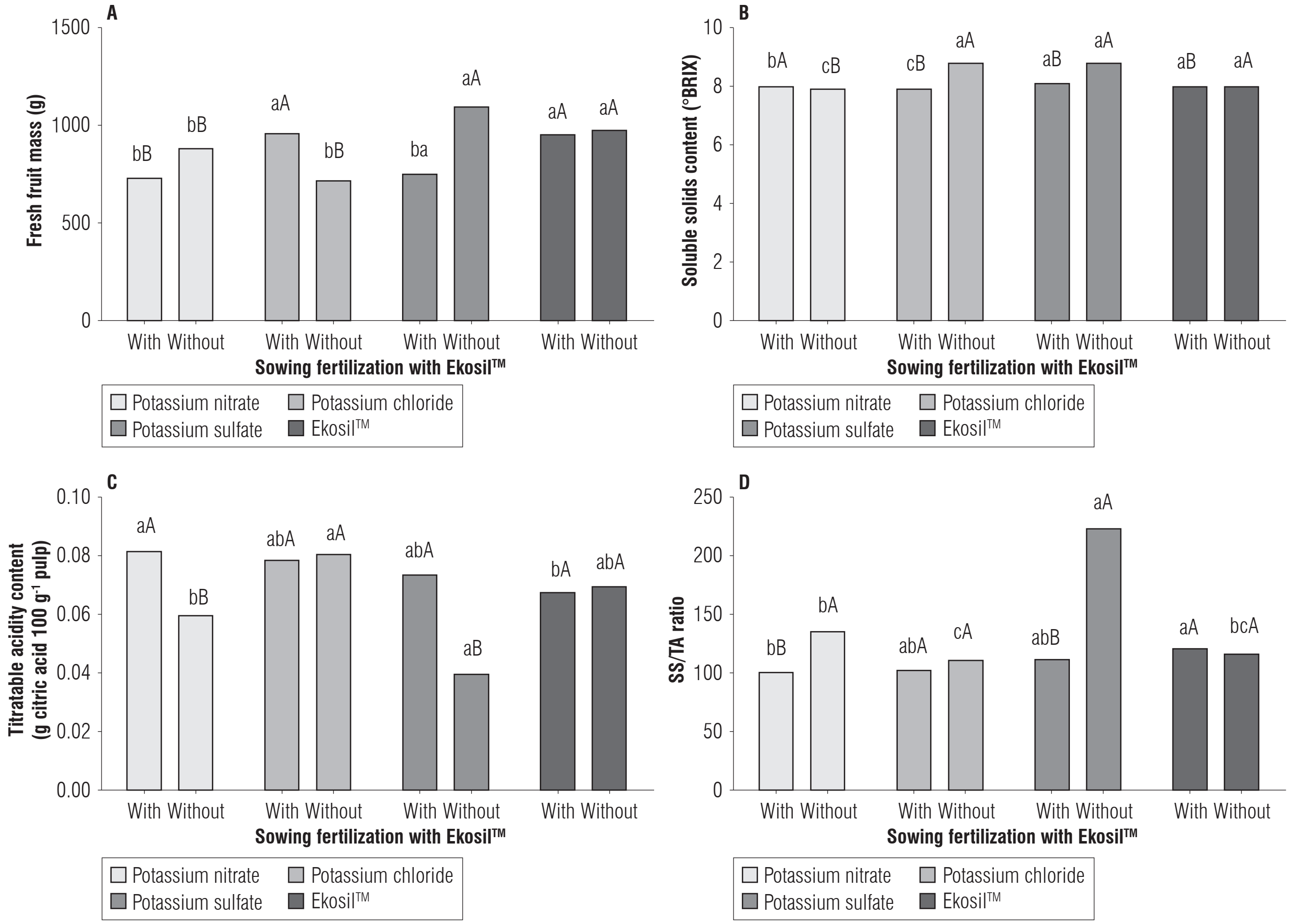


FIGURE 1. A) Fresh fruit mass; B) soluble solids content (SS); C) titratable acidity content (TA), and D) SS/TA ratio of cantaloupe cultivated with or without sowing fertilization with Ekosil™ and topdressing potassium sources (potassium nitrate, potassium chloride, potassium sulfate or Ekosil™). Means followed by different capital letters differ from each other regarding the use of sowing fertilization with Ekosil™ according to the Tukey's test (P<0.05). Means followed by different lowercase letters differ from each other regarding the source of potassium, according to the Tukey's test (P<0.05).

them) and PC when the sowing fertilization with Ekosil™ was performed. The effects were different when not using Ekosil™ at sowing, with greater SS contents for treatments PC and PS and lower for EK followed by PN. Effects of sowing fertilization with Ekosil™ within each topdressing potassium source were observed. For PC and PS, the greater values were observed when not using sowing fertilization with Ekosil™, whereas for PN the opposite was observed, and for EK no difference was seen (Fig. 1B).

The TA was altered by topdressing potassium sources and sowing fertilization with Ekosil™. The lowest TA content was observed for EK and the highest for PN, with PS and PC showing intermediate values ranging from 0.074 to 0.079 g citric acid 100 g^{-1} when using sowing fertilization with Ekosil™. When not using Ekosil™ at sowing, the lowest TA content was observed in PS and the highest in PC, with PN and EK ranging from 0.060 to 0.070 g citric acid 100 g^{-1}. The treatments PN and PS were affected similarly, showing greater TA content averages when sowing fertilization with Ekosil™ was used. No effect of sowing fertilization was observed for either PC or EK (Fig. 1C).

Although representing the SS/TA ratio, the results for RA were higher for EK and lower for PN when using sowing fertilization with Ekosil™. The results of this variable were greater for PS, followed by PN and EK (with similar values) and PC, when not using Ekosil™ at sowing (Fig. 1D).

The pH, CL, and CA were unaffected by sowing fertilization with Ekosil™ and topdressing potassium sources, with averages of 6.49, 0.23 mg g^{-1} FM, and 149.0 mg g^{-1} FM, respectively (Fig. 2A-C).

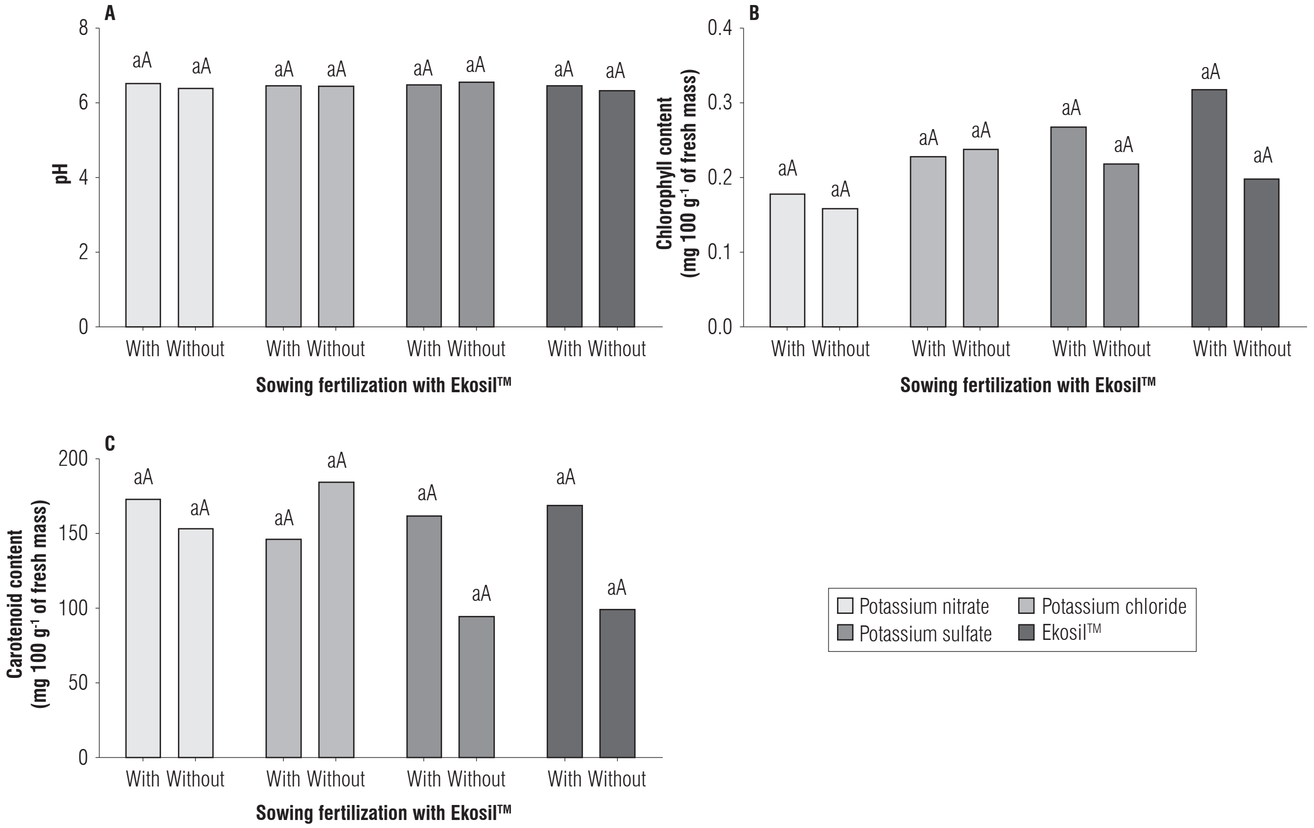


FIGURE 2. Values of A) pH; B) chlorophyll content; and C) carotenoid content of cantaloupes cultivated with or without sowing fertilization with Ekosil™ and topdressing potassium sources (potassium nitrate, potassium chloride, potassium sulfate or Ekosil™).

Discussion

The absorption of potassium by the roots is a highly efficient process for removing the nutrient from the soil, as long as there is an adequate amount of water for it to be transported via the xylem by mass flow (Römheld & Kirkby, 2010). The transport of nutrients by plants depends on the solubility of potassium sources, and this will influence the availability of this macronutrient for plants. Thus, in this experiment, it was seen that the PC and EK sources resulted in heavier fruits since the low solubility of the rock powder (Ekosil™) gradually provided nutrients to the plants that may result in larger fruits (Ehlers & Arruda, 2014). Feltrim (2010), evaluating fertilization with potassium nitrate and potassium chloride and spacing in watermelon, did not find significant differences in fruit mass. However, in our experiment, it was observed that PC application at sowing provided fruits with greater mass.

The application of soluble salts, such as potassium chloride, facilitates nutrient leaching due to water solubility and high doses applied to the soil (Van Raij, 2011). Potassium nitrate and sulfate are solubilized more gradually in the environment and have other elements, such as sulfur, magnesium, and/or nitrogen, in their composition. They also have a lesser saline effect and are less harmful to plants; however, they are less used since they are more expensive than potassium chloride that is the most commonly used source (Yamada & Roberts, 2005).

Another natural potassium source that can be used in agriculture is Ekosil™ that contains around 8% K_2O and 25% Si, and has slow solubility, releasing the potassium gradually into the soil. However, there is no evidence in the literature that the application of Ekosil™ in sowing fertilization could affect the topdressing fertilization requirements or how it affects the quality of the melons.

Kano *et al.* (2013), studying hybrids of cantaloupes, reported that the most extracted nutrients by the fruits are potassium, nitrogen, and calcium, and this will directly affect the quality of the fruits after harvest. The topdressing application of potassium sulfate and Ekosil™ without sowing fertilization with this natural product resulted in larger fruits since the nutrient was available to the plant during the period of greatest nutritional demand. This provided greater assimilation of photoassimilates due to the increase in the photosynthetic rate and greater water intake due to the lower osmotic potential in the presence of this nutrient.

Van Raij (2011) reported that potassium directly influences fruit characteristics due to the displacement of this nutrient throughout the plant. However, Wade *et al.* (2004), when applying potassium nitrate to melons, did not observe differences in fruit weight gain, corroborating the results found in our study.

According to Braga *et al.* (2010), fruits with values of soluble solids above 9.0 °Brix are considered quality fruits with good consumer acceptance. Asao *et al.* (2013) and Mohammadrezakhani *et al.* (2016) reported that potassium has a direct influence on the levels of soluble solids as it is a nutrient that is part of the metabolic process of melon fruits together with the ambient temperature that influences the flavor and fruit aroma, characteristics that are decisive at the time of marketing. Castoldi *et al.* (2008) found that lace melon hybrids with soluble solids ranging between 9.0 and 11.2 °Brix were fully accepted by the final consumers. Lester *et al.* (2010) observed that the levels of sucrose, glucose, and fructose in melons increased with the supplementary foliar fertilization with potassium.

The levels of organic acids normally decrease with the maturation of the fruits. These acids are also important sources of energy in the respiratory process and are converted or oxidized to sugars and used by the cells (Batista-Silva *et al.*, 2018). A climacteric fruit at harvesting will show greater sugar accumulation and less acidity. This fact was observed in cantaloupes fertilized with topdressing potassium sulfate without sowing fertilization with Ekosil™ that showed low acidity when compared to other treatments. This may have occurred because these potassium sources are slow-releasing, and the fruit absorbs the nutrient in the ideal phase of its reproductive process.

According to Chitarra and Chitarra (2005), the chemical indexes that better demonstrate the maturation point are pH, TA, and SS content. These authors also reported that RA is one of the best methods to evaluate the taste of fruits since the higher the value, the greater the amount of sugars, and the lower the acidity; this enables the perception of sweetness in the fruit that is one of the most relevant attributes for the consumer (Jordan & Seelye, 2009). The results of these reports agree with the SS and TA observed in this study, in which the treatment subjected to topdressing fertilization with potassium sulfate without sowing fertilization with Ekosil™ resulted in a greater content of SS and a lower content of TA, obtaining sweeter fruits. The increased potassium fertilization also increases its concentration in the fruits, improving its distribution and resulting in greater neutralization of acids and sweeter fruits (Grangeiro & Cecílio Filho, 2004). Feltrin *et al.* (2005) observed that the SS/TA ratio in tomatoes was not altered with topdressing fertilization with potassium chloride and potassium sulfate; however, the use of potassium sulfate here resulted in the increase of the ratio.

The potential of hydrogen is based on an index that represents the acidity, neutrality, or alkalinity of any medium. The pH value can indicate the time of fruit ripening and, thus, estimate the precocity. According to Batista-Silva *et al.* (2018), the acidity of the fruit generally tends to decrease due to the use of organic acids in the fruit's respiratory process that is intense as it grows and matures. For Max *et al.* (2010), the citric acid that initiates the reactions of the Krebs cycle and other organic acids used as intermediates in biochemical reactions may have their concentrations reduced in the pulp. In this trial, no pH variations were observed for cantaloupes fertilized with different topdressing potassium sources associated or not with sowing fertilization with Ekosil™. Malta *et al.* (2013) observed a reduction in acidity in guavas treated with the combination of cattle manure and rock powder, which is a desirable characteristic for fresh consumption.

Regarding chlorophyll and carotenoid contents, differences among the treatments were expected since chlorophyll degradation and carotenoid synthesis are processes that involve the differentiation of chloroplasts into chromoplasts that are plastids that accumulate carotenes (Li & Yuan, 2013). This occurs mainly during the ripening process of climacteric fruits. Carotenoids are divided into primary metabolites that are responsible for the survival of the plant, performing an active function in the processes of photosynthesis, respiration, and assimilation of nutrients and secondary metabolites responsible for the stabilization of the plasma membrane (Isah, 2019). In a study conducted by Lester *et al.* (2010), the beneficial effects of K, via the leaf, occurred due to the increase in the photosynthetic rate, improving the assimilation and translocation of photo-assimilates from leaves to fruits and causing greater activation of the lipoxygenase enzyme and availability of substrate for ascorbic acid and β-carotene biosynthesis. However, in this trial, we did not observe variations in pigments when various topdressing potassium sources were associated or not with sowing fertilization with Ekosil™. K acts as a cofactor for specific enzymes in the formation of pigments and, therefore, influences the increase in red coloration in the epidermis (Trevisan *et al*., 2006). However, this was not observed in this study. Medeiros *et al.* (2014) observed an increase in the content of carotenoids in passion fruit peel when associated with rock powder and bovine biofertilizers.

Conclusions

Under the edaphoclimatic conditions of the Pontal do Triângulo Mineiro region, the use of topdressing fertilization with potassium sulfate without sowing fertilization with Ekosil™ resulted in heavier and sweeter cantaloupes that are crucial factors for greater acceptability of the product by the consumer market. Further studies are needed to evaluate the fertilization with organic and natural products, especially regarding the absorption and assimilation of nutrients by plants and the interference in the quality and productivity of vegetables.

Conflict of interest statement

The authors declare that there is no conflict of interest regarding the publication of this article.

Author's contributions

ALG conceived and planned the trials, conducted the laboratory analysis, and wrote the manuscript. MBG, ALC and GPN conducted the laboratory analysis and wrote the manuscript. AS and ASSG conceived, planned the trials and conducted the field study and sampling. EHCBVC statistically analyzed data, translated the text, and wrote and edited the manuscript. VCG conceived and planned the trials, conducted the laboratory analysis, wrote, formatted, reviewed, and edited the manuscript. All authors reviewed the manuscript and approved the final version before submission.

Literature cited

AOAC. (1997). *Official methods of analysis of AOAC international* (16th ed.). Association of Official Analytical Chemists.

Arnon, D. I. (1949). Copper enzymes in isolated chloroplasts: polyphenoloxidase in *Beta vulgaris*. *Plant Physiology*, *24*(1), 1–15. https://doi.org/10.1104/pp.24.1.1

Asao, T., Asaduzzaman, M., Mondal, M. F., Tokura, M., Adachi, F., Ueno, M., Kawaguchi, M., Yano, S., & Ban, T. (2013). Impact of reduced potassium nitrate concentrations in nutrient solution on the growth, yield and fruit quality of melon in hydroponics. *Scientia Horticulturae*, *164*, 221–231. https://doi.org/10.1016/j.scienta.2013.09.045

Bardiviesso, D. M., Maruyama, W. I., Pessato, L. E., Pereira, A. C. B., & Modesto, J. H. (2015). Adubação potássica na produção de duas cultivares de meloeiro. *Revista de Agricultura Neotropical*, *2*(1), 32–40. https://doi.org/10.32404/rean.v2i1.244

Batista-Silva, W., Nascimento, V. L., Medeiros, D. B., Nunes-Nesi, A., Ribeiro, D. M., Zsögön, A., & Araújo, W. L. (2018). Modifications in organic acid profiles during fruit development and ripening: correlation or causation? *Frontiers in Plant Science*, *9*, Article 1689. https://doi.org/10.3389/fpls.2018.01689

Braga, M. B., Resende, G. M., Moura, M. S. B., Dias, R. C. S., Costa, N. D., Calgaro, M., Correia, J. S., & Silva, F. Z. (2010). Produtividade e qualidade do melão submetido a diferentes tipos de cobertura de solo. *Irriga*, *15*(4), 422–430. https://doi.org/10.15809/irriga.2010v15n4p422

Castoldi, R., Charlo, H. C. O., Vargas, P. F., & Braz, L. T. (2008). Qualidade de frutos de cinco híbridos de melão rendilhado em função do número de frutos por planta. *Revista Brasileira de Fruticultura*, *30*(2), 455–458. https://doi.org/10.1590/S0100-29452008000200032

Cavalcante, A. C. P., Cavalcante, L. F., Cavalcante, A. G., Bertino, A. M. P., Bertino, A. M. P., & Ferreira, N. M. (2018). Physiology of Paluma guava plants fertilized with potassium and calcium. *Idesia*, *36*(2), 163–172. https://doi.org/10.4067/S0718-34292018005000503

Chitarra, M. I. F., & Chitarra, A. B. (2005). *Pós-colheita de frutas e hortaliças: fisiologia e manuseio* (2nd ed.). Editora UFLA.

Ehlers, T., & Arruda, G. O. S. F. (2014). Utilização de pó de basalto em substratos para mudas de *Eucalyptus grandis*. *Floresta e ambiente*, *21*(1), 37–44. https://doi.org/10.4322/floram.2014.002

Feltrim, A. L. (2010). *Produtividade de melancia em função da adubação nitrogenada, potássica e população de plantas* [Doctoral dissertation, Universidade Estadual Paulista "Júlio de Mesquita Filho"]. Repositório Institucional UNESP. https://repositorio.unesp.br/handle/11449/105179?show=full

Feltrin, D. M., Pott, C. A., Furlani, P. R., & Carvalho, C. R. L. (2005). Produtividade e qualidade de frutos de cultivares de tomateiro fertirrigado com cloreto e sulfato de potássio. *Revista de Ciências Agroveterinárias*, *4*(1), 17–24.

Figueirêdo, M. C. B., Gondim, R. S., & Aragão, F. A. S. (Eds.). (2017). *Produção de melão e mudanças climáticas: sistemas conservacionistas de cultivo para redução das pegadas de carbono e hídrica*. Embrapa.

Grangeiro, L. C., & Cecílio Filho, A. B. (2004). Acúmulo e exportação de macronutrientes pelo híbrido de melancia Tide. *Horticultura Brasileira*, *22*(1), 93–97. https://doi.org/10.1590/S0102-05362004000100019

Isah, T. (2019). Stress and defense responses in plant secondary metabolites production. *Biological Research*, *52*, Article 39. https://doi.org/10.1186/s40659-019-0246-3

Jordan, R. B., & Seelye, R. J. (2009). Relationship between taste perception, density and soluble solids concentration in kiwifruit (*Actinidia deliciosa*). *New Zealand Journal of Crop and Horticultural Science*, *37*(4), 303–317. https://doi.org/10.1080/01140671.2009.9687585

Kano, C., Carmello, Q. A. C., Frizzone, J. A., & Cardoso, S. S. (2013). Teor e acúmulo de nutrientes pelo meloeiro rendilhado cultivado com potássio e CO_2 na água de irrigação. *Biotemas*, *26*(3), 19–28. https://doi.org/10.5007/2175-7925.2013v26n3p19

Lester, G. E., Jifon, J. L., & Makus, D. J. (2010). Impact of potassium nutrition on postharvest fruit quality: melon (*Cucumis melo* L.) case study. *Plant and Soil*, *335*, 117–131. https://doi.org/10.1007/s11104-009-0227-3

Li, L., & Yuan, H. (2013). Chromoplast biogenesis and carotenoid accumulation. *Archives of Biochemistry and Biophysics*, *539*(2), 102–109. https://doi.org/10.1016/j.abb.2013.07.002

Malta, A. O., Araújo, R. C., Medeiros, J. G. F., Costa, N. P., Azerêdo, L. P. M., & Dias, J. A. (2013). Características químicas dos frutos da goiabeira 'Paluma' em função da adubação orgânica e mineral. *Revista Educação Agrícola Superior*, *28*(2), 120–125. https://doi.org/10.12722/0101-756X.v28n02a07

Max, B., Salgado, J. M., Rodríguez, N., Cortés, S., Converti, A., & Domínguez, J. M. (2010). Biotechnological production of citric acid. *Brazilian Journal of Microbiology*, *41*, 862–875. https://doi.org/10.1590/S1517-83822010000400005

Medeiros, W. J. F., Oliveira, F. I. F., Cavalcante, L. F., Costa, L. C., Rocha, R. H. C., & Silva, A. R. (2014). Qualidade química em frutos de maracujazeiro amarelo cultivado em solo com biofertilizantes bovino. *Magistra*, *26*(2), 156–168.

Mohammadrezakhani, S., Pakkish, Z., & Rafeii, S. (2016). Role of brassinosteroid on qualitative characteristics improvement of strawberry fruit cv. Paros. *Journal of Horticultural Science*, *30*(2), 316–326. https://doi.org/10.22067/jhorts4.v30i2.42767

Robinson, R. W., & Decker-Walters, D. S. (1997). Evolution and exploitation. In R. W. Robinson, & D. S. Decker-Walters (Eds.), *Cucurbits*. CAB International.

Römheld, V., & Kirkby, E. A. (2010). Research on potassium in agriculture: needs and prospects. *Plant and Soil*, *335*, 155–180. https://doi.org/10.1007/s11104-010-0520-1

Salviano, A. M., Faria, C. M. B., Terao, D., Silva, D. J., Batista, D. C., Moreira, F. R. B., Resende, G. M., Yuri, J. E., Alencar, J. A., Oliveira, J. E. M., Araújo, J. L. P., Pinto, J. M., Grangeiro, L. C., Kiill, L. H. P., Lima, M. A. C., Silva, M. S. L., Lima, M. F., Costa, N. D., Ribeiro Júnior, P. M., Dias, R. C. S., Tavares, S. C. C. H., Costa-Lima, T. C., & Cunha, T. J. F. (2017). *Coleção plantar: melão* (3rd ed.). Embrapa.

Schönherr, J., & Luber, M. (2001). Cuticular penetration of potassium salts: effects of humidity, anions, and temperature. *Plant and Soil*, *236*, 117–122. https://doi.org/10.1023/A:1011976727078

Sousa, D. M. G., & Lobato, E. (Eds.). (2004). *Cerrado: correção do solo e adubação* (2nd ed.). Embrapa Informação Tecnológica.

Trevisan, R., Herter, F. G., Coutinho, E. F., Gonçalves, E. D., Silveira, C. A. P., & Freire, C. J. S. (2006). Uso de poda verde, plásticos refletivos, antitranspirante e potássio na produção de pêssegos. *Pesquisa Agropecuária Brasileira*, *41*(10), 1485–1490. https://doi.org/10.1590/S0100-204X2006001000005

Van Raij, B. (2011). *Fertilidade do solo e manejo de nutrientes*. International Plant Nutrition Institute.

Van Raij, B., Cantarella, H., Quaggio, J. A., & Furlani, A. M. C. (1997). *Recomendações de adubação e calagem para o Estado de São Paulo* (2nd ed.). Instituto Agronômico.

Van Raij, B., & Quaggio, J. A. (1983). *Métodos de análise de solo para fins de fertilidade (Boletim Técnico, 81)*. Instituto Agronômico de Campinas.

Wade, J., Holzapfel, B., Degaris, K., Williams, D., & Keller, M. (2004). Nitrogen and water management strategies for winegrape quality. *Acta Horticulturae*, *640*, 61–67. https://doi.org/10.17660/ActaHortic.2004.640.6

Yamada, T., & Roberts, T. L. (Eds.). (2005). *Potássio na agricultura brasileira*. KP Potafos.

Zörb, C., Senbayram, M., & Peiter, E. (2014). Potassium in agriculture - status and perspectives. *Journal of Plant Physiology*, *171*(9), 656–669. https://doi.org/10.1016/j.jplph.2013.08.008

Improvement of growth of common bean in phosphorus-deficient soils by phosphate-solubilizing and phytohormone-producing bacteria

Mejoramiento del crecimiento del frijol común en suelos deficientes en fósforo por bacterias solubilizadoras de fosfato y productoras de fitohormonas

Rosana Faria Vieira[1*], Vera Lúcia Ferracini[1], Adriana Parada Dias da Silveira[2], and Ricardo Antônio Almeida Pazianotto[1]

ABSTRACT

The use of phosphate fertilizers is generally required for normal growth and development of crops. The cost of manufactured phosphate fertilizers has increased drastically in recent years and these are becoming out of reach for low-income farmers. The objective of this research was to select phosphate-solubilizing and phytohormone-producing bacteria to improve the growth and P uptake by the common bean (IAC Alvorada) in Red-Yellow Oxisol without the addition of phosphate fertilizer. The experimental design was completely randomized with three replicates and the following treatments: non-inoculated bean plants and plants inoculated separately with seven bacterial isolates: K24, K36, K71, T30, T79, A24, and T22. The previously treated bean plants were grown in the presence and absence of phosphate fertilization. Only the plants inoculated with the isolates K36 and T79 showed higher values for the dry weight and the quantities of P and N uptake by the shoot when cultivated in the absence of phosphate fertilizer. These results demonstrate the potential use of the bacteria T79 and K36 as inoculants for bean plants grown in non-sterilized soil with low available P content, but also indicate that P solubilization may not be the only mechanism responsible for the positive growth response.

Key words: phosphate fertilizer, plant growth-promoting bacteria, legume.

RESUMEN

El uso de fertilizantes fosfatados se requiere generalmente para el crecimiento y desarrollo normal de los cultivos. El costo de estos fertilizantes ha incrementado drásticamente en los últimos años, y están fuera del alcance de los agricultores de bajos ingresos. El objetivo de esta investigación fue seleccionar bacterias solubilizadoras de fosfato y productoras de fitohormonas para mejorar el crecimiento y la absorción de P del frijol común (IAC Alvorada) en un Oxisol Rojo-Amarillo sin la adición de fertilizante fosfatado. El diseño experimental fue completamente al azar con tres repeticiones y los siguientes tratamientos: plantas de frijoles no inoculadas y plantas de frijoles inoculadas por separado con siete aislamientos bacterianos: K24, K36, K71, T30, T79, A24 y T22. Las plantas de frijoles previamente tratadas se cultivaron en presencia y ausencia de fertilización con fosfato. Solo las plantas inoculadas con los aislamientos K36 y T79 presentaron valores más altos para el peso seco y para las cantidades de captación de P y N por la parte aérea cuando se cultivaron en ausencia de fertilizante fosfatado. Estos resultados demuestran el uso potencial de los aislamientos bacterianos T79 y K36 como inoculantes para frijol cultivado en suelos no esterilizados con un bajo contenido de P disponible, pero también indican que la solubilización de P posiblemente no fue el único mecanismo responsable de la respuesta positiva del crecimiento.

Palabras clave: fertilizante de fosfato, bacterias promotoras del crecimiento de plantas, leguminosas.

Introduction

The common bean (*Phaseolus vulgaris* L.) is an essential protein source, principally for low-income farmers. The evolution of crop practices has allowed for an expressive gain in the productivity of common bean. However, there is still much subsistence farming characterized by low technology inputs, such as the application of fertilizers (Souza *et al*., 2013). Since the common bean is a plant with high nutritional demands, principally due to its superficial and little developed root system and short life cycle of about 90 d, the absence of fertilizer applications can have a significantly negative impact on its productivity (Oliveira *et al*., 2018).

Phosphorus plays an important role in all major metabolic processes in plants (Kouas *et al*., 2005; Khan *et al*., 2010). Although there is a substantial reserve of phosphorus in soils, in both inorganic and organic forms, its availability for plants is generally low, leading to a great demand for the

Received for publication: 3 May, 2021. Accepted for publication: 2 November, 2021. Doi: 10.15446/agron.colomb.v39n3.95461

[1] Environmental Embrapa, Jaguariúna, São Paulo (Brazil).
[2] Campinas Agronomic Institute, Campinas, São Paulo (Brazil).
[*] Corresponding author: rosana.vieira@embrapa.br

application of P-fertilizers. However, plants can only use a small amount of this element since 75-90% of the added P becomes immobile and unavailable for plant absorption due its fixation and precipitation in the soil (Sharma *et al.*, 2013). It should also be considered that the P reserves are finite non-renewable sources and may run out in 50-100 years due to anthropogenic exploitation (Cordell *et al.*, 2009). Thus, the use of this element is not fully in agreement with the principles of sustainable agriculture and, hence, environmentally friendly strategies for P utilization are required. The use of soil microorganisms with the ability to solubilize and/or mineralize soil P has been suggested as a promising tool to provide the plants with this element (Malboobi *et al.*, 2014). Phosphate-solubilizing bacteria (PSB) metabolize phosphate by producing enzymes or low molecular weight organic acids, which increases the availability of soluble phosphate in the soil (Sadiq *et al.*, 2013; Widdig *et al.*, 2019).

Different bacteria can also promote the growth of plants by producing phytohormones, which are important growth regulators. For example, indoleacetic acid (IAA) is an auxin produced by several bacteria and, in the plant, regulates a large number of root biological processes (Talboys *et al.*, 2014). Gibberellins have a vital role in seed germination, formation of floral organs, and lateral shoot growth (Olszewski *et al.*, 2002), while cytokinins regulate cell division and differentiation processes in the meristematic tissues of higher plants (Cassán *et al.*, 2014). The beneficial effect on plant growth of bacteria that produce IAA, gibberellins and cytokinins has been shown in various plant species (Liu *et al.*, 2013; Pereira & Castro, 2014).

The objective of this research was to isolate bacteria from the common bean-cultivating areas in the town of Campinas and characterize them according to their capacity to produce phytohormones and solubilize phosphate. We raised the hypothesis that bacteria with these characteristics could promote the growth of the common beans in soil with a low available P content.

Materials and methods

Isolation of bacteria

Eight bean (*Phaseolus vulgaris* L.) plants were uprooted along with a substantial amount of soil in a locality of the Campinas Agronomic Institute farm in the town of Campinas (22°54’20” S; 47°03’38” W), São Paulo State, Brazil. According to the Köppen classification, the climate of the region is Cfa, the mean annual temperature is 21.3°C, and the mean annual rainfall is 1462 mm.

In the field, the shoots were cut off, and the roots were placed in a container with ice and sent to the laboratory, where they were stored at 5°C for 24 h before being processed. The roots were first carefully washed with sterile distilled water to remove excess soil and then placed individually in 1 L beakers containing 500 ml of a sterile 10 mM $MgSO_4$ solution and shaken for 60 min. An aliquot of 10 ml was removed from each beaker and added to 90 ml of sterile distilled water, from which serial dilutions up to 10^{-8} were prepared. From each dilution, 100 µl were streaked over plates containing tryptic soy agar (TSA), a general-purpose medium (Atlas, 2004). Three plates were streaked for each dilution from each plant and incubated at 26°C. Microbial growth was monitored daily for about 20 d and isolated cultures were purified by streaking onto fresh agar. A total of 360 bacteria were isolated.

Selection of phosphate solubilizing bacteria

The capacity of the bacteria to solubilize phosphate was initially evaluated qualitatively by the standard dilution plating technique using the standard National Botanical Research Institute Phosphate (NBRIP) growth agar medium containing insoluble tricalcium phosphate as the sole source of insoluble P for strain growth (Nautiyal, 1999). The presence of a clear halo around the bacterial colony after 15 d of incubation, independent of its size, was used as a potential indicator of P-solubilization capacity. The bacterium was sent for a subsequent test in a liquid medium containing insoluble phosphate. The quantitative estimation of phosphate solubilization by bacteria showing halos was carried out in NBRIP medium. Portions of 25 ml of the medium contained in 125 ml Erlenmeyer flasks with a source of insoluble $Ca_3(PO_4)_2$ were inoculated with 0.5 ml of the bacterial suspension adjusted to 10^8 cells ml^{-1} (optical density (OD) = 600 nm), in triplicate. The flasks were incubated at 26°C for 15 d with shaking (160 rpm) and then centrifuged at 10,000 rpm for 10 min. The soluble P concentration was determined in the supernatant using the colorimetric method (Murphy & Riley, 1962) and an autoclaved non-inoculated medium served as control.

Selection of IAA, gibberellic acid and cytokinin (zeatin) producing bacteria

To determine the phytohormone-producing capacity, the isolates were first cultivated for 24 h in 125 ml Erlenmeyer flasks containing 25 ml tryptic soy broth, and then 100 µl of each culture containing 10^8 cells ml^{-1} (OD = 600) were inoculated into 100 ml of the same culture medium contained in 250 ml Erlenmeyer flasks. The flasks were incubated at 26°C for 10 d, with shaking at 160 rpm. After this growth period, the culture media were centrifuged at 10,000 rpm for 10 min and the extract was stored at 5°C until analyzed. Phytohormones were extracted with ethyl acetate and subjected to partitioning by adding salts. After another centrifugation at 10,000 rpm for 10 min, 1 ml of the supernatant was evaporated to dryness and the residue was re-suspended in the mobile phase (75% of 0.1% formic acid and 25% of methanol). The sample was injected into an ultra-performance liquid chromatograph (UPLC) with a Waters column (Acquity UPLCâ BEH, C18; 17 µm; 2.1 x 50 mm) coupled to an electrospray ionization mass spectrometer and triple quadrupole analyzer (UPLC-ESI-MS/MS, Quattro Premier XE, Waters, Milford, MA, USA). The total run time was 3 min. The instrument parameters were as follows: capillary (kV) 3.00; cone (V) 25.00; extractor (V) 4.00; source temperature of 120°C and desolvation temperature of 300°C. The multiple reaction monitoring (MRM) scanning mode was selected with the monitoring of two transitions per analyte. The limits of detection (LD) of the instrument were 0.01 µg ml^{-1} for cytokinins (zeatin) and IAA and 0.025 µg ml^{-1} for GA_3 gibberellic acid. The limits of quantification (LQ) of the method were 0.05 µg ml^{-1} for zeatin, IAA, and gibberellic acid, and the analytical curve was prepared in the range from 0.05 to 1.00 µg ml^{-1}. All the analyses were carried out in triplicate and the results for the phytohormones were given per ml of culture medium (Assalin *et al*., 2011).

Selection of bacteria for common bean growth promotion test in a greenhouse

Bacteria showing P solubilization values greater than 100 µg ml^{-1} were selected for the greenhouse experiment. Among these bacteria, those producing more IAA were chosen, *i.e.,* K36, K71, T22 and A24. Bacteria producing phosphate solubilization values greater than 100 µg ml^{-1} but not producing IAA were also selected (T79 and T30). The isolate K24 was chosen for comparison since it showed low phosphate solubilizing capacity and low IAA producing capacity. The production of other phytohormones was not considered since isolates capable of producing gibberellic acid and zeatin were not found.

Greenhouse experiment

The soil used in the greenhouse experiment (Red-Yellow Oxisol) was collected in the experimental area of Environmental Embrapa in the town of Jaguariúna, São Paulo State, Brazil (22°42'20" S; 46°59'09" W). According to the Köppen classification, the climate of the region is Cfa, the mean annual temperature is 21.3°C, and the mean annual rainfall is 1462 mm.

After collecting the soil at a depth of 0-20 cm, it was air dried, finely crushed, homogenized, and passed through a 2-mm sieve. The soil chemical characteristics were evaluated according to the methodology of Camargo *et al.* (2009) (Tab. 1). The texture analysis obtained the following results: 41.14% clay, 12.25% silt, 30.26% coarse sand, and 16.23% fine sand.

TABLE 1. Soil chemical characteristics.

Parameter	Unit	Value
pH $_{(CaCl_2)}$		4.2
OM	g kg^{-1}	28
K	mmol$_c$ dm^{-3}	21
Ca		0.5
Mg		7.0
SB		5.0
H+Al		12.5
CEC		80
V	%	14
P	mg dm^{-3}	21
S		12
B		0.23
Cu		1.2
Fe		50
Mn		3.3
Zn		0.2

OM - organic matter; SB - sum of bases; CEC - cation exchange capacity; V - base saturation.

The experimental design was completely randomized with three replicates and the following treatments: non-inoculated bean plants (C) and bean plants inoculated separately with the bacteria K24, K36, K71, T30, T79, A24 or T22. In all treatments, the plants were cultivated with two P doses: the recommended rate (P1) and zero (P0). The non-inoculated control treatment was denominated CP when the plants were cultivated with added P, and C0 when they were not cultivated with added P. Dolomitic limestone was applied 25 d before planting at a rate of 5.2 t ha^{-1}. For treatment P1, 30 kg P_2O_5 ha^{-1}, 50 kg K_2O ha^{-1}, 20 kg S ha^{-1} and 3 kg Zn ha^{-1} were applied as simple superphosphate, potassium chloride, ferrous sulfate, and zinc sulfate, respectively. The equivalent of 70 kg N ha^{-1} was also added as follows: 10 kg N ha^{-1} at sowing and two applications of 30 kg N ha^{-1} on the surface 15 and 30 d after seedling emergence (DAE), in the form of urea solution. For the P0 treatment, the same applications were performed except for the phosphate application. The experiment was carried out in pots each with a capacity for 2 kg of non-sterilized soil. Four bean seeds of the variety IAC Alvorada, widely grown in Brazil, were planted per pot, but only two seedlings were left after emergence. On the day of seeding, 1 ml of each isolate, re-suspended in 10 mM $MgSO_4$ at a concentration of 10^8 cells ml^{-1} (OD = 600), was applied to the seeds at a depth of about 1 cm. For the non-inoculated pots, the seeds only received 1 ml of 10 mM $MgSO_4$. The crop was irrigated with water to maintain 70% field capacity. The plants were harvested 48 d after emergence and taken to the laboratory to measure the plant growth parameters, *i.e.,* the dry weight of the shoot (DWS) and roots (DWR) and the quantities of P and N uptake by the shoot (QPUS, QNUS) (Bowman, 1989; Tedesco *et al*., 1995).

Data analysis

An entirely random design was used to evaluate the data on the growth promoting characteristics of the bacteria, and an analysis of variance was used to determine the probability according to the F-test. For the greenhouse experiment, the data were subjected to an analysis of variance and to the unfolding of the interaction between the factors when significant. All means were compared using the LSD test at 5% of probability. When necessary, the variables were transformed to guarantee the requisites of normality and homoscedasticity using the software R version 4.0.1. (R Core Team, 2020). Since QPUS did not guarantee these requirements, the means for this parameter were calculated after transformation of this variable using Equation 1:

$$1/\sqrt{QPUS} \quad (1)$$

Thus, lower values represented the greater P uptake by the plant.

Results and discussion

Phosphate solubilization and IAA production

To overcome dependency on P fertilizers and reduce the production costs of bean plants, non-identified bacterial isolates with different capacities to solubilize phosphate and produce IAA were tested for their capacities to promote the growth of bean plants in soil with low available P content. The results for phosphate solubilization and IAA production were only shown for those bacteria selected for the subsequent experiment in the greenhouse. The K36, T22 and T30 isolates were those exhibiting the greatest phosphate solubilizing capacity in a liquid medium, followed by the isolates T79, K71 and A24 (Tab. 2). The isolate K24 showed a phosphate solubilizing potential 263% lower than the mean obtained for the isolates K36, T22 and T30 (206.34 µg ml^{-1}) and 167% lower than the mean obtained for the isolates K71, T79 and A24 (151.44 µg ml^{-1}). The phosphate solubilizing levels found for the isolates K36, T22 and T30 were much lower than those found by Baig *et al*. (2012), Lee *et al*. (2012), and Li *et al*. (2017), but similar or higher than those found by Lavakush *et al*. (2014). None of the bacteria produced gibberellic acid or cytokinins (zeatin) under controlled conditions but did produce IAA. Masniawaty *et al*. (2019) found that bacteria isolated from the rhizosphere of chili peppers can produce gibberellic acid GA_3. The isolates K36 and A24 produced the maximum amounts of IAA followed by T22 and K71 (Tab. 3). The mean value found for the isolates K36 and A24 was 38.97 µg ml^{-1}, whereas the mean value obtained for the isolates T22 and K71 was 22.29 µg ml^{-1}. The lowest results were obtained for K24, T30 and T79 with a mean value of 1.55 µg ml^{-1}. The production of IAA by the isolates K36 and A24 was higher than the values found by Li *et al*. (2017) but lower than those found by Lee *et al*. (2012). The results for the production of this auxin were not related to the greater capacities of the microorganisms to solubilize phosphate in a liquid medium, except for the isolate K36, and, to a lesser extent, the isolate T22.

TABLE 2. Phosphate solubilization (PS) by the bacterial isolates.

Bacterial isolates	PS (µg ml^{-1})
K24	56.88 ± 1.02 e
T30	217.41 ± 0.34 a
K71	148.49 ± 6.05 d
T79	169.50 ± 9.99 c
A24	136.89 ± 0.75 d
T22	212.78 ± 0.43 a
K36	188.83 ± 0.16 b

Different letters in the column differ significantly from each other according to LSD multiple range test at a significance level of 5% ($P \leq 0.05$); ± standard error.

TABLE 3. Indoleacetic acid (IAA) production by the bacterial isolates.

Bacterial isolates	IAA production (µg ml^{-1})
K24	4.00 ± 0.15 d
T30	0.50 ± 0.08 d
K71	15.72 ± 3.26 c
T79	0.15 ± 0.03 d
A24	37.21 ± 0.27 a
T22	28.85 ± 5.08 b
K36	40.73 ± 1.87 a

Different letters in the column differ significantly from each other according to LSD multiple range test at a significance level of 5% ($P \leq 0.05$); ± standard error.

Greenhouse experiment

Table 4 shows the values for *P* for the different sources of variation. Only the variable DWR was not affected by the inoculation of plants with different bacteria (*P* = 0.48613) or by the interaction between the factors "bacteria" and "addition of phosphate" (*P* = 0.16168). However, the presence or absence of phosphate fertilization significantly affected DWR (*P* = 0.00008), with the highest value being obtained in the treatment P1 (Tab. 4).

TABLE 4. *P* values obtained in the analysis of variance.

Variables	Sources of variation		
	PF	Bacteria	Bacteria x PF
	P values		
DWR	0.00008	0.48613	0.16168
DWS	<2e-16	0.000284	0.000612
QPUS	<2e-16	1e-10	1.0725E-05
QNUS	0.000048	0.040699	0.000002

PF - Phosphate fertilizer; DWR - dry weight of the roots; DWS - dry weight of the shoot; QPUS - quantities of P uptake by the shoot; QNUS - quantities of N uptake by the shoot. e, base 10. The means for QPUS were calculated after the transformation of this variable using the equation 1 / √QPUS.

The absence of a response for DWR to the inoculation of plants with different bacterial isolates (Fig. 1) could be due to the difficulty in removing the roots from the soil, which resulted in a high value for the coefficient of variation (22.93%). Despite this, the results were greater in the P1 treatment.

The DWS showed significant effects for all the factors analyzed (Fig. 2). For the treatment P0, the highest values were obtained for plants inoculated with the isolates K24 (3.95 g per pot), T79 (4.16 g per pot), T22 (4.09 g per pot) and K36 (4.03 g per pot) (Fig. 2). On average, these results were 55% higher than those of the control (C0, 2.62 g per pot).

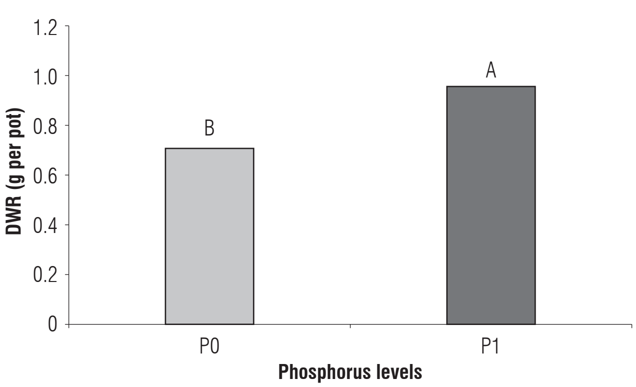


FIGURE 1. Effect of the addition of P-fertilizer on the dry weight of the roots (DWR) of common bean plants. P0: soil without added phosphate fertilizer; P1: soil with added phosphate fertilizer (30 kg P_2O_5 ha^{-1}). Different letters indicate significant differences by the LSD test at a significance level of 5% ($P \leq 0.05$).

Plants inoculated with the isolates T30 and A24 showed mean values for DWS 24.6% higher than those obtained for the treatment without the addition of phosphate fertilizer. There was no significant difference for this parameter between the plants of the control treatment (C0) and those inoculated with the isolate K71. In the treatment P1, all the values for DWS were higher than those of the treatment P0, although there were differences between the bacterial isolates for this level of P as can be seen from the higher values obtained for the control treatment (CP, 5.58 g per pot) and for the plants inoculated with the isolates A24 (6.13 g per pot) and T22 (5.54 g per pot). For the treatment P1, there were no significant differences compared to DWS for the plants inoculated with the isolates K24, K71, T79 and K36. The mean values of DWS for these bacteria were 8% lower than the means obtained for the control (CP) and for the isolates A24 and T22. The lowest values for DWS in the treatment P1 were obtained for the plants inoculated with the isolate T30 (4.67 g per pot).

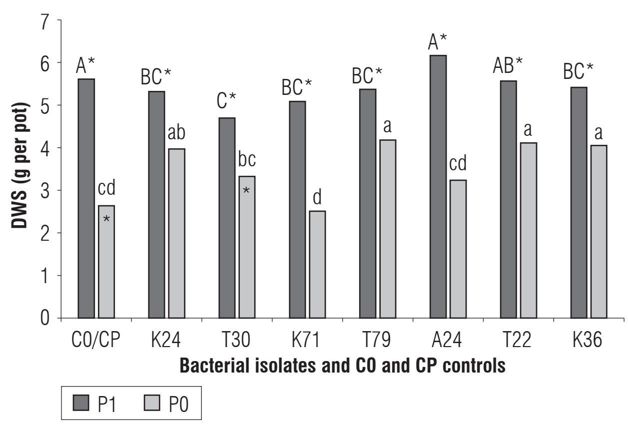


FIGURE 2. Dry weight of the shoot (DWS) of common bean plants. C0: control treatment for plants without added phosphate fertilizer; CP: control treatment for plants with added phosphate fertilizer. P0: soil without added phosphate fertilizer; P1: soil with added phosphate fertilizer (30 kg P_2O_5 ha^{-1}). Different uppercase letters in the treatment P1 or different lowercase letters in the treatment P0 differ significantly by the LSD test at a significance level of 5% ($P \leq 0.05$). *Significant differences between the treatments P0 and P1 within each inoculation treatment.

Due to the transformation of the data for QPUS using Equation 1, the lower values found for this parameter should be interpreted as a greater absorption of this element by the plants. In the soil without the addition of phosphate fertilizer, the lowest values found for QPUS were obtained in the plants inoculated with the isolates T79 and K36 with mean values of 0.265 mg per pot. The control treatment (C0) and the isolates T30 and T22 showed mean values of 0.31 mg per pot, the isolate K71 showed a value of 0.34 mg per pot, and the isolates K24 and A24 showed mean values of 0.365 mg per pot (Tab. 5). The mean increase in QPUS in the plants inoculated with the bacteria T79 and K36 compared to the control (C0) was 15.5%. These isolates can be considered as "real" PSB since the P plant nutrition increased directly (Bashan *et al*., 2013). Although the isolates T22 and T30 were the isolates that most solubilized the phosphate in the liquid culture medium, the amounts of P in the shoots did not increase, showing that the mode of action of the bacteria selected for their PS capacity is not fully understood. This agrees with other studies reporting that, although the ability to solubilize P frequently appears associated with plant growth-promoting rhizobacteria, it is not necessarily related to the ability to promote plant growth (De Freitas *et al*., 1997; Collavino *et al*., 2010). Li *et al*. (2017) also found that a bacterial strain did not induce the promotion of plant growth or an increase in total P despite its capacities for P solubilization and IAA production.

As expected for the soil fertilized with P, the values for QPUS were lower than those obtained in the treatment P0 for all the treatments with inoculation, although there were differences between the bacterial isolates. The lowest value obtained for QPUS in the treatment P1 was registered for the isolate K36 with a value of 0.22 mg per pot (Tab. 5). This shows that this bacterium exhibited the ability to solubilize phosphate even in the presence of phosphate fertilization. Other authors obtained bacteria with capacity to solubilize phosphate under high insoluble P conditions (De Bolle *et al*., 2013; Barra *et al*., 2019). According to Barra *et al*. (2019), this fact indicates that phosphorus solubilizing bacteria display an "enhancement effect" of the fertilization with triple superphosphate.

The values obtained for QNUS in the treatment P0 were higher in the plants inoculated with the isolates K24 (105.00 mg per pot), T79 (89.71 mg per pot), T22 (93.11 mg per pot), and K36 (92.52 mg per pot) (Tab. 5). These values were 45%, 24%, 29%, and 28% higher than those obtained in the C0 treatment. An increase in plant N uptake due to inoculation of the plant with phosphate solubilizing bacteria was also observed by Li *et al*. (2017). The results obtained for QNUS for the other isolates (T30, K71, and A24) were not significantly different from the value obtained for C0 (72.32 mg per pot). Although all QPUS values were lower in the treatment P1, this did not occur with the values obtained for QNUS as can be seen from the plant inoculated with the isolate K36, where the result for QNUS was 42% lower in the treatment P1 than that obtained in the treatment P0 (Tab. 5). In the presence of phosphorus, the highest values for QNUS were obtained for the plants inoculated with the isolates T30 (117.43 mg per pot) and A24 (102.74 mg per pot). The results for inoculation with the other isolates were significantly equal to those of the control (CP, 99.39 mg per pot). Also, regarding QNUS, there was no significant difference between the isolates K24, T79 and T22 when considering the two levels of phosphorus. The high N contents found in the plants inoculated with some of the bacteria could have been due to the ability of these microorganisms to fix atmospheric N_2 or to their capacities to influence the biological fixation of N_2 by the native rhizobia in a beneficial way. This last parameter was not evaluated, but all the plants showed nodules, although with reduced intensity in the control treatments (results not shown). Peix *et al*. (2001) reported that the bacterium *Burkholderia cepacia* (SAOCV2), a phosphate solubilizer, increased the growth of the bean plants and indirectly promoted nodulation, which may have led to an increase in N_2 fixation.

TABLE 5. Quantities of phosphorus and nitrogen uptake by the shoot (QPUS, QNUS) of common bean plants inoculated with different bacterial isolates and cultivated in soil without added phosphate fertilizer (P0) and with soil fertilized with phosphate fertilizer (P1).

Inoculation treatment	QPUS (mg per pot)	
	P0	P1
C0 or CP	0.32 ± 0.01 cA	0.24 ± 0.01 bcB
K24	0.37 ± 0.02 aA	0.26 ± 0.03 aB
T30	0.31 ± 0.02 cA	0.24 ± 0.02 bcB
K71	0.34 ± 0.008 bA	0.26 ± 0.01 aB
T79	0.27 ± 0.03 dA	0.23 ± 0.02 bcB
A24	0.36 ± 0.005 aA	0.24 ± 0.01 bcB
T22	0.31 ± 0.01 cA	0.24 ± 0.008 bcB
K36	0.26 ± 0.02 dA	0.22 ± 0.02 cB
Inoculation treatment	**QNUS (mg per pot)**	
	P0	**P1**
C0 or CP	72.32 ± 1.25 cdB	99.39 ± 5.30 bA
K24	105.00 ± 2.66 aA	97.38 ± 6.20 bA
T30	58.21 ± 1.41 dB	117.43 ± 4.01 aA
K71	70.13 ± 2.06 cdB	96.60 ± 6.56 bA
T79	89.71 ± 5.87 abA	86.33 ± 1.59 bcA
A24	76.25 ± 0.36 bcB	102.74 ± 3.85 aA
T22	93.11 ± 2.87 aA	98.62 ± 2.52 bA
K36	92.52 ± 6.21 abA	70.11 ± 5.96 cB

C0: control treatment for plants without added phosphate fertilizer; CP: control treatment for plants with added phosphate fertilizer. P0: soil without added phosphate fertilizer; P1: soil with added phosphate fertilizer (30 kg P_2O_5 ha^{-1}). The means for QPUS were calculated after transformation of this variable using the equation1 / √QPUS. Values with different lowercase letters in the column and uppercase letters in the row differ significantly from each other according to LSD multiple range test at a significance level of 5% ($P \leq 0.05$); ± standard error.

The inoculation of bean plants with the P-solubilizing *Bacillus megaterium* or the N_2-fixing *Bacillus subtilis* increased nodulation of the bean plants by the native soil *Rhizobium* population (Elkoca *et al*., 2010). However, as emphasized by some authors, an increase in the number of nodules does not always result in an increase in the N content or the dry matter of the plants, which do not depend on the number of nodules but on the efficiency of the strains (Peix *et al*., 2001). However, other hypotheses should also be considered since the plants received nitrogen fertilizers, which are known to interfere with the symbiotic fixation of N_2 by the rhizobia (Gentili *et al*., 2006). Oliveira-Longatti *et al*. (2015) found that the inoculation of common bean with *Burkholderia fungorum* UFLA 04-155 and *Azospirillum brasilense* BR 11001^T promoted nodulation by the indigenous rhizobia community in common bean plants when inoculated alone and without adding mineral nitrogen to the soil. Some authors reported that colonization by the fungus *Phomopsis liquidambari* could induce several genes related to N absorption and N metabolism to enhance N utilization efficiency in rice (Yang *et al*., 2014). Future studies are required to evaluate which processes are involved in both the growth promoting bacteria of inoculated bean plants and the increases in N uptake in soil with both low and high P content. This information could allow for a more sustainable use of nitrogen fertilizers since their production and use pose a significant environmental threat (Zhang *et al*., 2015).

In the treatment P0, only the plants inoculated with the isolates T79 and K36 obtained higher results for the traits to evaluate bean plant growth, as compared to the control (C0). Although the bacterium K36 was one of the isolates that most produced IAA and solubilized phosphate, this was not the case for the isolate T79. This last isolate was one of the bacteria that produced more solubilized phosphate in a liquid medium but that showed an almost non-existent capacity to produce IAA. Thus, factors other than P solubilization activity might have been involved. In the case of the bacterium K36, there may have been a synergistic effect between the plant growth promotion characteristics, *i.e.,* the production of IAA and phosphate solubilization, generating greater growth and nutrient uptake in bean plants. Some authors reported that *Bacillus* strains with two growth promoting characteristics, *i.e*., P solubilization and ACC deaminase activity, were superior in improving wheat plant growth as compared to the strains possessing only a single trait (Baig *et al*., 2012). Bacterial isolates having beneficial traits for plants like phosphate solubilization and IAA production were capable of improving the growth of wheat when used as inoculants and, therefore, qualified for the production of wheat crop biofertilizers (Tahir *et al*., 2013). Since the isolate K36 increased QPUS both in the presence and absence of phosphate fertilizer, it should be tested with increasing doses of the fertilizer. A low dose of the fertilizer associated with the inoculation of that isolate could increase the productivity of bean plants since the cost of this input is compensated in terms of yield for the small farmer. However, even using a low dose of the phosphate fertilizer, the N uptake by the plant would be affected, as observed in this study with the isolate K36. In this case, it would be pertinent to use this bacterium associated with another bacterium with the capacity to increase QNUS, such as T79 or T22, since both bacteria increased this parameter regardless of the amount of P added to the soil. Lavakush *et al*. (2014) reported that the combination of *Pseudomonas* culture (CPC) with *Azotobacter chroococcum*, *Azospirillum brasilense* and 30 kg P_2O_5 ha^{-1} would save 50% chemical fertilizer as compared to the treatment using a CPC with *A. chroococcum*, *A. brasilense* and 60 kg P_2O_5 ha^{-1}.

Conclusions

The results of this study demonstrated the feasibility of using some phosphate-solubilizing and phytohormone-producing bacteria to increase common bean growth in soil with low available P content. The bacterial isolates T79 and K36 showed great potential for this purpose. Field tests using the isolates T79 and K36 should further elucidate their effectiveness on the grain yields of common bean plants. These bacteria are being classified for further experiments in the field.

Acknowledgments

This study was funded by Brazilian Agricultural Research Company (EMBRAPA).

Conflict of interest statement

The authors declare that there is no conflict of interest regarding the publication of this article.

Author's contributions

RFV and APDS formulated the research goals and aims and managed the project. RFV, APDS and VLF developed and designed the methodology. RFV and VLF conducted the experiment. RAAP carried out the statistical analysis. RFV, APDS and VLF wrote the original draft. RFV, APDS, VLF and RAAP reviewed the draft and translated it into English.

Literature cited

Assalin, M. R., Piovesana, A. M., Rosa, M. A., Ferracini, V. L., & Vieira, R. F. (2011, December, 10–13). *Identificação e quantificação de fitohormônios produzidos por bactérias por UPLC-ESI-MSMS* [Conference presentation]. Congresso Brasileiro de Espectrometria de Massas, Campinas, Brazil.

Atlas, R. M. (2004). *Handbook of microbiological media* (3rd ed.). CRC Press.

Baig, K. S., Arshad, M., Shaharoona, B., Khalid, A., & Ahmed, I. (2012). Comparative effectiveness of *Bacillus* spp. possessing either dual or single growth-promoting traits for improving phosphorus uptake, growth and yield of wheat (*Triticum aestivum* L.). *Annals of Microbiology*, *62*, 1109–1119. https://doi.org/10.1007/s13213-011-0352-0

Barra, P. J., Pontigo, S., Delgado, M., Parra-Almuna, L., Duran, P., Valentine, A. J., Jorquera, M. A., & Mora, M. L. (2019). Phosphobacteria inoculation enhances the benefit of P-fertilization on *Lolium perenne* in soils contrasting in P-availability. *Soil Biology and Biochemistry*, *136*, Article 107516. https://doi.org/10.1016/j.soilbio.2019.06.012

Bashan, Y., Kamnev, A. A., & Bashan, L. E. (2013). A proposal for isolating and testing phosphate-solubilizing bacteria that enhance plant growth. *Biology and Fertility of Soils*, *49*, 1–2. https://doi.org/10.1007/s00374-012-0756-4

Bowman, R. A. (1989). A rapid plant digestion method for analysis of P and certain cations by inductively coupled plasma emission spectrometry. *Communications in Soil Science and Plant Analysis*, *20*(5–6), 539–553. https://doi.org/10.1080/00103628909368099

Camargo, O. A., Moniz, A. C., Jorge, J. A., & Valadares, J. M. A. S. (2009). *Métodos de análise química, mineralógica e física de solos do Instituto Agronômico de Campinas*. *Boletim Técnico 106*. Instituto Agronômico de Campinas.

Cassán, F., Vanderleyden, J., & Spaepen, S. (2014). Physiological and agronomical aspects of phytohormone production by model plant-growth-promoting rhizobacteria (PGPR) belonging to the genus *Azospirillum*. *Journal of Plant Growth Regulation*, *33*, 440–459. https://doi.org/10.1007/s00344-013-9362-4

Collavino, M. M., Sansberro, P. A., Mroginski, L. A., & Aguilar, O. M. (2010). Comparison of *in vitro* solubilization activity of diverse phosphate-solubilizing bacteria native to acid soil and their ability to promote *Phaseolus vulgaris* growth. *Biology and Fertility of Soils*, *46*, 727–738. https://doi.org/10.1007/s00374-010-0480-x

Cordell, D., Drangert, J. O., & White, S. (2009). The story of phosphorus: global food security and food for thought. *Global Environmental Change, 19*(2), 292–305. https://doi.org/10.1016/j.gloenvcha.2008.10.009

De Bolle, S., Gebremikael, M. T., Maervoet, V., & De Neve, S. (2013). Performance of phosphate-solubilizing bacteria in soil under high phosphorus conditions. *Biology and Fertility of Soils*, *49*, 705–714. https://doi.org/10.1007/s00374-012-0759-1

De Freitas, J. R., Banerjee, M. R., & Germida, J. J. (1997). Phosphate-solubilizing rhizobacteria enhance the growth and yield but not phosphorous uptake of canola (*Brassica napus* L.). *Biology and Fertility of Soils*, *24*, 358–364. https://doi.org/10.1007/s003740050258

Elkoca, E., Turan, M., & Donmez, M. F. (2010). Effects of single, dual and triple inoculations with *Bacillus subtilis*, *Bacillus megaterium* and *Rhizobium leguminosarum* bv. *phaseoli* on nodulation, nutrient uptake, yield and yield parameters of common bean (*Phaseolus vulgaris* L. cv. 'Elkoca-05'). *Journal of Plant Nutrition*, *33*(14), 2104–2119. https://doi.org/10.1080/01904167.2010.519084

Gentili, F., Wall, L. G., & Huss-Danell, K. (2006). Effects of phosphorus and nitrogen on nodulation are seen already at the stage of early cortical cell divisions in *Alnus incana*. *Annals of Botany*, *98*(2), 309–315. https://doi.org/10.1093/aob/mcl109

Khan, M. S., Zaidi, A., Ahemad, M., Oves, M., & Wani, P. A. (2010). Plant growth promotion by phosphate solubilizing fungi - current perspective. *Archives of Agronomy and Soil Science*, *56*(1), 73–98. https://doi.org/10.1080/03650340902806469

Kouas, S., Labidi, N., Debez, A., & Abdelly, C. (2005). Effect of P on nodule formation and N fixation in bean. *Agronomy for Sustainable Development*, *25*(3), 389–393. https://doi.org/10.1051/agro:2005034

Lavakush, Janardan, Y., Verma, J. P., Jaiswal, D. K., & Kumar, A. (2014). Evaluation of PGPR and different concentration of phosphorus level on plant growth, yield and nutrient content of rice (*Oryza sativa*). *Ecological Engineering*, *62*, 123–128. https://doi.org/10.1016/j.ecoleng.2013.10.013

Lee, S., Ka, J. O., & Song, H. G. (2012). Growth promotion of *Xanthium italicum* by application of rhizobacterial isolates of *Bacillus aryabhattai* in microcosm soil. *The Journal of Microbiology, 50*(1), 45–49. https://doi.org/10.1007/s12275-012-1415-z

Li, Y., Liu, X., Hao, T., & Chen, S. (2017). Colonization and maize growth promotion induced by phosphate solubilizing bacterial isolates. *International Journal of Molecular Sciences, 18*(7), Article 1253. https://doi.org/10.3390/ijms18071253

Liu, F., Xing, S., Ma, H., Du, Z., & Ma, B. (2013). Cytokinin-producing, plant growth-promoting rhizobacteria that confer resistance to drought stress in *Platycladus orientalis* container seedlings. *Applied and Microbiology and Biotechnology*, *97*, 9155–9164. https://doi.org/10.1007/s00253-013-5193-2

Malboobi, M. A., Zamani, K., Lohrasebi, T., Sarikhani, M. R., Samaian, A., & Sabet, M. S. (2014). Phosphate: the silent challenge. *Progress in Biological Sciences*, *4*(1), 1–32. https://doi.org/10.22059/pbs.2014.50302

Masniawaty, Mustari, K., Astuti, Gusmiaty, Larekeng, H., Yani, A., & Rahim, I. (2019). Exploration of bacteria associated with chili peppers' rhizosphere and their capacity to absorb and produce gibberellin hormone. *IOP Conference Series: Earth and Environmental Science*, *343*, Article 012059. https://doi.org/10.1088/1755-1315/343/1/012059

Murphy, J., & Riley, J. P. (1962). A modified single solution method for the determination of phosphate in natural waters. *Analytica Chimica Acta*, *27*, 31–36. https://doi.org/10.1016/S0003-2670(00)88444-5

Nautiyal, C. S. (1999). An efficient microbiological growth medium for screening phosphate solubilizing microorganisms. *FEMS Microbiology Letters*, *170*(1), 265–270. https://doi.org/10.1111/j.1574-6968.1999.tb13383.x

Oliveira, M. G. C., Oliveira, L. F. C., Wendland, A., Guimarães, C. M., Quintela, E. D., Barbosa, F. R., Carvalho, M. C. S., Lobo Junior, M., & Silveira, P. M. (2018). *Conhecendo a fenologia do feijoeiro e seus aspectos fitotécnicos*. Embrapa.

Oliveira-Longatti, S. M., Souza, P. M., Marra, L. M., Ferreira, P. A. A., & Moreira, F. M. S. (2015). *Burkholderia fungorum* promotes common bean growth in a dystrophic oxisol. *Annals of Microbiology*, *65*, 1825–1832. https://doi.org/10.1007/s13213-014-1020-y

Olszewski, N., Sun, T. P., & Gubler, F. (2002). Gibberellin signaling: biosynthesis, catabolism, and response pathways. *The Plant Cell*, *14*(suppl_1), S61–S80. https://doi.org/10.1105/tpc.010476

Peix, A., Mateos, P. F., Rodriguez-Barrueco, C., Martinez-Molina, E., & Velazquez, E. (2001). Growth promotion of common bean (*Phaseolus vulgaris* L.) by a strain of *Burkholderia cepacia* under growth chamber conditions. *Soil Biology and Biochemistry*, *33*(14), 1927–1935. https://doi.org/10.1016/S0038-0717(01)00119-5

Pereira, S. I. A., & Castro, P. M. L. (2014). Phosphate-solubilizing rhizobacteria enhance *Zea mays* growth in agricultural P-deficient soils. *Ecological Engineering, 73,* 526–535. https://doi.org/10.1016/j.ecoleng.2014.09.060

R Core Team. (2020). *R: a language and environment for statistical computing*. R Foundation for Statistical Computing. https://www.R-project.org/

Sadiq, H. M., Jahangir, G. Z., Nasir, I. A., Iqtidar, M., & Iqbal, M. (2013). Isolation and characterization of phosphate-solubilizing bacteria from rhizosphere soil. *Biotechnology & Biotechnological Equipment*, *27*(6), 4248–4255. https://doi.org/10.5504/BBEQ.2013.0091

Sharma, S. B., Sayyed, R. Z., Trivedi, M. H., & Gobi, T. A. (2013). Phosphate solubilizing microbes: sustainable approach for managing phosphorus deficiency in agricultural soils. *SpringerPlus*, *2*, Article 587. https://doi.org/10.1186/2193-1801-2-587

Souza, T. L. P. O., Faleiro, F. G., Dessaune, S. N., Paula-Junior, T. J., Moreira, M. A., & Barros, E. G. (2013). Breeding for common bean (*Phaseolus vulgaris* L.) rust resistance in Brazil. *Tropical Plant Pathology*, *38*(5), 361–374. https://doi.org/10.1590/S1982-56762013005000027

Tahir, M., Mirza, M. S., Zaheer, A., Dimitrov, M. R., Smidt, H., & Hameed, S. (2013). Isolation and identification of phosphate solubilizer *Azospirillum*, *Bacillus* and *Enterobacter* strains by 16SrRNA sequence analysis and their effect on growth of wheat (*Triticum aestivum* L.). *Australian Journal of Crop Science*, *7*(9), 1284–1292.

Talboys, P. J., Owen, D. W., Healey, J. R., Withers, P. J. A., & Jones, D. L. (2014). Auxin secretion by *Bacillus amyloliquefaciens* FZB42 both stimulates root exudation and limits phosphorus uptake in *Triticum aestivum*. *BMC Plant Biology*, *14*, Article 51. https://doi.org/10.1186/1471-2229-14-51

Tedesco, M. J., Gianello, C., Bissani, C. A., Bohnen, H., & Volkweiss, S. J. (1995). *Análises de solo, plantas e outros materiais*. UFRGS.

Widdig, M., Schleuss, P. M., Weig, A. R., Guhr, A., Biederman, L. A., Borer, E. T., Crawley, M. J., Kirkman, K. P., Seabloom, E. W., Wragg, P. D., & Spohn, M. (2019). Nitrogen and phosphorus additions alter the abundance of phosphorus-solubilizing bacteria and phosphatase activity in grassland soils. *Frontiers in Environmental Science*, *7*, Article 185. https://doi.org/10.3389/fenvs.2019.00185

Yang, B., Wang, X. M., Ma, H. Y., Jia, Y., Li, X., & Dai, C. C. (2014). Effects of the fungal endophyte *Phomopsis liquidambari* on nitrogen uptake and metabolism in rice. *Plant Growth Regulation*, *73*, 165–179. https://doi.org/10.1007/s10725-013-9878-4

Zhang, X., Davidson, E. A., Mauzerall, D. L., Searchinger, T. D., Dumas, P., & Shen, Y. (2015). Managing nitrogen for sustainable development. *Nature*, *528*, 51–59. https://doi.org/10.1038/nature15743

Effect of selected bacteria from biogas sludge on the growth and nutrition of upland rice

Efecto de las bacterias seleccionadas de los lodos de biogás en el crecimiento y la nutrición del arroz de tierras altas

Novilda Elizabeth Mustamu[1], Zulkifli Nasution[2*], Irvan[3], and Mariani Sembiring[2]

ABSTRACT

This study evaluated the influence of selected superior bacterial isolates (SBI), biogas sludge, and their interactions on growth and nutrient uptake of upland rice grown in Ultisols. We used a randomized block design with two factors and seven replicates from October 2020 to April 2021. The first factor used selected SBI (B0 = untreated, B1 = nitrogen-fixing bacteria isolate (N3), B2 = phosphate solubilizing bacteria isolate (P7), B3 = isolate combination (N3+P7)). The second factor was the dosage of biogas sludge (S0 = untreated, S1 = 157.5; S2 = 315; S3 = 630 ml/polybag). The parameters were determined by ANOVA and followed by Duncan's multiple range test at $P<0.05$. The results showed that the isolate P7 significantly increased the N uptake by 20.77% and crop growth rate (CGR) of upland rice 2.81 times. Biogas sludge doses from 315 to 630 ml/polybag significantly increased plant height, uptake of N and P, total fresh and dry weight, and CGR of upland rice. The interaction between N3 and biogas sludge dosage of 630 ml/polybag significantly increased the CGR of upland rice. The application of isolates N3 and P7 and their combination with biogas sludge of 630 ml/polybag has the potential to increase the CGR of upland rice in acidic soils.

Key words: acidic soil, crop growth rate, dosage, sludge potential.

RESUMEN

El presente estudio evaluó la influencia de aislamientos bacterianos superiores seleccionados (ABS), lodos de biogás y sus interacciones sobre el crecimiento y la absorción de nutrientes del arroz de tierras altas cultivado en ultisoles. Se utilizó un diseño de bloques al azar con dos factores y siete repeticiones desde octubre de 2020 hasta abril de 2021. El primer factor utilizado seleccionó ABS (B0 = sin tratamiento, B1 = aislamiento de bacterias fijadoras de nitrógeno (N3), B2 = aislamiento de bacterias solubilizadoras de fosfato (P7), B3 = combinación de aislamientos (N3+P7)). El segundo factor fue la dosificación del lodo de biogás (S0 = sin tratamiento, S1 = 157.5; S2 = 315; S3 = 630 ml/polybag). Los parámetros fueron determinados por análisis de varianza y seguidos de la prueba de rangos múltiples de Duncan a $P<0.05$. Los resultados mostraron que el aislamiento P7 aumentó significativamente la absorción de N en un 20.77% y la tasa de crecimiento del cultivo (TCC) de arroz de tierras altas 2.81 veces. Las dosis de lodos de biogás de 315 a 630 ml/polybag aumentaron significativamente la altura de la planta, la absorción de N y P, el peso fresco y seco total y el TCC de arroz de tierras altas. La interacción de N3 con la dosis de lodos de biogás de 630 ml/polybag aumentó significativamente la TCC del arroz de tierras altas. La aplicación de los aislamientos N3 y P7 y su combinación con lodos de biogás de 630 ml/polybag tiene el potencial de aumentar la TCC de arroz de tierras altas en suelos ácidos.

Palabras clave: suelo ácido, tasa de crecimiento de cultivos, dosis, potencial de lodo.

Introduction

Biogas sludge is the waste by-product from an anaerobic processing system (FAO, 1977) and has a high nutrient content that can be used as organic fertilizer to increase soil fertility and plant yield (Adela *et al.*, 2014). The following characteristics of the biogas sludge from palm oil waste have been reported: total N of 490 mg L^{-1}, total P of 110 mg L^{-1}, total K of 1.9 mg L^{-1} (Lubis *et al.*, 2014), C/N 8; 0.14% N, 1.12% C (Tepsour *et al.*, 2019), and N-NH_3 of 91-112 mg L^{-1} (Choorit & Wisarnwan, 2007). The pH may range from 6.8 to 8.3, with the highest bacterial population of 7.21×10^7 cells ml^{-1} and the lowest of 3.15×10^7 cells ml^{-1} (Alvionita *et al.*, 2019). Additionally, Mustamu and Triyanto (2020) reported that the biogas sludge has nitrogen-fixing and phosphate solubilizing bacteria that have the potential to increase the availability of nitrogen and phosphate in soils.

Received for publication: May 24, 2021. Accepted for publication: December 20, 2021. Doi: 10.15446/agron.colomb.v39n3.97583

[1] Doctoral Program in Agricultural Sciences, Faculty of Agriculture, Universitas Sumatera Utara, Sumatera Utara (Indonesia).
[2] Program of Agrotechnology, Faculty of Agriculture, Universitas Sumatera Utara, Sumatera Utara (Indonesia).
[3] Program of Chemical Engineering, Faculty of Engineering, Universitas Sumatera Utara, Sumatera Utara (Indonesia).
[*] Corresponding author: zulnasution@usu.ac.id

The diversity of beneficial bacteria such as nitrogen-fixing and phosphate solubilizing bacteria has a greater potential to increase soil fertility and plant growth. Zhang *et al.* (2013) reported that phosphate solubilizing bacteria play an important role in increasing soil fertility and plant yield, and reducing the use of chemical fertilizers. Sharma *et al.* (2013) described different *Bacillus* species, such as *B. circulans, B. cereus, B. fusiformis, B. pumilus, B. megaterium, B. mycoides, B. coagulans, B. chitinolyticus,* and *B. subtilis* as phosphate solubilizing microorganisms. Ambrosini *et al.* (2016) showed the highest nitrogenase activity in *Bacillus cereus* among 42 different strains of *Bacillus* spp. Lim *et al.* (2018) also reported the dominant bacteria found in the biogas sludge from anaerobic processing using the pyrosequencing and clone library methods, *i.e.*, *Proteobacteria, Firmicutes, Bacteroidetes*, and *Thermotogae.*

The application of bacteria from biogas sludge has never been reported in Indonesia for improving upland rice growth on acidic soils, including Ultisols. According to the Pusat Penelitian Tanah dan Agroklimat (Center for Soil and Agro-climate Research) (2000), the area in Indonesia covered by Ultisols is 45.8 million ha or 24% of the total area of the country. Furthermore, according to the Ministry of Agriculture, the area dedicated to rice cultivation in Indonesia is 15,712,025 ha with a yield of 81,148,617 t in 2017, and the contribution of upland rice yield is 4.66% (Kementerian Pertanian, 2017). The yield contribution of upland rice is classified as low and, therefore, it is necessary to find options to increase it. Therefore, it is necessary to test the potential of beneficial bacterial isolates from biogas sludge to increase the availability of nitrogen and phosphate and, thus, the growth response of upland rice in Ultisols. This study aimed to evaluate the influence of selected superior bacterial isolates, biogas sludge, and their interaction on the mineral nutrition of the upland rice grown in Ultisols.

Materials and methods

Study area

The concentration of total N and available P in Ultisols and in the plant tissue (N and P uptake) were analyzed in the Analytical Laboratory of Socfin Indonesia Inc., Medan (Indonesia). The bacterial isolates were applied to upland rice in the village of Padang Bulan (3°37.760' N, 98°38.898' E, and altitude of 18 m a.s.l.), Medan Selayang Subdistrict, Medan City, Indonesia, from October 2020 to April 2021. The average temperature was 27.4°C, the average air humidity was 82%, and the average rainfall was 228.5 mm per month.

Preparation of medium and upland rice seeds

The medium to grow upland rice plants was the Ultisol soil from the Simalingkar area, Medan Tuntungan Subdistrict, Medan City, collected at a depth of 0 to 20 cm. One hundred g of soil sample were taken and analyzed for chemical characteristics of pH H_2O, organic C by Walkley-Black, available P by Bray-II, total N using the Kjeldahl method, and cation exchange capacity (CEC) and base saturation (K, Ca, Na, Mg) by ammonium acetate pH7 method (Tab. 1). The soil was sterilized by drying at 100°C for 2 h. To prevent heat from the sterilization process, the soil was incubated for 1 d and then placed into a 10 kg polybag (18 cm × 18 cm). A basic NPK fertilizer (16-16-16) by Meroke Tetap Jaya Inc. (Medan, Indonesia) at a dose of 1.5 g/polybag was applied by stirring evenly with the soil. The seeds of upland rice (*Oryza sativa* L.) of the inbred variety Inpago-8 from the Indonesian Agency for Agricultural Research and Development were soaked in water for 24 h, followed by the application of the fungicide Propineb (70%) for 2 h. Upland rice was planted after 1 d of basic fertilization with two seeds per polybag at a depth of 2 cm.

TABLE 1. Chemical characteristics of the Ultisol soil samples after sterilization at 100°C.

Chemical characteristics	Method	Value	Category
Soil pH (H_2O)	Electrometry	4.80	Acid
Organic C (%)	Walkley-Black	0.44	Very low
Total N (%)	Kjeldahl	0.04	Very low
Available P (mg kg^{-1})	Spectrophotometry	870.25	Very high
CEC (meq 100 g^{-1})	Ammonium acetate pH 7	28.31	High
Base saturation (%)	Ammonium acetate pH 7	4.85	Very low
Exchangeable cations			
K (meq 100 g^{-1})	Ammonium acetate pH 7	0.60	High
Ca (meq 100 g^{-1})	Ammonium acetate pH 7	0.34	Very low
Mg (meq 100 g^{-1})	Ammonium acetate pH 7	0.32	Very low
Na (meq 100 g^{-1})	Ammonium acetate pH 7	0.09	Very low
Al (%)	Ammonium acetate pH 7	0.02	Very low

Criteria for pH (H_2O) = 4.5-5.5 (acid); organic C <1% (very low); total N <0.1% (very low); available P >60 mg kg^{-1} (very high); cation exchange capacity (CEC) = 25-40 meq 100 g^{-1} (high); base saturation <20% (very low); exchangeable K = 0.60-1.00 meq 100 g^{-1} (high); exchangeable Ca <2 meq 100 g^{-1} (very low); exchangeable Mg <0.4 meq 100 g^{-1} (very low); exchangeable Na <0.1 meq 100 g^{-1} (very low); exchangeable Al <5% (very low) (Balai Penelitian Tanah (Indonesia Soil Research Institute), 2009).

Preparation of superior bacterial isolates suspension and biogas sludge

One ml of the bacterial isolate suspension obtained from the characteristic stage was put into a test tube containing 9 ml of distilled water, homogenized and then diluted to 10^{-5}. A total of 0.1 ml of the suspension from the last dilution was spread over James nitrogen free malat bromothymol blue

(JNFB) medium (Kirchhof *et al.,* 1997) for the nitrogen-fixing bacterial isolates test and Pikovskaya (PVK) medium (Pikovskaya, 1948) for the phosphate solubilizing bacteria isolates. The culture medium was incubated for 2 to 3 d at 37°C. The nitrogen-fixing bacterial isolate test was characterized by the presence of colonies growing on the JNFB medium. The growth of phosphate solubilizing bacterial isolates was indicated by a halo zone around the microbial colonies on the PVK medium. Seven nitrogen-fixing and seven phosphate-solubilizing isolates were found to produce total N and available P. The isolates that showed the highest phosphate and nitrogen increasing abilities were selected, namely phosphate solubilizing bacteria (P7) and nitrogen-fixing bacteria (N3), which were confirmed by Mustamu *et al.* (2021a; 2021b).

The biogas sludge was collected from Nubika Jaya Inc., Pinang City, Labuhanbatu District, North Sumatra Province, Indonesia. The procedure for processing biogas sludge was the following: the palm oil mill removed the palm oil mill effluent (POME) waste from the second pond which has been mixed with oil and then separated at an optimal temperature of 35°C. Liquid waste was then pumped into the receiver tank of 10 m^3 volume and filtered on a fiber tank screen to separate solid waste such as fiber and other materials. Liquid waste from the receiver tank was further pumped into the tower tank. Then, it was distributed evenly to the fixed tank at a temperature of 35 to 37°C and a flow rate of 20 to 30 m^3/h. Finally, the biogas sludge was taken from the fixed tank. Bacterial isolates and biogas sludge were applied to the soil surface at the base of the plants at one week after planting. Biogas sludge samples of 500 ml volume were used to analyze the chemical and biological characteristics (Tab. 2).

Treatment application

This study used a randomized block design with two factors and seven replicates. The first factor was the type of superior bacterial isolates (B0 = untreated; B1 = nitrogen-fixing bacterial isolate (N3); B2 = phosphate solubilizing bacteria isolate (P7); B3 = combination of isolates N3+P7) at a similar dose, namely 10 ml/polybag. The second factor was the dosage of biogas sludge (S0 = untreated; S1 = 157.5; S2 = 315; S3 = 630 ml/polybag). Determination of biogas sludge based on the dose of liquid organic fertilizer at the oil palm was 126 m^3 ha^{-1} equal to 126,000 L ha^{-1} (Sutarta *et al.,* 2000), then converted to soil weight per polybag (Eq. 1). Each replicate was harvested at 4, 8, and 12 weeks after application (WAA) for determination of the crop growth rate (CGR).

$$\text{Biogas sludge} = \frac{\text{Dose of liquid organic fertilizer ha}^{-1}}{\text{Soil weight ha}^{-1}} \times \text{soil weight per polybag} \quad (1)$$

$$= \frac{126{,}000 \text{ L ha}^{-1}}{2{,}000{,}000 \text{ kg ha}^{-1}} \times 10 \text{ kg} = 630 \text{ ml}$$

Plant growth variables and data analysis

The growth variables were assessed by measuring the growth of upland rice (plant height, and total fresh and dry weights), contents and uptake of N and P in the aerial

TABLE 2. Chemical and biological characteristics of the biogas sludge.

Characteristics	Method	Value
pH	Electrometry	7.41
Chemical oxygen demand (mg L^{-1})	Spectrophotometry	4547.8
Biological oxygen demand (mg L^{-1})	Titrimetry	1127.5
Total N (%)	Kjeldahl	0.051
Total P (%)	Spectrophotometry	0.0097
Available P (%)	Spectrophotometry	0.013
Total K (%)	Graphite furnace - atomic absorption spectrophotometry (AAS)	0.18
Organic C (%)	Walkley-Black	0.14
Ca (%)	Graphite furnace-AAS	0.04
Mg (%)	Graphite furnace-AAS	0.04
Na (mg L^{-1})	Graphite furnace-AAS	44.41
Cu (%)	AAS	0.0001
Total nitrogen-fixing bacteria (CFU ml^{-1})	Plate count	29.4×10^5
Total phosphate solubilizing bacteria (CFU ml^{-1})	Plate count	7.0×10^4

Laboratory analysis based on the Balai Penelitian Tanah (Indonesia Soil Research Institute) (2009).

parts, and crop growth rate (CGR). The plant height was measured from the root apex to the tip of leaves using a measuring tape, and the total fresh weight was obtained by weighing the roots and shoots. The total dry weight (roots + shoots) was obtained after plant drying in an oven (VS-1202D3, Vision Scientific Co., Korea) at 60°C for 48 h and weighed using analytical scales. A 200 g sample of the second leaf from the shoots was collected and analyzed to determine the N content using the Kjeldahl method, and the P content was recorded using the destruction method through dry ashing (Bertramson, 1942). The N and P uptake were measured using Equation 2. The CGR was calculated as the dry weight related to the unit area at 4-8, 8-12, and 12-16 WAA using Equation 3 (Shon *et al.*, 1997):

$$\text{Nutrient uptake} = \text{nutrient content in the shoots} \times \text{total dry weight} \quad (2)$$

$$CGR = \frac{\Delta W}{\Delta t} \times \frac{W2-W1}{t2-t1} \quad (3)$$

where:

CGR = crop growth rate;

W1 = dry weight per unit area at t1;

W2 = dry weight per unit area at t2;

t_1 = first sampling;

t_2 = second sampling.

The parameters of the second phase of the study were analyzed by an ANOVA. If the treatment had a significant effect, the Duncan's multiple range test was applied at *P*<0.05 using SPSS v.20 software (IBM, 2011).

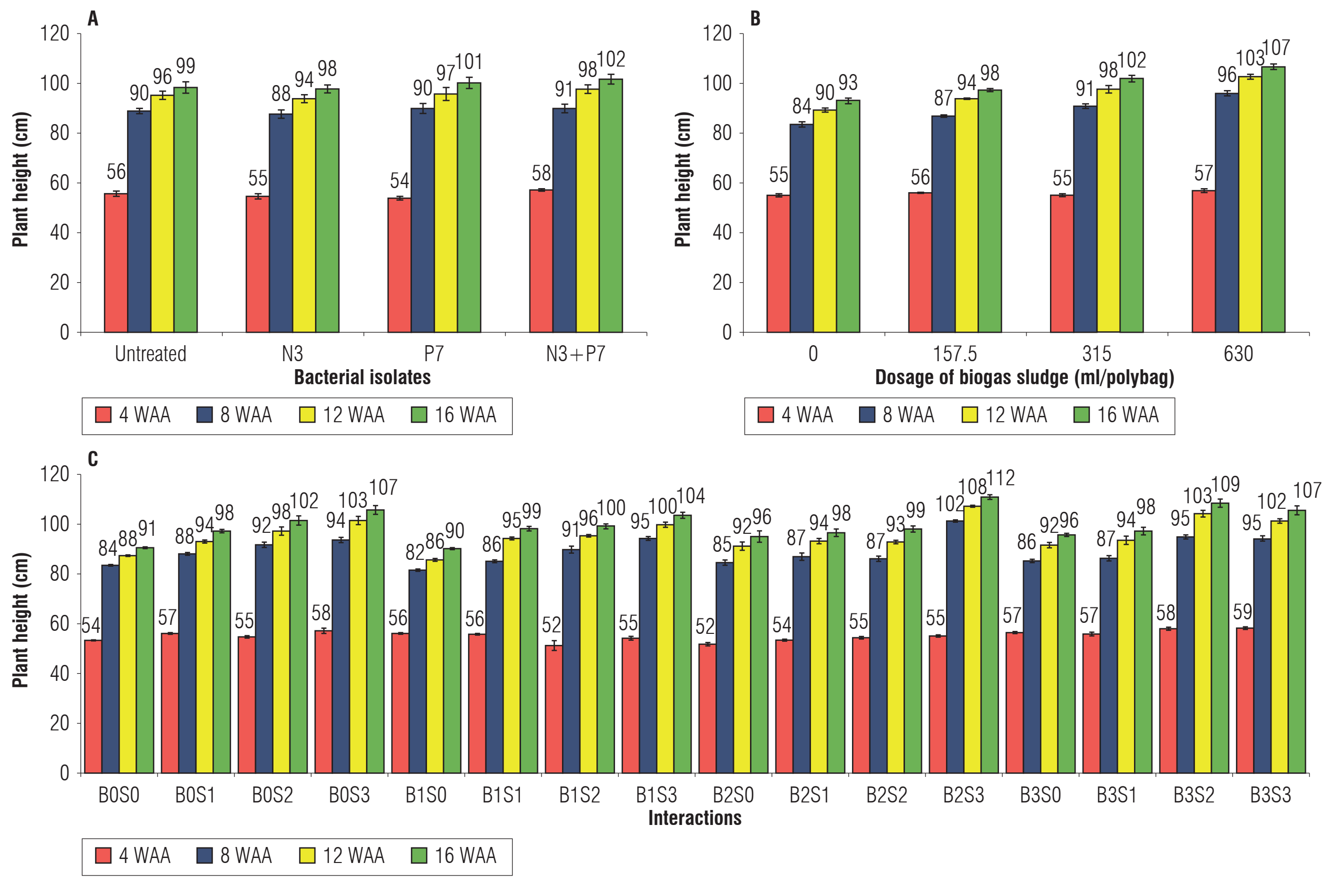


FIGURE 1. A) Effect of superior bacterial isolates, B) dosage of biogas sludge, and C) their interactions on plant height of upland rice at 4, 8, 12, and 16 weeks after application (WAA). Values followed by a different letter in the graph significantly differed according to the Duncan test at *P*<0.05. Vertical bars indicate the standard error. Dosage of biogas sludge (S0 = untreated; S1 = 157.5; S2 = 315; S3 = 630 ml/polybag). Superior bacterial isolates (B0 = untreated; B1 = isolate N3, B2 = isolate P7; B3 = isolates N3+P7).

Results

Effect of bacterial isolates and biogas sludge on upland rice growth

Plant height of upland rice

The effect of biogas sludge application was significant on the plant height of upland rice at 8, 12, and 16 WAA. Superior bacterial isolates and their interactions did not have a significant effect on the plant height of upland rice at 4, 8, 12, and 16 WAA (Fig. 1A-C). A significant increase in plant height of upland rice was observed with higher doses of biogas sludge of 630 ml/polybag at 8, 12, and 16 WAA, with the highest increase of 14.81% compared to the control at 16 WAA. Although the effect was not significant, the combination of isolates B3 and the interaction of B2S3 showed the highest increase in plant height of upland rice by 2.94% and 22.06%, respectively, compared to the control.

Biomass of upland rice

The effect of biogas sludge significantly increased the total fresh weight of upland rice at 8, 12, and 16 WAA. Superior bacterial isolates and their interactions did not have a significant effect on the total fresh weight of upland rice at 4-16 WAA (Tab. 3).

TABLE 3. Effect of superior bacterial isolates, biogas sludge, and their interactions on the total fresh weight (shoot + roots) of individual upland rice plants at 4, 8, 12, and 16 weeks after the application (WAA).

Treatments	Total fresh weight (g) ± standard error			
	4 WAA	8 WAA	12 WAA	16 WAA
Superior bacterial isolates (B)				
B0	4.15 ± 0.21	169.31 ± 8.90	215.27 ± 8.42	229.82 ± 8.94
B1	3.12 ± 0.12	194.50 ± 9.35	235.08 ± 10.32	252.02 ± 10.22
B2	4.52 ± 0.23	162.89 ± 11.15	201.85 ± 9.89	230.70 ± 9.28
B3	3.30 ± 0.25	173.91 ± 12.55	220.40 ± 15.96	245.03 ± 16.32
Biogas sludge (S)				
S0	3.72 ± 0.24	144.07 ± 9.37 b	182.67 ± 7.14 b	197.56 ± 6.58 b
S1	3.58 ± 0.27	153.41 ± 7.93 b	190.70 ± 8.90 b	215.65 ± 7.03 b
S2	3.64 ± 0.27	199.68 ± 10.30 a	258.70 ± 9.63 a	280.15 ± 9.25 a
S3	4.15 ± 0.25	203.45 ± 1.36 a	240.52 ± 2.81 a	264.21 ± 2.42 a
Interactions (B×S)				
B0S0	4.99 ± 0.33	124.08 ± 5.60	185.64 ± 3.32	192.78 ± 2.96
B0S1	3.47 ± 0.26	160.43 ± 1.16	188.60 ± 5.76	207.05 ± 3.97
B0S2	3.42 ± 0.42	185.97 ± 6.80	232.60 ± 8.75	250.84 ± 7.40
B0S3	4.71 ± 0.42	206.76 ± 5.49	254.23 ± 10.27	268.61 ± 8.85
B1S0	2.80 ± 0.18	155.79 ± 1.12	183.96 ± 5.20	202.88 ± 2.88
B1S1	3.74 ± 0.29	174.82 ± 9.01	227.91 ± 6.38	236.60 ± 6.32
B1S2	3.28 ± 0.40	241.17 ± 5.25	283.60 ± 7.76	296.08 ± 8.05
B1S3	2.67 ± 0.22	206.20 ± 7.23	244.85 ± 6.26	272.52 ± 4.34
B2S0	3.19 ± 0.18	190.90 ± 7.77	215.36 ± 7.67	229.11 ± 6.75
B2S1	4.85 ± 0.38	106.74 ± 13.42	143.16 ± 13.02	179.61 ± 10.36
B2S2	5.20 ± 0.24	148.40 ± 11.59	219.65 ± 5.26	248.72 ± 6.94
B2S3	4.82 ± 0.45	205.53 ± 10.50	229.21 ± 16.57	265.34 ± 9.58
B3S0	3.91 ± 0.30	105.53 ± 3.94	145.72 ± 1.96	165.45 ± 1.11
B3S1	2.25 ± 0.09	171.63 ± 4.90	203.14 ± 7.07	239.34 ± 12.07
B3S2	2.66 ± 0.14	223.17 ± 7.84	298.95 ± 1.51	324.94 ± 3.03
B3S3	4.37 ± 0.07	195.31 ± 6.77	233.79 ± 8.40	250.38 ± 8.16
CV (%)	56.09	29.68	26.31	20.78

Values followed by a different letter in the column significantly differed according to the Duncan test at $P<0.05$. CV - coefficient of variation. Dosage of biogas sludge (S0 = untreated; S1 = 157.5; S2 = 315; S3 = 630 ml/polybag). Superior bacterial isolates (B0 = untreated; B1 = isolate N3, B2 = isolate P7, B3 = isolates N3+P7).

A significant increase in the total fresh weight of upland rice was observed with the higher dose of biogas sludge of 315 ml/polybag at 16 WAA, with the highest increase of 41.81% compared to the control. Although the effect was not significant, B1 and the interaction of B3S2 showed the highest increase in the total fresh weight of upland rice with 9.66% and 68.55%, respectively, compared to the control.

The effect of biogas sludge significantly increased the total dry weight of upland rice at 12 and 16 WAA. Superior bacterial isolates and their interactions had an insignificant effect on the total dry weight of upland rice at 4-16 WAA (Tab. 4).

A significant increase in the total dry weight of upland rice was observed with the increase in the dosage of biogas sludge of 630 ml/polybag at 16 WAA, with the highest increase of 50.55% compared to the control. Although the effect was not significant, B1 and the interaction of B3S3 showed the highest increase in the total dry weight of upland rice with 20.84% and 81.53%, respectively, compared to the control.

Crop growth rate of upland rice

The effect of superior bacterial isolates, biogas sludge, and their interactions significantly increased the crop growth rate of upland rice at 12-16 WAA but did not have a significant effect at 4-8 and 8-12 WAA (Tab. 5).

TABLE 4. Effect of superior bacterial isolates, biogas sludge, and their interactions on the total dry weight (shoot + roots) of individual upland rice plants at 4, 8, 12, and 16 weeks after the application (WAA).

Treatments	Total dry weight (g) ± standard error			
	4 WAA	8 WAA	12 WAA	16 WAA
Superior bacterial isolates (B)				
B0	1.38 ± 0.06	48.01 ± 1.29	73.60 ± 3.99	82.52 ± 4.18
B1	1.13 ± 0.05	54.09 ± 2.41	76.83 ± 2.66	99.72 ± 4.15
B2	1.49 ± 0.06	47.30 ± 3.30	73.20 ± 2.28	98.25 ± 3.90
B3	1.15 ± 0.07	52.32 ± 3.39	77.18 ± 4.90	98.47 ± 4.56
Biogas sludge (S)				
S0	1.26 ± 0.06	45.51 ± 2.63	62.88 ± 2.19 b	76.78 ± 1.63 c
S1	1.23 ± 0.08	44.47 ± 1.71	68.52 ± 2.00 ab	87.65 ± 2.84 bc
S2	1.26 ± 0.08	55.36 ± 3.43	85.69 ± 1.08 a	98.95 ± 1.86 b
S3	1.40 ± 0.06	56.38 ± 1.05	83.73 ± 3.44 a	115.59 ± 2.11 a
Interactions (B×S)				
B0S0	1.58 ± 0.08	41.73 ± 2.78	58.08 ± 1.54	67.23 ± 0.96
B0S1	1.12 ± 0.08	45.87 ± 0.83	62.74 ± 1.83	71.08 ± 1.91
B0S2	1.20 ± 0.12	52.25 ± 2.07	81.39 ± 5.48	88.28 ± 5.02
B0S3	1.60 ± 0.12	52.18 ± 0.29	92.20 ± 3.05	103.49 ± 2.43
B1S0	0.97 ± 0.04	46.64 ± 1.39	69.53 ± 4.90	80.30 ± 4.51
B1S1	1.40 ± 0.07	48.13 ± 2.78	78.91 ± 0.53	96.23 ± 1.50
B1S2	1.12 ± 0.10	67.79 ± 1.44	91.05 ± 2.25	101.80 ± 2.40
B1S3	1.02 ± 0.08	53.81 ± 3.76	67.84 ± 1.77	120.54 ± 2.15
B2S0	1.17 ± 0.05	59.32 ± 2.33	70.92 ± 4.20	81.43 ± 3.82
B2S1	1.54 ± 0.10	34.47 ± 2.16	61.69 ± 1.97	89.84 ± 1.41
B2S2	1.73 ± 0.05	37.37 ± 3.74	83.10 ± 1.19	105.46 ± 1.37
B2S3	1.53 ± 0.10	58.05 ± 1.76	77.07 ± 4.27	116.28 ± 1.30
B3S0	1.30 ± 0.07	34.35 ± 7.04	52.98 ± 0.73	78.16 ± 0.48
B3S1	0.85 ± 0.03	49.40 ± 0.08	70.72 ± 1.29	93.44 ± 2.19
B3S2	0.99 ± 0.05	64.05 ± 4.68	87.22 ± 2.90	100.26 ± 1.93
B3S3	1.44 ± 0.02	61.48 ± 2.47	97.80 ± 0.77	122.04 ± 0.20
CV (%)	43.80	31.22	26.54	18.38

Values followed by a different letter in the column significantly differed according to the Duncan test at $P<0.05$. CV - coefficient of variation. Dosage of biogas sludge (S0 = untreated; S1 = 157.5; S2 = 315; S3 = 630 ml/polybag). Superior bacterial isolates (B0 = untreated; B1 = isolate N3, B2 = isolate P7, B3 = isolates N3+P7).

TABLE 5. Effect of superior bacterial isolates, biogas sludge, and their interactions on the crop growth rate of the upland rice 4, 8, 12, and 16 weeks after the application (WAA).

Superior bacterial isolates (B)	Biogas sludge (S)				Average
	S0	S1	S2	S3	
			4-8 WAA		
B0	1.434	1.598	1.823	1.806	1.665
B1	1.631	1.669	2.381	1.885	1.892
B2	2.077	1.176	1.273	2.019	1.636
B3	1.180	1.734	2.252	2.144	1.828
Average	1.580	1.544	1.932	1.964	CV = 32.28%
			8-12 WAA		
B0	0.584	0.602	1.041	1.430	0.914
B1	0.818	1.099	0.831	0.501	0.812
B2	0.414	0.972	1.633	0.679	0.925
B3	0.665	0.761	0.828	1.297	0.888
Average	0.620	0.859	1.083	0.977	CV = 56.17%
			12-16 WAA		
B0	0.327 fgh	0.298 gh	0.246 h	0.403 b-h	0.318 b
B1	0.385 c-h	0.619 a-h	0.384 d-h	1.882 a	0.817 a
B2	0.375 e-h	1.005 a-h	0.798 a-h	1.400 a-h	0.895 a
B3	0.899 a-h	0.811 a-h	0.466 a-h	0.866 a-h	0.761 a
Average	0.496 b	0.683 b	0.474 b	1.138 a	CV = 51.07%

Values followed by a different letter in the column significantly differed according to the Duncan test at $P<0.05$; CV - coefficient of variation. Dosage of biogas sludge (S0 = untreated; S1 = 157.5; S2 = 315; S3 = 630 ml/polybag). Superior bacterial isolates (B0 = untreated; B1 = isolate N3, B2 = isolate P7, B3 = isolates N3+P7).

The biogas sludge dose of 630 ml/polybag (S3) significantly increased the highest crop growth rate for upland rice at 12-16 WAA by 129.44% compared to the control. The isolates B1-B3 significantly increased the crop growth rate of upland rice with the highest increase for B2 of 181.45% compared to the controls at 12-16 WAA. The interaction of B1S3 significantly increased the crop growth rate of upland rice, showing values 5.76 times greater than those of the control.

Effect of bacterial isolates and biogas sludge on upland rice nutrition

Contents of N and P in the upland rice

The effect of biogas sludge, superior bacterial isolates, and their interactions did not have a significant effect on the content of N and P in the upland rice (Fig. 2A-C). The biogas sludge doses of 315 and 630 ml/polybag (S2 and S3) increased P and N in the plant tissue of upland rice by 33.33% and 4.53%, respectively, compared to the control. The isolate B2 showed the highest content of N in the plant tissues of upland rice with values 1.63% higher than those of the control. However, all isolates (B1-B3) showed a similar level of P in the plant tissues of upland rice compared to the control.

Uptake of N and P in the upland rice

The effect of biogas sludge significantly increased the uptake of N and P. The superior bacterial isolates significantly increased the uptake of nitrogen. The interaction of biogas sludge with superior bacterial isolates did not show a significant effect on the uptake of N and P in the upland rice (Fig. 2D-F).

A significant increase in the uptake of N and P in upland rice was observed with a higher dose of biogas sludge of 630 ml/polybag, with the highest increases of 54.11% and 65.20%, respectively, compared to the control. The bacterial isolates B1-B3 also significantly increased the uptake of N in the upland rice with the highest increase with B2 of 20.77% compared to the control. Although the effect was not significant, B3 showed the highest uptake of P in the upland rice of 19.93% compared to the control.

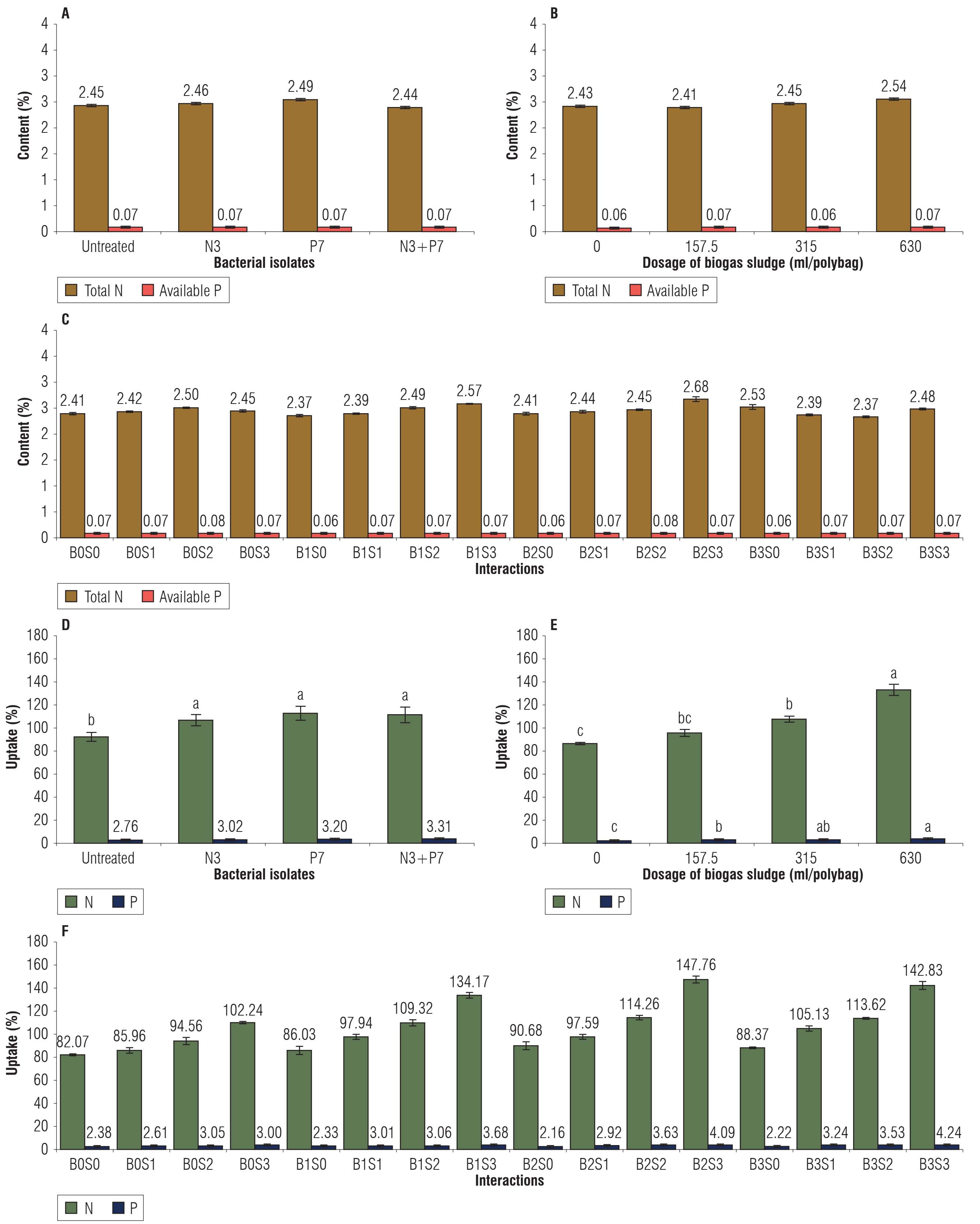


FIGURE 2. Effect of superior bacterial isolates, dosage of biogas sludge, and their interactions on the nutrient content (A-C) and uptake of N and P (D-F) of upland rice. Values followed by different letters significantly differed according to the Duncan test at $P<0.05$. Vertical bars indicate the standard error. Dosage of biogas sludge (S0 = untreated; S1 = 157.5; S2 = 315; S3 = 630 ml/polybag). Superior bacterial isolates (B0 = untreated; B1 = isolate N3, B2 = isolate P7, B3 = N3+P7 isolates).

Discussion

Effect of selected superior bacterial isolates

The selected superior bacterial isolates (N3 and P7) significantly increased the uptake of nitrogen and the crop growth rate of upland rice in an Ultisol at 12-16 WAA but did not have a significant effect on plant height, total fresh weight, total dry weight, content of N and P in leaves, uptake of phosphorus by the plants, and crop growth rate of upland rice from 4 to 12 WAA. The superior bacterial isolates (N3, P7, and N3+P7) increased the uptake of nitrogen in upland rice by 14.64%, 20.77%, and 20.68%, respectively, compared to the control (Fig. 2). Similar results are also shown in Table 5, with the crop growth rate of upland rice at 12-16 WAA increased by 2.57, 2.81, and 2.39 times, respectively, due to the selected superior bacterial isolates (N3, P7, N3+P7) compared to the control. The results indicate that a single P7 bacterial isolate increased the nitrogen uptake and crop growth rate of upland rice compared to a single N3 isolate and the combination of N3+P7 isolates. This was due to the presence of several organic acids and hormones produced by the isolate P7 that can increase the uptake of nitrogen and crop growth rate of upland rice. This result is supported by Mustamu *et al.* (2021a) who found that the phosphate solubilizing bacterial isolate (P7) from the biogas sludge contains organic acids, such as lactic, oxalic, acetic, and citric, and had the highest ability to solubilize phosphate from calcium triphosphate and rock phosphate with values 4.62 and 2.66 times higher, respectively, compared to the control. Meena *et al.* (2016) reported that the availability of nitrogen and phosphorus in soils slightly increased with the application of bio fertilization with *Bacillus cereus*. This was due to the production of organic acids and other substances, including citric, tartaric, and oxalic acids, that can stimulate plant growth and nutrient availability. Youssef and Eissa (2017) reported that the increase in vegetative growth and total biomass was due to increased photosynthesis, translocation, and accumulation of mineral nutrients. Khan *et al.* (2020) reported that *Bacillus cereus* strain SA1 can produce the hormones gibberellin, indole-acetic acid (IAA), and organic acids. Ferrara *et al.* (2012) reported that gibberellin and IAA can increase plant growth under stressful conditions. Kang *et al.* (2014) indicated that the plant growth-promoting bacteria (PGPB) has several mechanisms to increase plant growth with nitrogen-fixation and phosphate solubilization, increasing nutrient availability. Suksong *et al.* (2016) reported that bacteria of palm oil solid waste from an anaerobic digester include *Ruminococcus* sp., *Thiomargarita* sp., *Clostridium* sp. *Anaerobacter* sp., *Bacillus* sp., *Sporobacterium* sp., *Saccharofermentans* sp., *Oscillibacter* sp., *Sporobacter* sp., and *Enterobacter* sp. Liaquat *et al.* (2017) also reported abundance of *Bacillus, Clostridium*, and *Enterobacter* spp. in an anaerobic digester of wastewater when producing biogas.

Effect of biogas sludge

The dose of biogas sludge significantly increased the plant height, total fresh weight (8, 12, and 16 WAA), total dry weight (12 and 16 WAA), uptake (N and P), and crop growth rate of upland rice at 8-12 WAA. However, it did not have a significant effect on the content (N and P) in leaf tissue, and crop growth rate of upland rice (4-8 and 8-12 WAA). An increase in plant height, total dry weight, uptake of nitrogen and phosphorus, and also crop growth rate of upland rice in an Ultisol with a higher dose of biogas sludge of 630 ml/polybag was seen at the end of this study (16 WAA). In contrast, the total fresh weight increased along with the higher dose of biogas sludge of 315 ml/polybag and then decreased at the dose of 630 ml/polybag. This result is due to the chemical characteristics of the biogas sludge such as pH (7.41), total N (0.051%), available P (0.013%), organic C (0.14%), total K (0.18%), and the biological characteristics such as total nitrogen-fixing bacteria (29.4×10^5 CFU ml^{-1}) and total phosphate solubilizing bacteria (7.0×10^4 CFU ml^{-1}) (Tab. 2). The organic C content and the total population of nitrogen-fixing and phosphate solubilizing bacteria from the biogas sludge could increase the uptake of nitrogen and phosphorus in upland rice with an increasing dose of biogas sludge of 630 ml/polybag (Fig. 2). Therefore, the nutrients absorbed are used for plant metabolic processes and stimulate the plant height, biomass, and crop growth rate of the upland rice. A similar result was reported by Mustamu and Triyanto (2020), who determined the macro and micronutrients from the biogas sludge and the population of nitrogen-fixing and phosphate solubilizing bacteria of 480×10^4 and 42×10^4 CFU ml^{-1}, respectively. Ndubuisi-Nnaji *et al.* (2020) reported that the total phosphate solubilizing bacteria (1.6 to 2.5 CFU ml^{-1}) was significantly higher than nitrogen-fixing bacteria (0.5-1.4 CFU ml^{-1}), showing a significant increase in nutrient concentration in the order of N>K>P>Ca>Mg>S in all anaerobic digester bioreactors. Möller and Müller (2012) reported an increase in concentrations of NH_4^+-N from 45% to 80% in the anaerobic waste.

Interaction of selected superior bacterial isolates and biogas sludge

The interaction of biogas sludge and superior bacterial isolates only significantly increased the crop growth rate of upland rice in Ultisols at 12-16 WAA but did not have a significant effect on the other parameters in this study. The interaction of B1 with biogas sludge at the dose of 630 ml/polybag (B1S3) showed the highest crop growth rate of upland rice compared to other interactions and was 5.76 times greater compared to the control. This was caused by the application of biogas sludge that could have increased soil organic matter and the total population of beneficial bacteria. Likewise, the biogas sludge contained organic C (0.14%), total nitrogen-fixing bacteria (29.4×10^5 CFU ml^{-1}), and total phosphate solubilizing bacteria (7.0×10^4 CFU ml^{-1}) (Tab. 2) that could improve soil quality and increase the CGR. This result is supported by Urra *et al.* (2019) who found that the application of sewage sludge in the long term significantly increases the organic matter contents in the soil, causing a decrease in soil pH due to the nitrification of ammonium in sewage sludge and the production of organic acids along with the decomposition of the organic matter. Bhardwaj *et al.* (2014) and Carvajal-Muñoz and Carmona-Garcia (2012) showed that the application of a biofertilizer had advantages for the plants such as availability of nutrients balanced for plant health. It also stimulates nutrient mobilization that can increase soil biological activity and nutrient availability for microorganisms to encourage the growth of beneficial microorganisms, increasing the soil organic matter content and, therefore, the cation exchange capacity. Siswanti and Lestari (2019) indicated that the interaction of biogas sludge + biofertilizer (36 ml + 10 L ha^{-1}) significantly increased the plant height, number of leaves, and capsaicin content in chili pepper compared to a single treatment of biogas sludge and biofertilizer.

Conclusions

The isolates N3, P7, N3+P7 from the biogas sludge significantly increased the uptake of nitrogen (20.77%) and crop growth rate (2.81 times higher than the control) of upland rice in Ultisols with the highest increase found with the P7 isolate. The dose of biogas sludge significantly increased plant height (14.81%), total dry weight (50.55%), uptake of nitrogen (54.11%) and phosphorus (65.20%), and also crop growth rate (129.44%) of upland rice in Ultisols with the highest increase at a dose of 630 ml/polybag. Likewise, the dose of biogas sludge significantly increased the total fresh weight of upland rice by 41.81% with the highest increase at the dose of 315 ml/polybag. The interaction of isolates N3, P7, N3+P7 with the dose of biogas sludge only significantly increased the crop growth rate of upland rice in Ultisols 5.76 times with the highest increase found with B1S3.

Conflict of interest statement

The authors declare that there is no conflict of interest regarding the publication of this article.

Author's contributions

All authors formulated the overarching research goals and aims, provided the study materials, and developed the methodology. NEM analyzed and interpreted the study data. NEM and MS wrote the initial draft, managed and coordinated the research activity in the field, and collected the data. ZN and I verified the overall reproducibility of the results and the other research outputs. All authors conducted the critical review of the manuscript.

Literature cited

Adela, B. N., Muzzammil, N., Loh, S. K., & Choo, Y. M. (2014). Characteristics of palm oil mill effluent (POME) in an anaerobic biogas digester. *Asian Journal of Microbiology, Biotechnology and Environmental Sciences*, *16*(1), 225–231.

Alvionita, F., Faizal, M., Komariah, L. N., & Said, M. (2019). Biogas production from palm oil mill effluent with indigenous bacteria. *International Journal on Advanced Science, Engineering and Information Technology*, *9*(6), 2060–2066. https://doi.org/10.18517/ijaseit.9.6.10462

Ambrosini, A., Stefanski, T., Lisboa, B. B., Beneduzi, A., Vargas, L. K., & Passaglia, L. M. P. (2016). Diazotrophic bacilli isolated from the sunflower rhizosphere and the potential of *Bacillus mycoides* B38V as biofertiliser. *Annals of Applied Biology*, *168*(1), 93–110. https://doi.org/10.1111/aab.12245

Balai Penelitian Tanah. (2009). *Petunjuk teknis 2: Analisis kimia tanah, tanaman, air, dan pupuk*. Kementerian Pertanian.

Bertramson, B. R. (1942). Phosphorus analysis of plant material. *Plant Physiology*, *17*(3), 447–454. https://doi.org/10.1104%2Fpp.17.3.447

Bhardwaj, D., Ansari, M. W., Sahoo, R. K., & Tuteja, N. (2014). Biofertilizers function as key player in sustainable agriculture by improving soil fertility, plant tolerance and crop productivity. *Microbial Cell Factories*, *13*, Article 66. https://doi.org/10.1186/1475-2859-13-66

Carvajal-Muñoz, J. S., & Carmona-Garcia, C. E. (2012). Benefits and limitations of biofertilization in agricultural practices. *Livestock Research for Rural Development*, *24*(3), Article 43.

Choorit, W., & Wisarnwan, P. (2007). Effect of temperature on the anaerobic digestion of palm oil mill effluent. *Electronic Journal of Biotechnology*, *10*(3), 376–385. https://doi.org/10.2225/vol10-issue3-fulltext-7

FAO. (1977). *FAO soils Bulletin 40 - China: recycling of organic wastes in agriculture*. Food and Agriculture Organization of the United Nations. https://www.fao.org/publications/card/en/c/34d03d32-bd9f-5d08-aa08-ed2499349eb1/

Ferrara, F. I. S., Oliveira, Z. M., Gonzales, H. H. S., Floh, E. I. S., & Barbosa, H. R. (2012). Endophytic and rhizospheric enterobacteria isolated from sugar cane have different potentials for producing plant growth-promoting substances. *Plant and Soil*, *353*, 409–417. https://doi.org/10.1007/s11104-011-1042-1

IBM. (2011). *IBM SPSS statistics for Windows version 20.0*. International Business Machines Corporation.

Kang, S. M., Radhakrishnan, R., You, Y. H., Joo, G. J., Lee, I. J., Lee, K. E., & Kim, J. H. (2014). Phosphate solubilizing *Bacillus megaterium* mj1212 regulates endogenous plant carbohydrates and amino acids contents to promote mustard plant growth. *Indian Journal of Microbiology*, *54*, 427–433. https://doi.org/10.1007/s12088-014-0476-6

Kementerian Pertanian. (2017). *Produksi tanaman pangan di Indonesia*.

Khan, M. A., Asaf, S., Khan, A. L., Jan, R., Kang, S. M., Kim, K. M., & Lee, I. J. (2020). Thermotolerance effect of plant growth-promoting *Bacillus cereus* SA1 on soybean during heat stress. *BMC Microbiology*, *20*, Article 175. https://doi.org/10.1186/s12866-020-01822-7

Kirchhof, G., Reis, V. M., Baldani, J. I., Eckert, B., Döbereiner, J., & Hartmann, A. (1997). Occurrence, physiological and molecular analysis of endophytic diazotrophic bacteria in gramineous energy plants. In J. K. Ladha, F. J. de Brujin, & K. A. Malik (Eds.), *Opportunities for biological nitrogen fixation in rice and other non-legumes* (pp. 45–55). Springer. https://doi.org/10.1007/978-94-011-7113-7_6

Liaquat, R., Jamal, A., Tauseef, I., Qureshi, Z., Farooq, U., Imran, M., & Ali, M. I. (2017). Characterizing bacterial consortia from an anaerobic digester treating organic waste for biogas production. *Polish Journal of Environmental Studies*, *26*(2), 709–716. https://doi.org/10.15244/pjoes/59332

Lim, J. W., Ge, T., & Tong, Y. W. (2018). Monitoring of microbial communities in anaerobic digestion sludge for biogas optimisation. *Waste Management*, *71*, 334–341. https://doi.org/10.1016/j.wasman.2017.10.007

Lubis, F. S., Irvan, Anwar, D., Harahap, B. A., & Trisakti, B. (2014). Kajian awal pembuatan pupuk cair organik dari effluent pengolahan lanjut limbah cair pabrik kelapa sawit (LCPKS) skala pilot. *Jurnal Teknik Kimia USU*, *3*(1), 32–37. https://doi.org/10.32734/jtk.v3i1.1499

Meena, V. S., Bahaur, I., Maurya, B. R., Kumar, A., Meena, R. K., Meena, S. K., & Verma, J. P. (2016). Potassium-solubilizing microorganism in evergreen agriculture: an overview. In V. S. Meena, B. R. Maurya, J. P. Verma, & R. S. Meena (Eds.), *Potassium solubilizing microorganisms for sustainable agriculture* (pp. 1–20). Springer. https://doi.org/10.1007/978-81-322-2776-2_1

Möller, K., & Müller, T. (2012). Effects of anaerobic digestion on digestate nutrient availability and crop growth: a review. *Engineering in Life Sciences*, *12*(3), 242–257. https://doi.org/10.1002/elsc.201100085

Mustamu, N. E., Nasution, Z., Irvan, & Sembiring, M. (2021a). Isolation of phosphate solubilizing bacteria from anaerobic digestion sludge of palm oil mill effluent on ultisols. *Plant Cell Biotechnology and Molecular Biology*, *22*(35–36), 220–230.

Mustamu, N. E., Nasution, Z., Irvan, & Sembiring, M. (2021b). Potential and phylogenetic of superior bacterial isolates in biogas sludge from anaerobic digestion of palm oil mill effluent. *IOP Conference Series: Earth and Environmental Science*, *913*, Article 012065.

Mustamu, N. E., & Triyanto, Y. (2020). Nature of chemical and biological sludge biogas liquid waste oil palm. *International Journal of Innovative Science and Research Technology*, *5*(2), 955–957.

Ndubuisi-Nnaji, U. U., Ofon, U. A., Ekponne, N. I., & Offiong, N. A. O. (2020). Improved biofertilizer properties of digestate from codigestion of brewer's spent grain and palm oil mill effluent by manure supplementation. *Sustainable Environment Research*, *30*, Article 14.

Pikovskaya, R. I. (1948). Mobilization of phosphorus in soil in connection with the vital activity of some microbial species. *Mikrobiologiya*, *17*, 362–370.

Pusat Penelitian Tanah dan Agroklimat. (2000). *Atlas peta tanah Indonesia*. Jakarta Puslittanak.

Sharma, S. B., Sayyed, R. Z., Trivedi, M. H., & Gobi, T. A. (2013). Phosphate solubilizing microbes: sustainable approach for managing phosphorus deficiency in agricultural soils. *SpringerPlus*, *2*, Article 587. https://doi.org/10.1186/2193-1801-2-587

Shon, T. K., Haryanto, T. A. D., & Yoshida, T. (1997). Dry matter production and utilization of solar energy in one year old *Bupleurum falcatum*. *Journal of the Faculty of Agriculture Kyushu University*, *41*(3–4), 133–139.

Siswanti, D. U., & Lestari, M. F. (2019). Growth rate and capsaicin level of curly red chili (*Capsicum annum* L.) on biofertilizer and biogas sludge application. *Jurnal Biodjati*, *4*(1), 126–137. https://doi.org/10.15575/biodjati.v4i1.4216

Suksong, W., Kongjan, P., Prasertsan, P., Imai, T., & O-Thong, S. (2016). Optimization and microbial community analysis for production of biogas from solid waste residues of palm oil mill industry by solid-state anaerobic digestion. *Bioresource Technology*, *214*, 166–174. https://doi.org/10.1016/j.biortech.2016.04.077

Sutarta, E. S., Winarna, P. L., & Sufianto, T. (2000, June 13–14). *Aplikasi limbah cair pabrik kelapa sawit pada perkebunan kelapa sawit* [Conference presentation]. Pertemuan Kelapa Sawit II, Medan, Indonesia.

Tepsour, M., Usmanbaha, N., Rattanaya, T., Jariyaboon, R., O-Thong, S., Prasertsan, P., & Kongjan, P. (2019). Biogas production from oil palm empty fruit bunches and palm oil decanter cake using solid-state anaerobic co-digestion. *Energies*, *12*(22), Article 4368. https://doi.org/10.3390/en12224368

Urra, J., Alkorta, I., Mijangos, I., Epelde, L., & Garbisu, C. (2019). Application of sewage sludge to agricultural soil increases the abundance of antibiotic resistance genes without altering the composition of prokaryotic communities. *Science of the Total Environment*, *647*, 1410–1420. https://doi.org/10.1016/j.scitotenv.2018.08.092

Youssef, M. A., & Eissa, M. A. (2017). Comparison between organic and inorganic nutrition for tomato. *Journal of Plant Nutrition*, *40*(13), 1900–1907. https://doi.org/10.1080/01904167.2016.1270309

Zhang, A. M., Zhao, G. Y., Gao, T. G., Wang, W., Li, J., Zhang, S. F., & Zhu, B. C. (2013). Solubilization of insoluble potassium and phosphate by *Paenibacillus kribensis* CX-7: a soil microorganism with biological control potential. *African Journal of Microbiology Research*, *7*(1), 41–47. https://doi.org/10.5897/AJMR12.1485

Weed control and selectivity of different pre-emergence active ingredients in a soybean crop

Control de malezas y selectividad de diferentes principios activos preemergentes en un cultivo de soya

Marcelo de Sousa da Silva[1*], José Augusto Lima Furtado[1], Janine Quadros Castro[1], Iasmim Leite dos Santos[1], Edmilson Igor Bernardo Almeida[1], Leonardo Bernardes Taverny de Oliveira[1], Washington da Silva Sousa[1], and Rodolfo César de Albuquerque Araújo[2]

ABSTRACT

This study aimed to analyze the efficacy of different pre-emergence active ingredients on the suppression of the weed seed bank and the growth of soybeans. The experiments were carried out on a commercial farm located in Brejo (MA, Brazil), during the 2019/2020 harvest. The experiment was designed in randomized blocks with nine treatments and four replicates. The treatments consisted of control (without pre-emergence application), s-metolachlor, flumioxazin + imazethapyr, flumioxazin, imazethapyr, trifluralin, diclosulam, diclosulam + imazethapyr, and clomazone + carfentrazone-ethyl. Phytosociological surveys were carried out in pre- and post-planting (10 and 36 d after application - DAA) to control weed competition. Nineteen species of weeds were identified, distributed in 17 genera and 13 botanical families. The species *Scoparia dulcis, Richardia scabra*, and *Cyperus iria* exhibited the highest phytosociological indices (123.77, 28.62, and 28.29, respectively), estimated at 36 DAA. Flumioxazin and diclosulam were the most efficient in suppressing weed competition, with only 15.63 and 16.13 plants m^{-2}. The highest phytotoxicity scores (3.0) were found at 10 DAA with the application of s-metolachlor, flumioxazin + imazethapyr, trifluralin, and diclosulam + imazethapyr. The pre-emergent control using flumioxazin and diclosulam is recommended for the edaphoclimatic conditions in the Eastern mesoregion of the state of Maranhão, Brazil.

Key words: *Glycine max* (L.) Merrill, diclosulam, phytosociology, pre-emergence herbicides, weed competition.

RESUMEN

Este estudio tuvo como objetivo analizar la eficacia de diferentes principios activos de preemergencia sobre la supresión del banco de semillas de malezas y el crecimiento de la soya. Los experimentos se llevaron a cabo en una finca de producción agrícola comercial ubicada en el municipio de Brejo (MA, Brasil), durante la cosecha 2019/2020. El diseño experimental fue en bloques al azar con nueve tratamientos y cuatro repeticiones. Los tratamientos consistieron en el control (sin aplicación de preemergencia), S-metolaclor, flumioxazin + imazetapir, flumioxazin, imazetapir, trifluralin, diclosulam, diclosulam + imazetapir, y clomazona + carfentrazone-etil. Se realizaron estudios fitosociológicos antes y después de la siembra (10 y 36 d después de la aplicación - DDA) para controlar la competencia entre malezas. Se identificaron 19 especies de malezas, distribuidas en 17 géneros y 13 familias botánicas. Las especies *Scoparia dulcis*, *Richardia scabra* y *Cyperus iria* obtuvieron los índices fitosociológicos más altos (123.77, 28.62 y 28.29, respectivamente), estimados a los 36 DDA. La flumioxazina y el diclosulam fueron los más eficientes en la supresión de la competencia de malezas, con solo 15.63 y 16.13 plantas m^{-2}. Las puntuaciones más altas de fitotoxicidad (3.0) se encontraron a los 10 DDA con la aplicación de S-metolaclor, flumioxazina + imazetapir, trifluralina y diclosulam + imazetapir. Se recomienda el control preemergente con flumioxazina y diclosulam para las condiciones edafoclimáticas en la mesorregión este del estado de Maranhão, Brasil.

Palabras clave: *Glycine max* (L.) Merrill, diclosulam, fitosociología, herbicidas de preemergencia, competencia de malezas.

Introduction

The soybean (*Glycine max* (L.) Merrill) is one of the most important oilseed crops in the world. Brazil has a relevant role in this scenario, with a record harvest in 2019/2020, with production estimated at 120.4 million t. This amount was 4.7% higher than that obtained in the 2018/2019 harvest

Received for publication: 2 June, 2021. Accepted for publication: 27 December, 2021. Doi: 10.15446/agron.colomb.v39n3.96334

[1] Center for Studies and Research in Phytotechnics, Center for Agrarian and Environmental Sciences, Federal University of Maranhão - UFMA, Chapadinha, Maranhão (Brazil).
[2] Department of Vegetable Production, Federal University of Grande Dourados - UFGD, Dourados, Mato Grosso do Sul (Brazil).
[*] Corresponding author: marcelo.ss@discente.ufma.br

and 0.9% higher than the last record of 119.3 million t reached in the 2017/18 harvest (CONAB, 2020).

Soybeans were introduced in the 1970s in the southern mesoregion of the state of Maranhão. At the beginning of 2000s, the crop was expanded to the Chapadinha microregion and nearby microregions, such as the Baixo Parnaíba Maranhense, Coelho Neto, and recently, to the microregion of Caxias (Conte *et al*., 2018). Nowadays, Maranhão has a planted area of 1,000,300 ha, with an estimated production of 3,029,900 t (CONAB, 2020), confirming the importance of soybean cultivation in the state.

Despite being a relatively exploited crop with outstanding socio-economic importance, the soybean still presents significant losses in productivity due to several factors, especially weed competition (Gazola *et al*., 2016). Weeds harm the crop, mainly through competition for water, light, and nutrients; moreover, depending on the cultivar and infestation level, they might hamper the harvesting operation and affect the quality of the grains (EMBRAPA, 2013; Fried *et al*., 2017).

The phytosociological survey of weeds is a strategic tool used to assess the potential impact of agricultural practices and management systems on the dynamics of weed infestation (Fialho *et al*., 2011). These studies allow obtaining information on density, frequency, abundance, and index of relative importance of weeds in crops, which are indispensable for efficient chemical control, especially in the pre-emergence phase (Cardoso *et al*., 2013).

Pre-emergence chemical control uses herbicides with a prolonged half-life in the soil. These products provide control of germinating seeds, a phenological phase in which weed species are more susceptible to the herbicide. The seed bank is reduced in the period before interference (PBI), avoiding initial competition and reducing the number of post-emergence applications and, consequently, the cost of production (Gonçalves *et al*., 2018). The efficiency of pre-emergence herbicides should be assessed according to the conditions of soil, climate, humidity, and weed species present in the area (Silva *et al*., 2021).

Some initial studies indicated that weed management predominantly adopted in this mesoregion had probably shown a low level of efficiency as plants resistant to chemical control have been selected, particularly with sequential applications of glyphosate at post-emergence. Hence, the expansion of soybean cultivation in these areas may show several economic, phytosanitary, or environmental problems due to the adoption of inadequate control practices in the medium and long term. Therefore, this study is based on the hypothesis that for the proposal and implementation of integrated weed management (IWM) in the east of Maranhão (Brazil), it is necessary to identify and define adequate control methods of the species present at this site.

In this context, the aim of this study was to analyze the efficiency of different pre-emergence active ingredients on the suppression of the weed seed bank and the growth of soybeans to present strategies for rotating molecules and efficient management of weed competition in commercial soybean farming in the state of Maranhão.

Materials and methods

The study was conducted on a commercial grain farm, located in the municipality of Brejo, in the eastern mesoregion of the state of Maranhão, Brazil (03°42' S, 42°57' W and at an altitude of 90 m a.s.l.) between January and April 2020.

The soil of the experimental area was classified as a typical Distrocoeso Yellow Argisol (Dantas *et al*., 2014). According to the Köppen classification, the region's climate is tropical wet and dry (Aw), with an average annual temperature above 27°C and average annual rainfall ranging from 1600 to 2000 mm. The rainy season occurs between January and June and the dry season between July and December, with annual relative humidity between 73 and 79% (Passos *et al*., 2016).

The data of rainfall, maximum and minimum temperatures during the months of the experiment is shown in Figure 1, according to INMET (Portuguese acronym for Brazilian National Institute of Meteorology) (INMET, 2019).

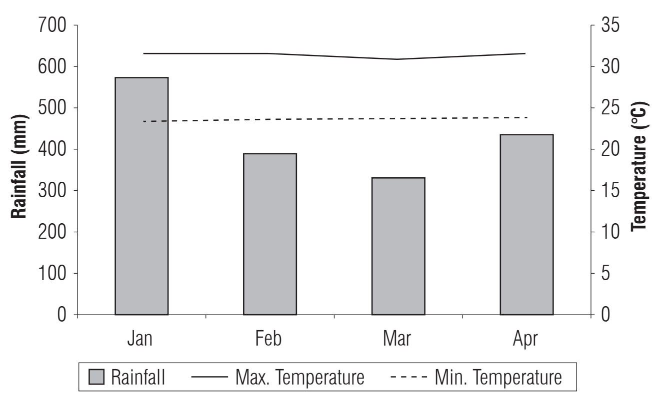


FIGURE 1. Data of rainfall (mm) and minimum and maximum air temperature (°C) registered for the eastern mesoregion of the state of Maranhão between January and April 2020.

The experiment was conducted in a randomized block design, consisting of nine treatments, four replicates, and 40 useful plants per plot. The treatments consisted of control (without pre-emergence application), s-metolachlor (1,200 g of active ingredient (a.i.) ha^{-1}), flumioxazin + imazethapyr (50 + 100 g a.i. ha^{-1}), flumioxazin (50 g a.i. ha^{-1}), imazethapyr (100 g a.i. ha^{-1}), trifluralin (1,112.5 g a.i. ha^{-1}), diclosulam (45 g a.i. ha^{-1}), diclosulam + imazethapyr (30 + 100 g a.i. ha^{-1}) and clomazone + carfentazone-ethyl (720 + 18 g a.i. ha^{-1}). The plot occupied an area of 18 m^2 (6 m x 3 m), spaced 2 m apart. For crop evaluation, the three central lines of 1 linear meter of each plot were assessed, leaving a border of marginal lines.

Pre-planting desiccation was carried out 15 d before planting by applying glyphosate (2,477.1 g a.i. ha^{-1}) + 2,4-D (1,476 g a.i. ha^{-1}). Additionally, the seeds were treated with the insecticide thiamethoxam (500 g a.i. L^{-1}), at a dose of 0.002 L kg^{-1}; fungicide (20 g a.i. L^{-1} metalaxil-M + 150 g a.i. L^{-1} tiabendazole + 25 g a.i. L^{-1} fludioxonil), at a dose of 0.002 L kg^{-1}; and the insecticide cyantraniliprole (600 g a.i. L^{-1}), at a dose of 0.0012 L kg^{-1} of seeds.

The soybean cultivar FTR 3190 IPRO was used. Sowing was carried out on January 28, 2020, with a density of 16.5 seeds per linear meter and spacing of 0.5 m between rows. The treatments were applied 1 d after sowing as suggested by the "plant and apply" method according to the manufacturer recommendations. For the application of the treatments, we used a knapsack sprayer with a bar with six flat fan nozzles spaced 0.5 m apart, with pressure of 207 kPa and a rate of 150 L ha^{-1}.

At 36 d after application (DAA), plant height, number of leaflets, and canopy closure were assessed in 10 plants set at three central lines of each plot. Additionally, the number of plants per linear meter in each evaluated line was estimated. The parameters evaluated were: plant height (cm), by measuring the distance from the ground level up to the last apical bud using a graduated measuring tape; the number of leaflets, by counting the open leaflets; canopy closure (%), defined as the percentage area covered by the foliage, by using a graduated ruler (the half canopy (25 cm) covered by the leaves of each adjacent line was considered as 100% closure); and the number of plants per meter, by counting the number of plants in the rows of each useful plot.

To determine the effects of herbicides on weed flora, three phytosociological surveys were conducted. The first was carried out at pre-planting 5 d before desiccation and the others at 10 DAA and 36 DAA. In the first survey, 49 launches of an inventory square with an area of 1 m^2 were carried out in 3 m x 5 m intervals, in the extension of seven sample lines.

In each sampling, weeds were identified with the help of specialized literature (Lorenzi, 2014) and quantified for estimates of phytosociological indices. The other surveys (10 and 36 DAA) were performed within the experimental plots, in areas of 4 m^2, pre-defined and monitored throughout the study.

Based on this information, the phytosociological indices were estimated: density = total number of individuals per species/unit of area (m^2); relative density = density of the species x 100/total density of all species; frequency = number of squares containing the species/total number of squares; relative frequency = species frequency x 100/total species frequency; abundance = total number of individuals per species/total number of squares that contain the species; relative abundance = species abundance x 100/total abundance of all species; importance value index (IVI) = relative frequency + relative density + relative abundance. After species identification, the occurrence of individuals by class (monocots and eudicots) for each treatment was quantified.

In this study, the phytotoxic effects of herbicides on soybean cultivation were analyzed. The analysis was conducted at 10, 17, 21, and 36 DAA following the Conceptual Scale Model of the European Weed Research Community (Johannes, 1964); the scores ranged from one for plants that did not show phytotoxicity symptoms to nine for plant death (Tab. 1).

TABLE 1. Scale of phytotoxicity scores proposed by the European Weed Research Community).

Score	Phytotoxicity symptoms
1	Absence of phytotoxicity symptoms
2	Minor changes (discoloration, deformation), visible in some plants
3	Minor changes (chlorosis and shriveling), visible in many plants
4	Strong discoloration or reasonable deformation, without necrosis
5	Necrosis of some leaves, accompanied by deformation in leaves and buds
6	Reduced plant size, shriveling, and leaf necrosis
7	More than 80% of destroyed leaves
8	Extremely serious damage, leaving small green areas in the plants
9	Plant death

Phytosociological data were analyzed using descriptive statistics. The variables related to soybean growth were subjected to analysis of variance by the F test ($P<0.05$) and the means compared by the Tukey's test. Additionally, a multidimensional test was applied using the software Statistica® 7.0.

Results and discussion

In the surveys carried out before and after planting, 19 weed species were identified, distributed in 17 genera and 13 botanical families (Tab. 2).

The Fabaceae family had three identified species, followed by Amaranthaceae (2), Cyperaceae (2), and Poaceae (2). The families and species found were similar to those identified by Lopes, Silva, Costa, Oliveira, *et al.* (2020) when studying the phytosociology of the seed bank in experimental farming located in Chapadinha (MA, Brazil).

In the phytosociological survey carried out in the pre-desiccation period, the species *Scoparia dulcis* showed the highest importance value index (85.35) (Fig. 2). Such

TABLE 2. List of weeds identified in pre- and post-planting.

Family	Scientific name	Common name in Brazil
Amaranthaceae	*Amaranthus deflexus* L.	Bredo-rasteiro
	Amaranthus hybridus L.	Caruru, bredo, caruru-roxo
Boraginaceae	*Heliotropium indicum* L.	Crista-de-galo
Comelinaceae	*Commelina benghalensis* L.	Trapoeraba
Convolvulaceae	*Ipomoea triloba* L.	Corda-de-viola
Cyperaceae	*Cyperus iria* L.	Tiririca, tiririca-do-brejo, juquinho
	Cyperus odoratus L.	Tiririca-comum, tiririca-de-três-quinas
Euphorbiaceae	*Euphorbia hirta* L.	Erva-de-santa-luzia, quebra-pedra
Fabaceae	*Mimosa pudica* L.	Malícia, dormideira
	Desmodium tortuosum (Sw.) DC.	Carrapicho-beiço-de-boi, pega-pega
	Neptunia plena (L.) Benth	Dorme-dorme, jurema-d'água
Malvaceae	*Sida rhombifolia* L.	Guanxuma, vassourinha
Molluginaceae	*Mollugo verticillata* L.	Molugo, capim-tapete
Poaceae	*Cenchrus echinatus* L.	Capim-carrapicho, carrapicho
	Eleusine indica (L.) Gaertn	Capim-pé-de-galinha, pé-de-galinha
Rubiaceae	*Richardia scabra* L.	Poaia-do-cerrado, poaia
	Spermacoce verticillata L.	Vassourinha-de-botão, falsa-poaia
Scrophulariaceae	*Scoparia dulcis* L.	Vassourinha
Turneraceae	*Turnera subulata* Sm.	Chanana

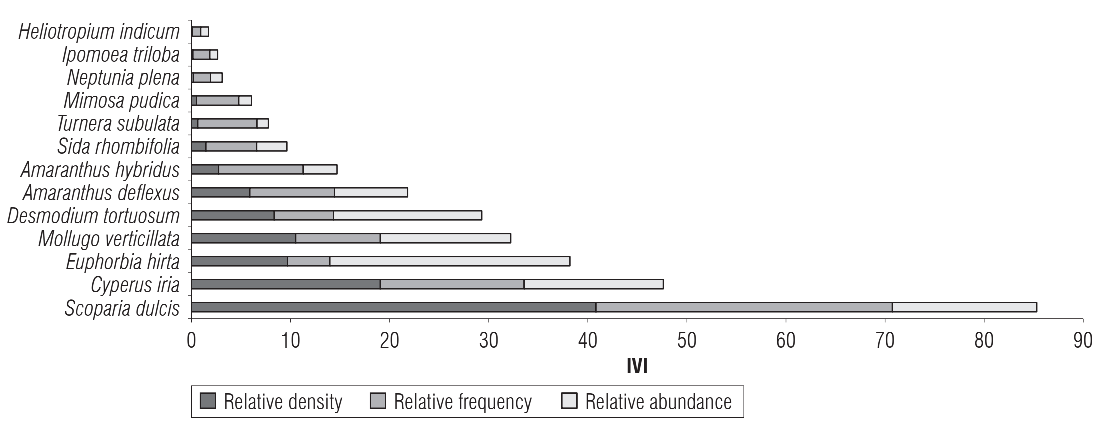


FIGURE 2. Importance value index (IVI) of the main weed species in the pre-desiccation period.

results are similar to those found by Lopes, Silva, Costa, Silva, *et al.* (2020) in studies on the spatial distribution of the seed bank in an area with a history of soybean crops in Chapadinha.

According to Oliveira and Freitas (2008), the importance value index indicates which species within the weed community has the greatest potential to cause damage to the crop. Thus, the species *Scoparia dulcis* could be considered the weed with the highest potential, followed by the species *Cyperus iria* (47.61), *Euphorbia hirta* (38.21), *Mollugo verticillata* (32.21), and *Desmodium tortuosum* (29.28), which demand constant monitoring during the crop cycle and later harvests.

When assessing the class of weeds, it was observed that the highest infestation, estimated as the number of plants per square meter in the pre-desiccation period was by the eudicotyledonous (Fig. 3). According to Rizzardi and Silva (2014), to define adequate suppression tools, it is necessary to have information about the class of weeds existing in a determined location, its distribution, and population.

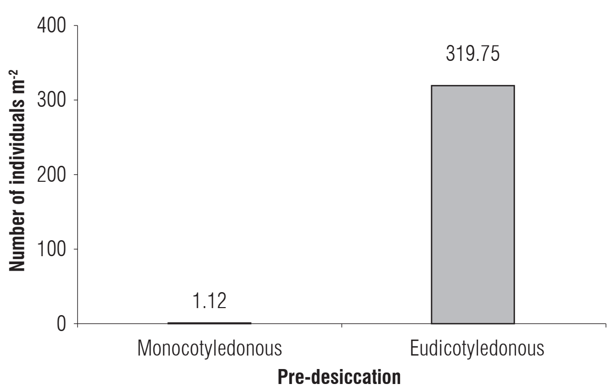


FIGURE 3. Characterization of weed infestation, by class, in the pre-desiccation period.

Takano *et al.* (2013) recommended pre-planting desiccation with broad-spectrum herbicides or latifolicides, such as glyphosate and 2,4-D, respectively, which is in line with the management employed by the producer in this study. However, Placido *et al.* (2016) emphasized that it is relevant to rotate the use of mechanisms of action at different stages of chemical weed control. These two molecules must be used in a planned way, especially glyphosate, since their sequential use in high doses has induced the occurrence of resistant genotypes in several countries, including Brazil.

The phytosociological survey conducted at 10 DAA revealed a reduction in the number of infesting species compared to infestation during pre-planting (pre-desiccation). This result confirms the importance of well-planned pre-emergence control and desiccation to obtain a cleaner crop, particularly at the critical growth phase of the crop (Fig. 4).

All pre-emergence treatments significantly reduced the number of weed species compared to the control. Although the whole plot was uniformly desiccated at pre-planting, the plots not treated with pre-emergence herbicides became more vulnerable to weed infestation. Thus, these non-treated plots demanded post-emergence applications to reduce the weed competition effects on soybean cultivation, with imminent risks on productive yield.

Among the identified species, *Scoparia dulcis* showed the highest IVI in the control (66.30) and treatments with s-metolachlor (184.23) and diclosulam (150). *Cyperus iria* had high values of IVI in the treatments flumioxazin + imazethapyr (212.38), flumioxazin (300), imazethapyr (300), trifluralin (213.33), and diclosulam + imazethapyr (300), whereas *Cyperus odoratus* showed similar values for treatment with clomazone + carfentrazone-ethyl (158.21). These results show the capacity of these species to form large seed banks in the soil as well as to adapt to the edaphoclimatic conditions of the studied region, even when subjected to chemical control with different molecules at pre- and post-planting (Lopes, Silva, Costa, Oliveira, *et al.*, 2020).

In the phytosociological survey carried out at 36 DAA, 12 species were examined in the control, mainly *Scoparia dulcis* (185.68). The finding corroborates the high infestation of this species in the pre-desiccation period (IVI: 85.35), which indicates that the absence of application at pre-emergence, as a complementary practice, can stimulate weed competition at the growth phase of the crop. In this context, *Scoparia dulcis* was effectively controlled by diclosulam, in accordance with the results obtained by Martins and Christoffoleti (2014) (Fig. 5).

In all pre-emergence treatments, except for trifluralin, there was a reduction in the number of weed species. In treatments using trifluralin, the reductions were observed only in 13 species (Fig. 5F). According to data from the University of Hertfordshire (2020), this herbicide shows a high volatilization capacity at 20°C, which may have decreased its pre-emergent control efficiency under the study conditions (27.7°C), demonstrating the limitations for its use in this phase in the local productive sector.

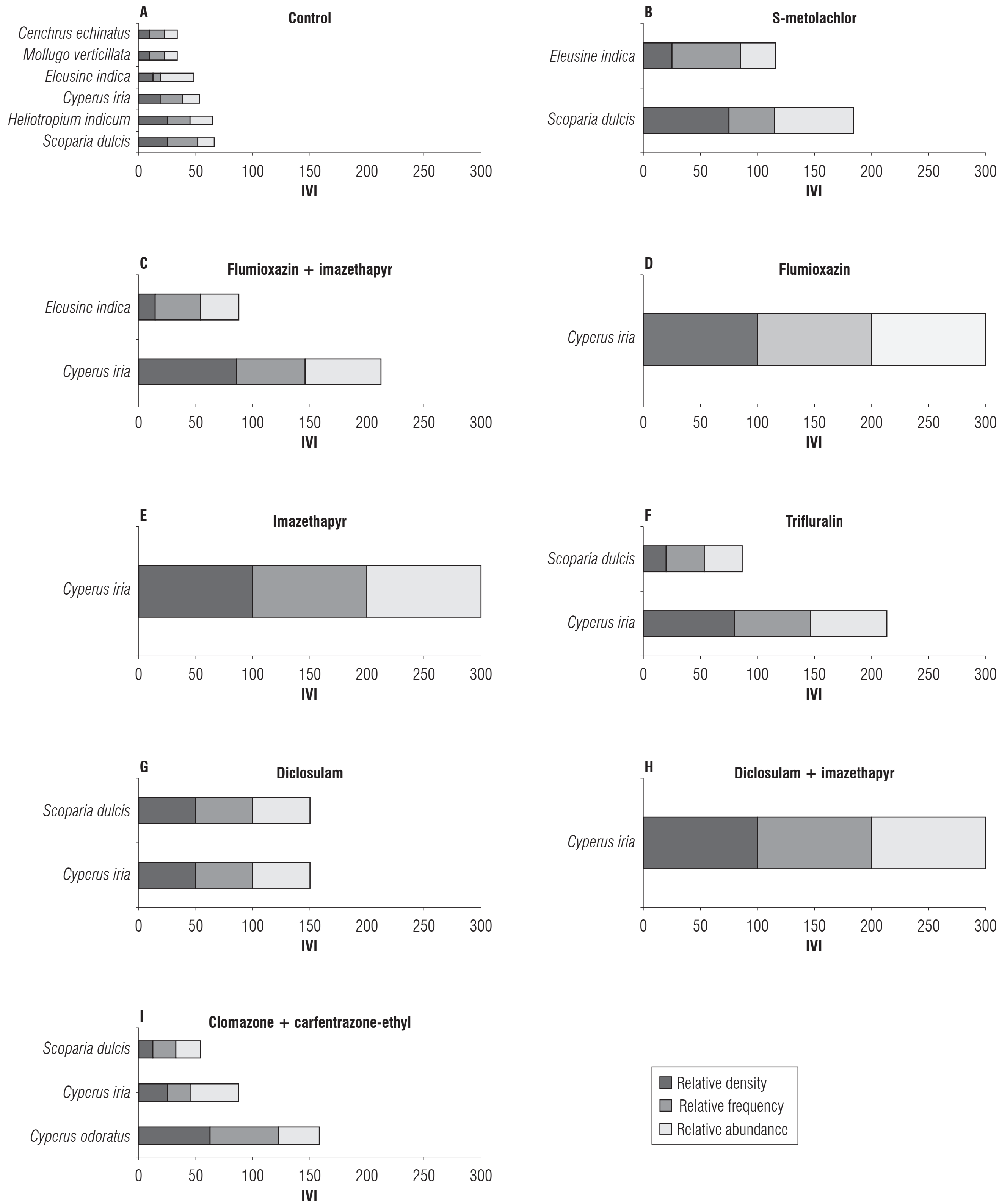


FIGURE 4. Importance value index (IVI) of weeds in soybean crops, 10 d after application (DAA), under the effect of different pre-emergence molecules: A) control; B) s-metolachlor; C) flumioxazin + imazethapyr; D) flumioxazin; E) imazethapyr; F) trifuralin; G) diclosulam; H) diclosulam + imazethapyr; I) clomazone + carfentrazone-ethyl.

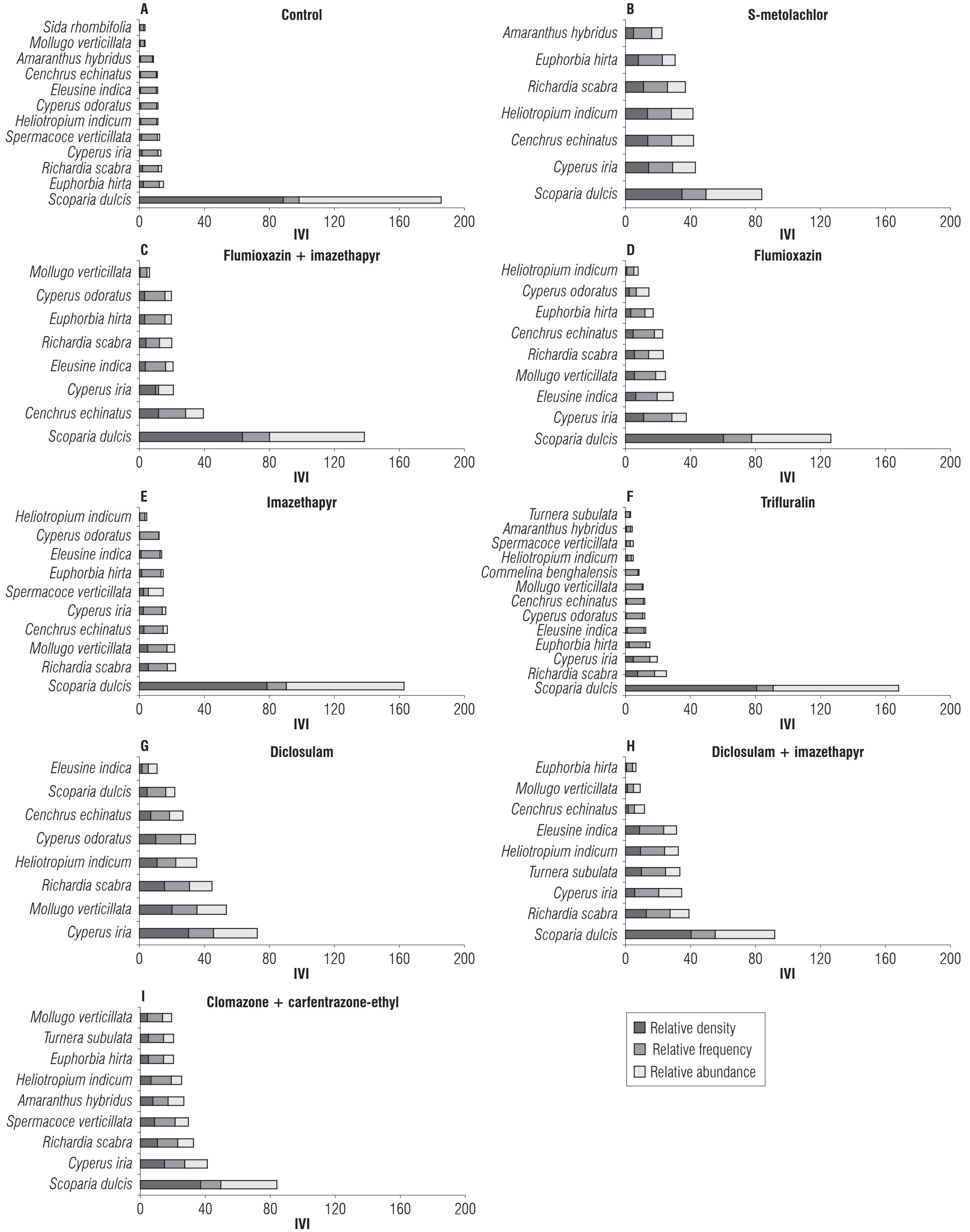


FIGURE 5. Importance value index (IVI) of weeds in soybean crops 36 d after application (DAA), under the effect of different pre-emergence molecules: A) control; B) s-metolachlor; C) flumioxazin + imazethapyr; D) flumioxazin; E) imazethapyr; F) trifluralin; G) diclosulam; H) diclosulam + imazethapyr; I) clomazone + carfentrazone-ethyl.

A reduction in the infestation of mono and eudicotyledonous weeds at 10 and 36 DAA was observed for all treatments compared to the control, which highlights the importance of pre-emergence chemical control as an alternative to pre-planting desiccation (Fig. 6). This result corroborates the findings of Andrade Junior *et al.* (2018) that demonstrated pre-emergence chemical control as an indispensable tool to obtain control of weed competition in the critical period of prevention of interference and to provide maximum productive potential of the soybean crop.

The pre-emergence herbicides have demonstrated, in their majority, the adequate control of monocots. Similar results were obtained by Alonso *et al.* (2013), who reported the selective, graminicide, and residual effect of these molecules on soybeans. Thus, these herbicides possibly provided an efficient suppression of the emergence flow of weeds in the critical phase of crop establishment for most of the treatments analyzed.

According to Jadhav *et al.* (2013), the performance of pre-emergence herbicides may vary depending on diverse factors, such as edaphic and climatic conditions and the species that will be controlled. In this sense, s-metolachlor, which is one of the molecules most used by local producers, did not show the residual and selective efficiency expected concerning the control of monocotyledonous weeds (Fig. 6B).

Different results were found by Machado *et al.* (2016), who obtained satisfactory control of monocotyledonous weeds up to 46 DAA under the effect of s-metolachlor at a dose of 768 g a.i. ha^{-1} in Rio Verde (GO, Brazil). This may be due to the conditions mentioned by Jadhav *et al.* (2013), which affect the behavior of molecules in the soil and in the plant and, therefore, affect their efficiency in suppressing weed competition.

The application of flumioxazin in a mixture with imazethapyr or alone exhibited significant results, being efficient in controlling weed competition but with superior efficiency when applied alone (Fig. 6C-D). This efficiency could be associated with the interactions that occur between the mixed herbicides, which, according to Gazziero (2015), can result in synergistic or antagonistic interactions. In a study conducted by Vidal *et al.* (2010), an antagonistic effect of imazethapyr was found in a mixture with clomazone and glyphosate.

Imazethapyr applied alone was efficient to control the monocotyledonous infestation up to 36 DAA. There was no effect on eudicotyledonous infestation (Fig. 6E), even though this is an herbicide with a broad spectrum of control (Rodrigues & Almeida, 2011). These results are in line with those of Marchioretto and Magro (2018), who did not find adequate control of imazethapyr on eudicotyledonous weeds in the bean crop for the same tested dose (100 g a.i. ha^{-1}).

Treatments with trifluralin and clomazone + carfentrazone-ethyl did not show effective control of weed competition at 36 DAA, mainly in eudicots. These treatments exhibited infestations of 205.25 and 154.00 plants per square meter, respectively (Fig. 6F and I), only lower than the control. According to Sofiatti *et al.* (2012), trifluralin is a selective herbicide indicated for the control of monocots and some small-sized eudicots, a fact that explains the greater control of monocots by this herbicide.

A lower level of eudicotyledonous infestation was observed after the application of clomazone + carfentrazone-ethyl. This result is in line with those obtained by Sanchotene *et al.* (2017), who tested the effectiveness of pre-emergence herbicide control on the weed competition in the soybean crop. These authors did not obtain effective control of this mixture on eudicotyledonous weeds.

According to Willingham *et al.* (2008), clomazone is a selective and efficient herbicide used for controlling annual grasses in different crops. When mixed with carfentrazone-ethyl, clomazone increases the control spectrum over weed competition. In this study, there was no control effect on the broadleaf weeds, which may be associated with the behavior of the product in the soil and the plant and with the application techniques adopted.

The use of diclosulam alone showed an adequate control of weed competition, mainly of eudicotyledonous weeds up to 36 DAA (Fig. 6G). However, when applied in a mixture with imazethapyr, a reduction in latifolicidal selectivity was observed, which is in line with the study conducted by Vidal *et al.* (2010) that indicated antagonistic interactions (Fig. 6H).

Regarding the effect of pre-emergence treatments on selectivity in soybean cultivation, there was a significant effect ($P<0.05$) of treatments on plant height, canopy closure, number of leaflets, and number of plants per linear meter. The plants treated with diclosulam showed the highest plant height (50.00 cm), which was statistically similar to the other pre-emergence treatments, except for s-metolachlor (40.30 cm) (Tab. 3).

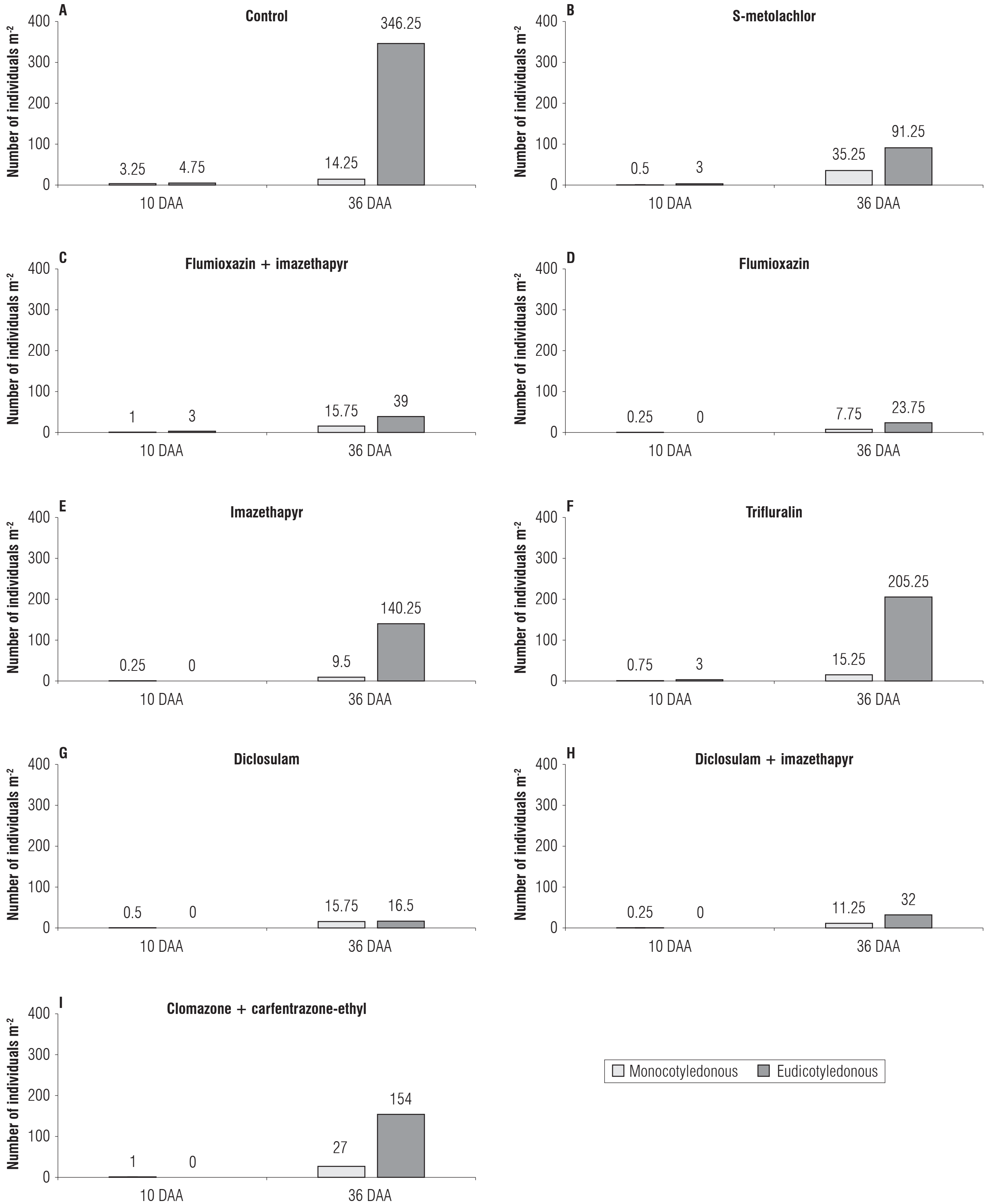


FIGURE 6. Monocotyledonous and eudicotyledonous weed infestation in a commercial soybean plot at 10 and 36 days after application (DAA), under the effect of different pre-emergence molecules: A) control; B) s-metolachlor; C) flumioxazin + imazethapyr; D) flumioxazin; E) imazethapyr; F) trifluralin; G) diclosulam; H) diclosulam + imazethapyr; I) clomazone + carfentrazone-ethyl.

TABLE 3. Effect of pre-emergence treatments on plant height (PH), canopy closure (CC), number of leaflets (NL), and number of plants per linear meter (NPm) at 36 d after application (DAA) in soybean plants.

Treatment	PH (cm)	CC (%)	NL	NPm
Control	44.96AB	68.26C	7.46C	16.33AB
S-metolachlor	40.30B	93.73A	8.00BC	16.66AB
Flumioxazin + Imazethapyr	44.59AB	87.26AB	9.00B	14.00B
Flumioxazin	46.93AB	77.36BC	8.20BC	15.00AB
Imazethapyr	45.76AB	93.43A	8.36AB	15.00AB
Trifluralin	41.73AB	93.06A	8.96AB	17.00A
Diclosulam	50.00A	86.66AB	8.86AB	15.66AB
Diclosulam + Imazethapyr	42.61AB	88.66AB	9.40A	14.66AB
Clomazone + carfentrazone-ethyl	45.08AB	94.93A	8.26ABC	14.33AB
CV (%)	15.42	27.46	1.88	5.47

CV - Coefficient of variation; the means were subjected to the Tukey's test at 5% probability. Equal capital letters indicate the treatments that do not differ between themselves in the column.

According to Santos *et al.* (2012), s-metolachlor is a nonionizable compound that acts as an inhibitor of cell division of the plant's shoot. The results indicated that the herbicide probably produced an unexpected antagonistic effect on the initial growth of soybean simultaneously with the control of the weed seed bank in the soil.

Regarding canopy closure, the control showed the lowest average (68.26%), with the high weed infestation occurring in this treatment at 10 and 36 DAA (Tab. 3; Fig. 7). According to Carvalho *et al.* (2010), the intense competition for water, light, and nutrients in the critical phase of the crop can cause a delay in its growth with negative consequences for the canopy closure and suppression of weed competition. Evident losses may occur at the time the post-emergence application is carried out. Thus, pre-emergence control is recommended as an effective practice to complement chemical control at pre-planting (desiccation) and post-planting (post-emergence).

The effect of flumioxazin on canopy closure and number of leaflets was similar to the control (Tab. 3). According to Gonçalves *et al.* (2018), flumioxazin is a latifolicidal that acts as an inhibitor of the enzyme protoporphyrinogen oxidase, playing a role in the oxidation of protoporphyrinogen IX to protoporphyrin IX, which are precursors of chlorophyll biosynthesis. In this study, there was likely a phytotoxic effect due to the unexpected synthesis of reactive oxygen species and lipid peroxidation, leading to negative impacts on soybean growth.

In relation to the number of plants per linear meter, the lowest average of plants per linear meter was observed for the mixture flumioxazin + imazethapyr, which indicates a negative effect of this mixture on the germination or initial growth of soybeans (Tab. 3). Corroborating these findings, Drehmer *et al.* (2015) demonstrated that the spectrum of control of these active ingredients leads to phytotoxicity effects on legumes when mixed or at high doses.

The analysis of the main components of soybean growth showed a significant correlation between canopy closure and number of leaves, considering that the first component accounts for 39.58% of the total variance of the data; that is, as one of these variables decreases, the other one also decreases (Fig. 7).

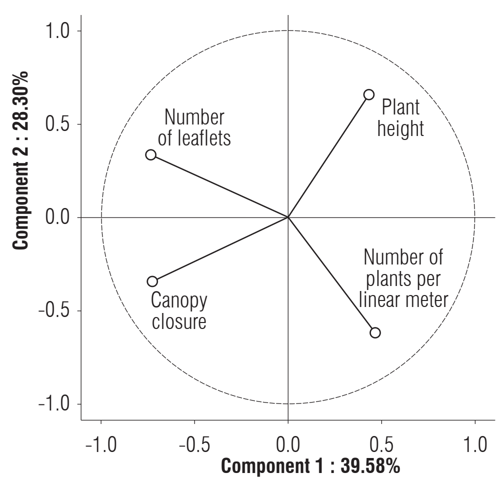


FIGURE 7. Pearson correlation chart for plant height, canopy closure, number of leaflets, and number of plants per linear meter.

These results demonstrate that the components of the crop are linked to the degree of weed interference in such a way that the faster the vegetative growth of the crop, the faster the canopy closure will occur, favoring the suppression of weed competition. According to Pereira *et al*. (2014), this occurs due to the faster soil shading and the competitive potential of the crop by the limiting factors of the environment, which interferes with the germination and establishment of weeds.

Lopes, Silva, Costa, Silva, *et al*. (2020) emphasized that suppression is intensified by the use of pre-emergence chemical control, which produces a chemical barrier on the soil surface and delays the development of the dissemination weed bank. This result is well within the findings in this study for most of the pre-emergence herbicides tested in this research.

Regarding the phytotoxic effect of pre-emergence herbicides on soybean cultivation, the highest scores (3) were attributed, at 10 DAA, to treatments s-metolachlor, flumioxazin + imazethapyr, trifluralin, and diclosulam + imazethapyr with some plants at the useful lines analyzed showing slight visible changes (such as chlorosis and shriveling). At 17 DAA, the persistence of this manifestation in the plots treated with flumioxazin + imazethapyr was noted. This may have influenced the smaller number of plants per linear meter and corroborated the hypothesis previously presented (Tab. 4).

Sanchotene *et al*. (2017) observed the phytotoxic effect of flumioxazin + imazethapyr on the soybean crop in two tested doses (50 + 150 and 50 + 104 g a.i. ha^{-1}). The results indicated a low selectivity of that mixture for pre-emergence application on soybean cultivation, although it showed effective control of weed competition.

Clomazone + carfentrazone-ethyl mixture was the pre-emerging treatment that exhibited the highest selectivity on soybean culture. This result was similar to the control (without pre-emergence application) and, therefore, the absence of phytotoxicity at 10, 17, 24, and 36 DAA. These results corroborate those found by Sanchotene *et al*. (2017) when testing the performance of different pre-emergence herbicides for the control of *Euphorbia heterophylla* in soybean. Moreover, these authors did not observe expressions of phytotoxicity of this herbicide at a dose of 600 + 15 g a.i. ha^{-1}.

Conclusions

In this study, 19 species of weeds were identified, distributed in 17 genera and 13 botanical families. The species *Scoparia dulcis, Richardia scabra*, and *Cyperus iria* exhibited the highest phytosociological indices (123.77, 28.62, and 28.29, respectively), estimated at 36 DAA.

Flumioxazin and diclosulam were the most efficient in suppressing weed competition, with only 15.63 and 16.13 plants m^{-2}. The highest phytotoxicity scores (3.0) were found at 10 DAA, with the application of s-metolachlor, flumioxazin + imazethapyr, trifluralin, and diclosulam + imazethapyr.

The pre-emergent control using flumioxazin and diclosulam is recommended for the edaphoclimatic conditions in the Eastern mesoregion of the state of Maranhão, Brazil.

Acknowledgments

The authors would like to thank the Foundation for Research and Scientific and Technological Development of Maranhão (FAPEMA) for the financial resources, and the Nationwide Grant Program (universal call) no. 002/2019, process 795/19. We would also like to thank the Brazilian Association of Soybean Growers (Aprosoja Brasil) of Maranhão.

TABLE 4. Scores attributed to the visual effects of phytotoxicity of treatments on soybean cultivation at 10, 17, 24, and 36 d after application (DAA).

Pre-emergence	Dose (g a.i. ha^{-1})	Phytotoxicity degree			
		10 DAA	17 DAA	24 DAA	36 DAA
Control	-	1	1	1	1
S-metolachlor	1.200	3	2	2	1
Flumioxazin + Imazethapyr	50 + 100	3	3	2	1
Flumioxazin	50	2	1	1	1
Imazethapyr	100	2	2	1	1
Trifluralin	1.112,5	3	2	2	1
Diclosulam	45	2	1	1	1
Diclosulam + imazethapyr	30 + 100	3	2	2	1
Clomazone + carfentrazone-ethyl	720 + 18	1	1	1	1

Conflict of interest statement

The authors declare that there is no conflict of interest regarding the publication of this article.

Author's contributions

MSS and JALF wrote the article. WSS and EIBA formulated the overarching research goals and aims and obtained the financial support for the project leading to this publication. JQC and ILS performed field data collection. LBTO applied statistical, mathematical, and computational techniques to analyze and synthesize study data. RCAA contributed to the writing of the article. All authors reviewed the manuscript.

Literature cited

Alonso, D. G., Oliveira Jr., R. S., & Constantin, J. (2013). Potencial de *carryover* de herbicidas com atividade residual usados em manejo outonal. In J. Constantin, R. S. Oliveira Jr., & A. M. Oliveira Neto (Eds.), *Buva: fundamentos e recomendações para manejo* (pp. 91–104). Omnipax Editora.

Andrade Junior, E. J., Barroso, A. L. L., Moraes, V. H., Gomes, F. H. F., Bastos, A. V. S., & Lopes Filho, L. C. (2018). Controle residual de capim amargoso na soja cultivada em região de Cerrado. *Científic@ Multidisciplinary Journal*, *5*(3), 48–55. https://doi.org/10.29247/2358-260X.2018v5i3.p48-55

Cardoso, A. D., Viana, A. E. S., Barbosa, R. P., Teixeira, P. R. G., Cardoso Júnior, N. S., & Fogaça, J. J. N. L. (2013). Levantamento fitossociológico de plantas daninhas na cultura da mandioca em Vitória da Conquista, Bahia. *Bioscience Journal*, *29*(5), 1130–1140.

Carvalho, L. B., Bianco, S., & Guzzo, C. D. (2010). Interferência de *Euphorbia heterophylla* no crescimento e acúmulo de macronutrientes da soja. *Planta Daninha*, *28*(1), 33–39. https://doi.org/10.1590/S0100-83582010000100004

CONAB. (2020). *Acompanhamento da safra brasileira: grãos. V. 7 - safra 2019/20 - n. 12 - décimo segundo levantamento setembro 2020.* Companhia Nacional de Abastecimento. https://www.conab.gov.br/info-agro/safras/graos/boletim-da-safra-de-graos/item/download/33275_6780e71910d3f0d489c5f171231b65cd

Conte, O., Prando, A. M., Castro, C., Balbinot Junior, A. A., Campos, L. J. M., Hirakuri, M. H., Lima, D., Oliveira, A. B., Tavares, L. C. V., Oliveira Junior, A., Debiasi, H., Bortolon, L., Vieira, P. F. M. J., Souza, H. A., Procópio, S. O., Alves, L. W. R., Santos, J. C., Sena, A. L. S., & El Husny, J. C. (2018). A evolução da produção de soja na macrorregião sojícola 5. In M. H. Hirakuri, O. Conte, A. M. Prando, C. Castro, & A. A. Balbinot Junior (Eds.), *Diagnóstico da produção de soja na macrorregião sojícola 5* (pp. 23–61). Embrapa Soja.

Dantas, J. S., Marques Júnior, J., Martins Filho, M. V., Resende, J. M. A., Camargo, L. A., & Barbosa, R. S. (2014). Gênese de solos coesos do leste maranhense: relação solo-paisagem. *Revista Brasileira de Ciência do Solo*, *38*, 1039–1050. https://doi.org/10.1590/S0100-06832014000400001

Drehmer, M. H., Zagonel, J., Ferreira, C., & Senger, M. (2015). Eficiência de herbicidas aplicados em pré-emergência para o controle de *Digitaria insularis* na cultura do feijão. *Revista Brasileira de Herbicidas*, *14*(2), 148–154. https://doi.org/10.7824/rbh.v14i2.395

EMBRAPA. (2013). *Sistemas de produção 16 - tecnologias de produção de soja - Região Central do Brasil 2014.* Embrapa Soja. https://ainfo.cnptia.embrapa.br/digital/bitstream/item/95489/1/SP-16-online.pdf

Fialho, C. M. T., Santos, J. B., Freitas, M. A. M., França, A. C., Silva, A. A., & Santos, E. A. (2011). Fitossociologia da comunidade de plantas daninhas na cultura da soja transgênica sob dois sistemas de preparo do solo. *Scientia Agraria*, *12*(1), 9–17. https://doi.org/10.5380/rsa.v12i1.33634

Fried, G., Chauvel, B., Reynaud, P., & Sache, I. (2017). Decreases in crop production by non-native weeds, pests, and pathogens. In M. Vilà, & P. E. Hulme (Eds.), *Impact of biological invasions on ecosystem services* (pp. 83–101). Springer.

Gazola, T., Dias, M. F., Belapart, D., Castro, E. B., & Bianchi, L. (2016). Efeitos do diclosulam na soja cultivada em solos de diferentes classes texturais. *Revista Brasileira de Herbicidas*, *15*(4), 353–361. https://doi.org/10.7824/rbh.v15i4.483

Gazziero, D. L. P. (2015). Misturas de agrotóxicos em tanque nas propriedades agrícolas do Brasil. *Planta Daninha*, *33*(1), 83–92. https://doi.org/10.1590/S0100-83582015000100010

Gonçalves, F. A. R., Melo, C. A. D., Queiroz, P. C., Endo, R. T., Silva, D. V., & Reis, M. R. (2018). Atividade residual de herbicidas nas culturas do milho e da soja. *Revista de Ciências Agrárias*, *61*, 1–6. https://doi.org/10.22491/rca.2018.2570

INMET. (2019). *Dados históricos anuais - ano 2019*. Instituto Nacional de Meteorologia. https://portal.inmet.gov.br/dadoshistoricos

Jadhav, J., Amaregouda, A., Chetti, M. B., Hiremath, S. M., Nawalgatti, C. M., & Gali, S. K. (2013). Effect of herbicides on weed growth, yield and yield components of soybean (*Glycine max* L.). *Karnataka Journal of Agricultural Sciences*, *26*(2), 314–315.

Johannes, H. (1964). Report of the third and fourth meetings of the European Weed Research Council Committee on methods. *Weed Research*, *4*(1), 79. https://doi.org/10.1111/j.1365-3180.1964.tb00271.x

Lopes, K. A. L., Silva, M. S., Costa, L. S., Oliveira, A. K. S., Silva, E. A., Almeida, E. I. B., Oliveira, I. R., Oliveira, L. B. T., Sousa, W. S., & Freitas, J. R. B. (2020). Fitossociologia do banco de sementes de plantas daninhas em campo agrícola e vegetação de cerrado. *Revista Ibero-Americana de Ciências Ambientais*, *11*(4), 362–370. https://doi.org/10.6008/CBPC2179-6858.2020.004.0029

Lopes, K. A. L., Silva, M. S., Costa, L. S., Silva, T. F., Costa, T. V., Almeida, E. I. B., Oliveira, I. R., Freitas, J. R. B., Sousa, W. S., & Oliveira, L. B. T. (2020). Spatial distribution of weed seed banks in the agricultural field and anthropized Cerrado. *Journal of Agricultural Studies*, *8*(2), 480–497. https://doi.org/10.5296/jas.v8i2.16031

Lorenzi, H. (2014). *Manual de identificação e controle de plantas daninhas: plantio direto e convencional*. Plantarum.

Machado, F. G., Jakelaitis, A., Gheno, E. A., Oliveira Jr., R. S., Rios, F. A., Franchini, L. H. M., & Lima, M. S. (2016). Performance de herbicidas para o controle de plantas daninhas no sorgo. *Revista Brasileira de Herbicidas*, *15*(3), 281–289. https://doi.org/10.7824/rbh.v15i3.476

Marchioretto, L. D. R., & Magro, T. D. (2018). Efeito protetor do bentazon sobre a fitotoxicidade de herbicidas inibidores de ALS em duas cultivares de feijoeiro. *Revista de Ciências Agroveterinárias*, *17*(1), 77–82. https://doi.org/10.5965/223811711712018077

Martins, B. A. B., & Christoffoleti, P. J. (2014). Herbicide efficacy on *Borreria densiflora* control in pre- and post-emergence conditions. *Planta Daninha*, *32*(4), 817–825. https://doi.org/10.1590/S0100-83582014000400017

Oliveira, A. R., & Freitas, S. P. (2008). Levantamento fitossociológico de plantas daninhas em áreas de produção de cana-de-açúcar. *Planta Daninha*, *26*(1), 33–46. https://doi.org/10.1590/S0100-83582008000100004

Passos, M. L. V., Zambrzycki, G. C., & Pereira, R. S. (2016). Balanço hídrico e classificação climática para uma determinada região de Chapadinha-MA. *Revista Brasileira de Agricultura Irrigada*, *10*(4), 758–766. https://doi.org/10.7127/rbai.v10n400402

Pereira, F. C. M., Barroso, A. A. M., Albrecht, A. J. P., & Alves, P. L. C. A. (2014). Interferência de plantas daninhas: conceitos e exemplos na cultura do eucalipto. *Journal of Agronomic Sciences*, *3*(n. especial), 236–255.

Placido, H. F., Albrecht, A. J. P., Santos, R. F., Albrecht, L. P., Becker, A. S., Barroso, A. A. M., & Victória Filho, R. (2016). Identificação e manejo de biótipos de *Chloris polydactyla* com resistência ou suscetibilidade diferencial ao glyphosate no Estado do Paraná. *Revista Brasileira de Herbicidas*, *15*(3), 251–262. https://doi.org/10.7824/rbh.v15i3.449

Rizzardi, M. A., & Silva, L. (2014). Manejo de plantas daninhas eudicotiledôneas na cultura da soja Roundup Ready®. *Planta Daninha*, *32*(4), 683–697.

Rodrigues, B. N., & Almeida, F. S. (2011). *Guia de herbicidas*. Edição dos Autores.

Sanchotene, D. M., Dornelles, S. H. B., Bolzan, T. M., Voss, H. M. G., Escobar, O. S., Leon, C. B., Muller, E. N., & Shimóia, E. P. (2017). Desempenho de diferentes herbicidas pré-emergentes para controle de *Euphorbia heterophylla* na cultura da soja. *Perspectiva*, *41*(155), 7–15.

Santos, G., Francischini, A. C., Constantin, J., & Oliveira Jr., R. S. (2012). *Carryover* proporcionado pelos herbicidas s-metolachlor e trifluralin nas culturas de feijão, milho e soja. *Planta Daninha*, *30*(4), 827–834. https://doi.org/10.1590/S0100-83582012000400017

Silva, M. S., Costa, T. V., Furtado, J. A. L., Souza, J. B. C., Silva, E. A., Ferreira, L. S., Silva, C. A. A. C., Almeida, E. I. B., Sousa, W. S., Oliveira, L. B. T., Freitas, J. R. B., & Oliveira, J. T. (2021). Performance of pre-emergence herbicides in weed competition and soybean agronomic components. *Australian Journal of Crop Science*, *15*(4), 610–617.

Sofiatti, V., Severino, L. S., Silva, F. M. O., Silva, V. N. B., & Brito, G. G. (2012). Pre and postemergence herbicides for weed control in castor crop. *Industrial Crops and Products*, *37*(1), 235–237. https://doi.org/10.1016/j.indcrop.2011.12.019

Takano, H. K., Oliveira Jr., R. S., Constantin, J., Biffe, D. F., Franchini, L. H. M., Braz, G. B. P., *Rios, F. A., Gheno, E. A.,* & Gemelli, A. (2013). Efeito da adição do 2,4-D ao glyphosate para o controle de espécies de plantas daninhas de difícil controle. *Revista Brasileira de Herbicidas*, *12*(1), 1–13. https://doi.org/10.7824/rbh.v12i1.207

University of Hertfordshire. (2020). *PPDB: pesticide properties database - trifluralin*. http://sitem.herts.ac.uk/aeru/ppdb/en/Reports/667.htm

Vidal, R. A., Rainero, H. P., Kalsing, A., & Trezzi, M. M. (2010). Prospección de las combinaciones de herbicidas para prevenir malezas tolerantes y resistentes al glifosato. *Planta Daninha*, *28*(1), 159–165. https://doi.org/10.1590/S0100-83582010000100019

Willingham, S. D., Falkenberg, N. R., McCauley, G. N., & Chandler, J. M. (2008). Early postemergence clomazone tank mixes on coarse-textured soils in rice. *Weed Technology*, *22*(4), 565–570. https://doi.org/10.1614/WT-08-051.1

Co-inoculation with a product based on native microorganisms improves germination and seedling growth of *Phaseolus vulgaris* L.

La co-inoculación con un producto a base de microorganismos nativos mejora la germinación y el crecimiento de plántulas de *Phaseolus vulgaris* L.

Maykelis Díaz Solares[1], Leticia Fuentes Alfonso[2], Madyu Matos Trujillo[2], Ramón Liriano González[2], Liliet González Sierra[1], and Yunel Pérez Hernández[2*]

ABSTRACT

Agricultural products based on native microorganisms represent an ecological alternative to traditional chemical fertilizers for enhancing growth and crop yield. This study aimed to evaluate the effect of the product based on native microorganisms IHPLUS® on germination, emergence, and primary leaf formation of *Phaseolus vulgaris* L. seedlings. The treatments consisted of a control (immersion in distilled water) and nine treatments that were the combination of IHPLUS® at three concentrations and three immersion times. The application of IHPLUS® significantly increased the percentage of germination, mainly in the first days. After 7 d, the germination rate and root and hypocotyl lengths increased in almost all treatments compared to control seedlings. The beneficial effects of IHPLUS® on the germination of *P. vulgaris* may be partially attributed to changes on seedling metabolism due to an increase in α-amylase activity and in the content of reducing sugars and soluble proteins. Results suggest that IHPLUS® may act as an enhancer of germination in common beans that might lead to rapid seed germination, uniform seedling growth, and better seedling establishment and crop productivity.

Key words: agroecology, biochemistry, common bean, efficient microorganisms.

RESUMEN

Los productos agrícolas a base de microorganismos nativos constituyen una alternativa ecológica al uso tradicional de fertilizantes químicos para potenciar el crecimiento y el rendimiento de los cultivos. Este estudio tuvo como objetivo evaluar el efecto del producto a base de microorganismos nativos IHPLUS® en la germinación, emergencia y formación de hojas primarias de plántulas de *Phaseolus vulgaris* L. Los tratamientos consistieron en un control (inmersión en agua destilada) y nueve tratamientos con la combinación de tres concentraciones de IHPLUS® y tres tiempos de inmersión. La aplicación de IHPLUS® incrementó significativamente el porcentaje de germinación principalmente en los primeros días. Después de 7 d, la tasa de germinación y la longitud de la raíz y del hipocótilo aumentaron en casi todos los tratamientos con relación a las plántulas control. El efecto benéfico de IHPLUS® sobre la germinación de *P. vulgaris* se puede atribuir, en parte, a cambios en el metabolismo de las plántulas debidos a un aumento en la actividad de la α-amilasa y en el contenido de azúcares reductores y las proteínas solubles. Los resultados sugieren que el IHPLUS® puede actuar como un potenciador de la germinación en el frijol común, lo que puede contribuir a elevar la velocidad de germinación, el crecimiento uniforme de las plántulas y a un mejor establecimiento y productividad del cultivo.

Palabras clave: agroecología, bioquímica, frijol común, microorganismos eficientes.

Introduction

The excessive use of hazardous agrochemicals in the world to achieve high crop yields has caught the attention of the scientific community and consumers, because of the negative effects on the ecosystems and human health. The indiscriminate application of agrochemicals may increase waterway eutrophication and soil contamination with toxic heavy metals. Additionally, synthetic pesticides negatively affect biodiversity and reinforce pest resistance (Alori *et al*., 2017). As a viable alternative to conventional production systems, agroecological and sustainable agriculture represents a tool for reducing the use of synthetic fertilizers and pesticides to preserve the environment.

The development of new bio-agrochemicals based on soil microorganisms has emerged as a promising approach to achieve ecological management of agroecosystems (Sözer

Received for publication: October 25, 2020. Accepted for publication: December 27, 2021.

Doi: 10.15446/agron.colomb.v39n3.91841

[1] Experimental Station of Pasture and Forage "Indio Hatuey", University of Matanzas, Matanzas (Cuba).
[2] Center for Biotechnology Studies, Faculty of Agronomy, University of Matanzas, Matanzas (Cuba).
[*] Corresponding author: yunel.perez@umcc.cu

Bahadir *et al*., 2018). The technology of effective microorganisms (EM), also known as native or autochthonous microorganisms, consists of the inoculation of a mixed culture of beneficial microorganisms into the soil, to establish a favorable environment for crop growth and health (Olle & Williams, 2015).

Effective microorganisms promote plant growth and development in different ways. Direct mechanisms involve the production of plant hormones such as auxins, cytokinins, and gibberellins (Felestrino *et al*., 2017), the solubilization of minerals (Thakur *et al*., 2017), biological nitrogen fixation (Nushair *et al*., 2018), and the synthesis of iron-chelating siderophores. Indirect mechanisms include the production of antimicrobial agents and lytic enzymes that reduce populations of plant pathogens (Maan *et al*., 2019). Soil microorganisms are currently used to reduce the organic contaminants that result from industrial activities (Tarekegn *et al*., 2020) and to alleviate abiotic stresses like drought and salinity (Jochum *et al*., 2019).

The common bean (*Phaseolus vulgaris* L.) is considered one of the most cultivated and consumed dry grain legumes in developing countries and constitutes a staple food for Cuban populations. The nutritional and health benefits of this grain, rich in proteins, carbohydrates, minerals, vitamins, and dietary fiber, have been well documented (Celmeli *et al*., 2018; Rezende *et al*., 2018).

Research has focused on improving vegetative growth and yield of the common bean, using beneficial microorganisms shown to be effective in enhancing plant morphological and physiological parameters, such as number of leaves, dry weight, and yield components (Vasallo Cristia *et al*., 2018; Calero Hurtado *et al*., 2019; Calero-Hurtado *et al*., 2020; Gabre *et al*., 2020). However, the beneficial effects of EM on seed germination may also play a pivotal role in the development of the common bean and other important crops since seed inoculation with plant growth-promoting microorganisms (PGPM) leads to an improvement of vigor and seedling growth uniformity (Ayala-Villegas *et al*., 2014).

Additionally, seed germination is considered the most vulnerable stage in the life cycle of plants and its performance defines crop establishment and yield (Channaoui *et al*., 2017). Various studies have been conducted to enhance *P. vulgaris* germination and seedling growth by using PGPM as well as to elucidate the biochemical and physiological mechanisms by which those microorganisms stimulate seed germination (Yadav *et al*., 2013; Talaat *et al*., 2015; Kumar *et al*., 2016).

IHPLUS® is an EM-based product shown to be versatile for improving crop productivity and animal health and production (Blanco-Betancourt *et al*., 2017; Tellez-Soria & Orberá-Ratón, 2018). The hypothesis that IHPLUS® could improve germination and seedling growth of the common bean was tested under controlled conditions. The objective of this study was to evaluate the effect of IHPLUS® on germination and seedling growth of *P. vulgaris* L. cv. Tomeguín.

Materials and methods

Inoculum of IHPLUS®

This research was developed in the Center for Biotechnology Studies of the University of Matanzas, Cuba. The germination experiment was performed under controlled laboratory conditions. The liquid inoculum of IHPLUS® was produced at the Pasture and Forage Experimental Station, Perico, Cuba and is a natural product based on native microorganisms. The composition of the main groups of beneficial microorganisms are shown in Table 1. The pH of the product was 3.45 and microbiological analysis showed no pathogenic microorganisms in the medium.

TABLE 1. Microbiological composition of the liquid inoculum of IHPLUS®.

Content of microorganisms (CFU ml^{-1})	
Aerobic bacteria	$1x10^6$
Anaerobic bacteria	$1x10^5$
Fungi and yeasts	$1x10^4$ - $1x10^5$
Lactobacillus	$1x10^4$

CFU - Colony-forming unit.

Germination test

Seeds of *P. vulgaris* L. cv. Tomeguín were first immersed in concentrations of IHPLUS® (2, 4, and 6%) during three immersion times (2, 4, 6 h) plus the control (distilled water) (Tab. 2). Ten seeds per Petri dish (9 cm diameter) were placed on Whatman no. 1 filter paper containing sterile distilled water in a proportion of three times the weight of the dry substrate (ISTA, 2014). Four replicates of each treatment were performed. The seeds were kept in a growing room at 25 ± 2°C with a photoperiod of 16 h (35 µmol m^{-2} s^{-1}). Germination was evaluated daily for 7 d and the results were expressed as percentages of normal seedlings. Seeds were considered germinated when a radicle of at least 2 mm length emerged.

TABLE 2. Combinations of different concentrations of IHPLUS® and time of immersion used as experimental treatments.

Seed treatments	IHPLUS® (%)	Immersion time (h) into IHPLUS®
T1 (control)	0	6 (water)
T2	2	2
T3	2	4
T4	2	6
T5	4	2
T6	4	4
T7	4	6
T8	6	2
T9	6	4
T10	6	6

Germination rate and root and shoot length

Germination rate (GR) was determined using the formula by Djavanshir and Pourbeik (1976).

$$GR = \left(\sum_{i=1}^{n} Sdei\right)\left(\frac{Ef}{10N}\right) \quad (1)$$

where *Sdei* is the speed of daily radicle emergence, calculated as the ratio between the percentage of accumulated emergence and the number of days since the beginning of the test. *N* is the frequency or number of *Sde* determined during the test, and *Ef* is the percentage of seedling emergence at the end of the experiment. Root and hypocotyl length (cm) were determined by using a sheet of graph paper.

Biochemical assays

Crude enzymatic extract preparation

Roots and shoots of 7-d-old seedlings were used to prepare the crude enzymatic extracts. Extracts were carried out by homogenizing plant material in a cold solution of sodium citrate buffer pH = 5.0 in a proportion of 1:2 (w/v). The homogenates were centrifuged at 10000 rpm at 4°C and the supernatants were collected and stored at -20°C for the biochemical assays.

α-amylase activity

The α-amylase activity in the seeds was determined by the procedure described by Díaz Solares *et al*. (2019). Enzymatic activity was expressed as a unit of α-amylase ml^{-1}. One unit of α-amylase is defined as the amount of the enzyme that liberates 1.0 μmol of reducing sugars per min with D-glucose as a standard under the enzyme activity conditions.

Content of reducing sugars

The content of reducing sugars was determined by the method of 3.5-dinitrosalicylic acid (Miller, 1959). D-glucose (Sigma-Aldrich, Steinheim, Germany) was used as a standard, and the absorbance was measured at 456 nm.

Content of soluble proteins

Soluble protein content was determined according to Lowry *et al*. (1951) using bovine serum albumin (BSA) as the standard. Absorbance values were recorded at 750 nm and the concentration (mg ml^{-1}) was calculated using a standard curve. All the spectrophotometric readings were performed in a UV/VIS spectrophotometer (Ultrospec 2000, Pharmacia Biotech, Sweden).

Experimental design and statistical analysis

The germination assay was conducted using a completely randomized design with four replicates (Petri dishes) per treatment. Five seedlings per each treatment were randomly selected to carry out the biochemical assays. Morphological parameters were evaluated by measuring 10 seedlings per treatment. Data were processed with the statistical program SPSS version 18.0 for Windows. The significance for each evaluated parameter was established by one-way analysis of variance (ANOVA) and the means were separated by the Tukey's test ($P \leq 0.05$), once the normal distribution of data and the homogeneity of variance were determined using the Shapiro-Wilk and Levene's tests, respectively.

Results and discussion

Effect of IHPLUS® on the germination process

The results indicated that IHPLUS® had a significant effect on the germination of *P. vulgaris* during the first days of this process (Fig. 1). The greatest difference among treatments was observed at d 1, when all the treatments containing IHPLUS® (except for T2) led to an increase in the percentage of germination when compared to the control. Values in those treatments ranged from 63% (T9) up to 88% (T6), with non-statistical differences, whereas T1 (Control, 30%) and T2 (43%) showed the lowest percentages without differences between them. The highest increases were observed in T6 (2.93-fold), T5 and T8 (2.66-fold) compared to the control.

After 3 d, T6 (98%), T5 and T8 (95%), and T4 and T7 (93%) showed the highest germination percentages among the evaluated treatments, compared to the control with 73%. These results agree with other reports in *P. vulgaris* L. (Saxena *et al*., 2013) and *Capsicum annuum* L. (Marquina *et al*., 2018), where seed inoculation with PGPM also increased germination during the first days of the experiment. This is an important result since EM-based IHPLUS® seems to quickly activate seed metabolism that could be of interest in areas where water is limited.

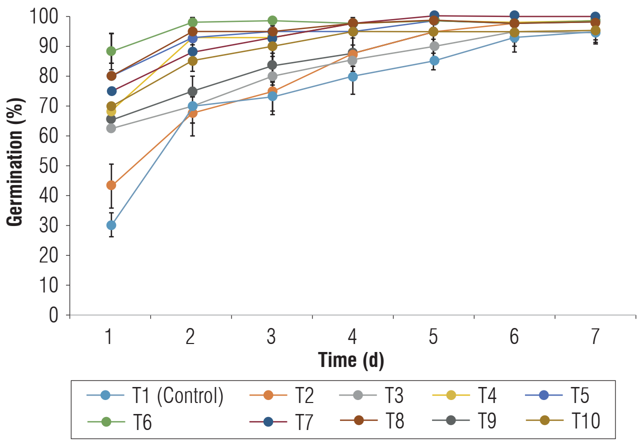


FIGURE 1. Effect of IHPLUS® on the percentage of germination of *P. vulgaris* L. cv. Tomeguín. T1: Control (immersion in distilled water for 6 h); T2: 2% IHPLUS® - immersion for 2 h; T3: 2% IHPLUS® - immersion for 4 h; T4: 2% IHPLUS® - immersion for 6 h; T5: 4% IHPLUS® - immersion for 2 h; T6: 4% IHPLUS® - immersion for 4 h; T7: 4% IHPLUS® - immersion for 6 h; T8: 6% IHPLUS® - immersion for 2 h; T9: 6% IHPLUS® - immersion for 4 h, and T10: 6% IHPLUS® - immersion for 6 h. Data are the mean of four replicates and vertical bars represent the standard error ($P \leq 0.05$).

After 7 d of the experiment, the results were similar for all the treatments and control with germination percentages above 95%. This is consistent with data reported by Wangdi *et al.* (2020), who obtained germination percentages higher than 95% in *P. vulgaris* L. after seed inoculation with beneficial microorganisms and with no differences between untreated (control) and treated seeds. This may be associated with higher viability and seed vigor as well as suitable conditions of humidity and oxygen that ensure an efficient germination process (Finch-Savage & Bassel, 2016).

Similar studies in *P. vulgaris* have shown the ability of PGPM to improve the final germination percentage (Custodio *et al.*, 2013; Yadav *et al.*, 2013; Kumar *et al.*, 2016; Romero-García *et al.*, 2016). However, in those studies, control seeds showed percentages of germination lower than 75% that were suitable for improvement.

The beneficial effect of IHPLUS® on the germination of *P. vulgaris* might be attributed to the presence of plant growth promoter substances such as auxins, gibberellins, and cytokinins that have been reported as responsible for the growth-promoting response during embryo elongation and seedling growth of *Capsicum annuum* L. during the first days of germination (Marquina *et al.*, 2018). These phytohormones are commonly produced by a large number of soil microorganisms (Taiwo *et al.*, 2017).

Among plant hormones, the natural auxin indole 3-acetic acid (IAA) is synthetized by 80% of the soil bacteria and other microbial species (Abri *et al.*, 2015). Recently, IAA was reportedly produced by bacillus strains isolated from IHPLUS® (Pérez-Hernández *et al.*, 2020). Gibberellins (GAs) are involved in triggering the germination process by inducing the expression of α-amylase that hydrolyzes the starch reserve into glucose required to produce metabolic energy for embryo growth (Taiz & Zeiger, 2010). On the other hand, auxins and cytokinins act by enhancing cell division and elongation (Thakur *et al.*, 2017).

Germination rate and seedling length

Table 3 shows the effect of IHPLUS® on the germination rate and seedling length of *P. vulgaris* L. The application of IHPLUS® increased the germination rate in all treatments, except for T2 that showed no difference when compared to control seedlings. The highest germination rate was observed when seeds were immersed into 4% IHPLUS® for 4h (T6) with a value of 3.39 (1.61-fold higher than control seeds immersed in distilled water at 2.11).

The positive effect of beneficial microorganisms on germination rate was reported in *P. vulgaris* L. (Saxena *et al.*, 2013) and other crops such as *Solanum lycopersicon* L. and *Zea mays* L. (Mahadevamurthy *et al.*, 2016). The application of IHPLUS® also brought forward the day at which the higher number of germinated seeds was recorded (d 1), compared to the control that showed the higher germination on the second day after experimental initiation. This could be an important result, since those seeds that germinate in a short period of time show higher vigor and seedling growth uniformity (Ayala-Villegas *et al.*, 2014). IHPLUS® promoted root and hypocotyl growth (Tab. 3). The highest increases in root length were reported in T6 (26.6%), T4 (21.4%) and T9 (19.7%), with non-statistical differences among them, but significantly higher compared to the control. The rest of the treatments showed similar results to those observed in the control. IHPLUS® significantly increased hypocotyl length in all the tested treatments, although the maximum values were recorded for T8 (33.8%), T5 (32.2%), and T6 (29.8%) when compared to control.

The growth-promoting effect of beneficial microorganisms on roots and shoots has been previously reported in *P. vulgaris* L. (Yadav *et al.*, 2013; Kumar *et al.*, 2016;

TABLE 3. Effect of IHPLUS® on the germination rate and seeding growth of *P. vulgaris* L. cv. Tomeguín.

Treatments	Germination rate	Seedling length (cm)	
		Root	Hypocotyl
T1 (Control)	2.11 c ± 0.161	10.05 b ± 0.513	6.84 c ± 0.512
T2	2.40 bc ± 0.123	11.40 ab ± 0.452	8.84 ab ± 0.455
T3	2.89 ab ± 0.117	10.98 ab ± 0.410	8.19 b ± 0.410
T4	3.06 ab ± 0.185	12.20 a ± 0.425	8.65 ab ± 0.423
T5	3.23 ab ± 0.154	11.49 ab ± 0.588	9.04 a ± 0.581
T6	3.39 a ± 0.161	12.73 a ± 0.576	8.88 ab ± 0.576
T7	3.21 ab ± 0.214	11.98 ab ± 0.691	8.25 b ± 0.693
T8	3.26 ab ± 0.162	11.65 ab ± 0.717	9.15 a ± 0.772
T9	2.93 ab ± 0.177	12.03 a ± 0.790	8.53 ab ± 0.794
T10	2.90 ab ± 0.143	11.62 ab ± 0.582	8.15 b ± 0.584

Different letters indicate statistical differences among treatments for each column according to the Tukey's test ($P<0.05$). T1: Control (immersion in distilled water for 6 h); T2: 2% IHPLUS® - immersion for 2 h; T3: 2% IHPLUS® - immersion for 4 h; T4: 2% IHPLUS® - immersion for 6 h; T5: 4% IHPLUS® - immersion for 2 h; T6: 4% IHPLUS® - immersion for 4 h; T7: 4% IHPLUS® - immersion for 6 h; T8: 6% IHPLUS® - immersion for 2 h; T9: 6% IHPLUS® - immersion for 4 h, and T10: 6% IHPLUS® - immersion for 6 h. Data are the mean of four replicates ± standard error.

Romero-García *et al*., 2016; Ugochi *et al*., 2016). IHPLUS® was also found to increase vegetative growth of *Beta vulgaris* L., alone or in combination with *Brevibacillus borstelensis* B65 (Tellez-Soria & Orberá-Ratón, 2018). Since germinating seeds receive most of the nutrients from reserve food material available in the endosperm, the growth-promoting effect of IHPLUS® on the root and hypocotyl of *P. vulgaris* can be attributed to the action of phytohormones, nutrients and/or metabolites present in the IHPLUS® inoculum. Nevertheless, other experiments need to be carried out to determine the agents involved in the growth-promoting effect observed.

The recorded variations among IHPLUS® treatments may be associated with the balance among phytohormones established inside the seed tissues, after their treatment with IHPLUS® inoculum at different concentrations and times of immersion. The specific amount of endogenous and exogenous plant hormones inside the seed tissues may interact with secondary metabolites that can interfere with these hormonal pathways, resulting in a specific plant growth-promoting response. The balance between auxins and cytokinins is fundamental for plant organogenesis, and the balance shapes root architecture (Taiwo *et al*., 2017). Low levels of IAA can enhance primary root elongation, whereas high IAA concentration promotes the formation of lateral roots, decreases primary root length, and increases the formation of root hairs (Remans *et al*., 2008). Active GAs not only stimulate germination but also increase plant growth by inducing primary root elongation and lateral root extension (Yaxley *et al*., 2001).

Biochemical parameters

α-amylase activity

Although the beneficial effect of PGPM on the germination and vegetative growth of the common bean has been well-documented under laboratory and field conditions, little is known about the impact of single microorganisms or consortia on the amylotytic activity of *P. vulgaris* during germination and seedling growth. The effect of IHPLUS® on the α-amylase activity in root and hypocotyl is shown in Figure 2. The enzymatic activity in roots increased with the application of 2% IHPLUS® for 2 h (T2) and 4 h (T3) compared to control (Fig. 2). The amylolytic activity for the rest of the treatments was not significantly different between them and the control, except for T6, T9 and T10 that showed a decrease when compared to the control. The α-amylase activity in the hypocotyl significantly increased in T2, T3, T5, T9 and T10 as compared to the control. This result is consistent with other reports in *Oryza sativa* L., where seeds treated with biofertilizers based on rhizobacteria increased in amylolytic activity (Mohd Din *et al*., 2014). These authors also demonstrated that α-amylase activity is determined by several factors, such as plant genotype, the composition and concentration of the microorganisms present in the biofertilizer, as well as the physiological stage of the plant.

The increase in α-amylase activity might result from the higher GA/abscisic acid (ABA) ratio inside the seed tissues that, in turn, depends on the endogenous GA synthetized by the embryo and the amount of exogenous GA contained

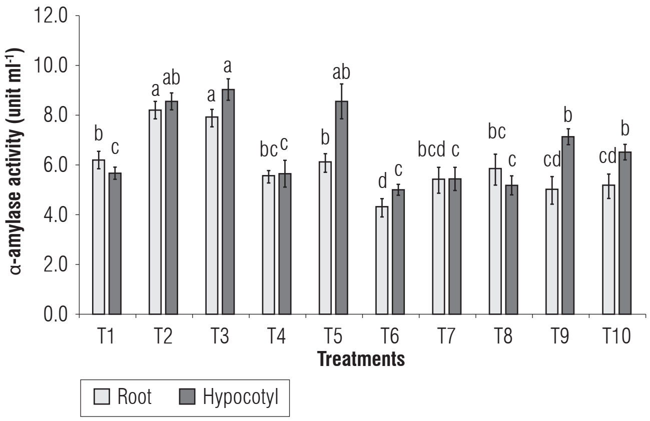


FIGURE 2. Effect of IHPLUS® on α-amylase activity of roots and shoots of *P. vulgaris* L. cv. Tomeguín. Different letters indicate statistical differences among the treatments according to the Tukey's test ($P<0.05$). T1: Control (immersion in distilled water for 6 h); T2: 2% IHPLUS® - immersion for 2 h; T3: 2% IHPLUS® - immersion for 4 h; T4: 2% IHPLUS® - immersion for 6 h; T5: 4% IHPLUS® - immersion for 2 h; T6: 4% IHPLUS® - immersion for 4 h; T7: 4% IHPLUS® - immersion for 6 h; T8: 6% IHPLUS® - immersion for 2 h; T9: 6% IHPLUS® - immersion for 4 h, and T10: 6% IHPLUS® - immersion for 6 h. Data are the mean of four replicates and vertical bars represent the standard error ($P<0.05$).

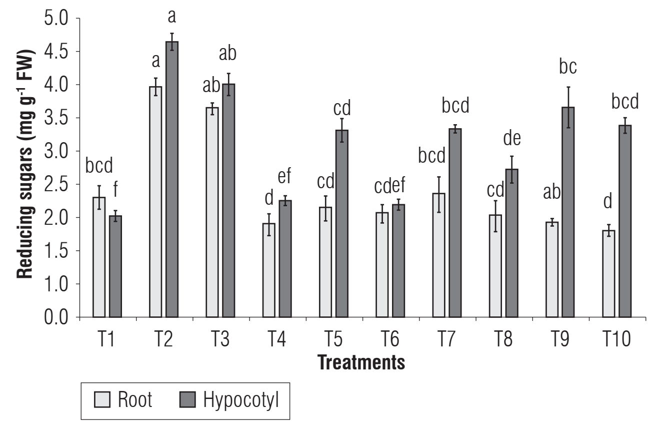


FIGURE 3. Effect of IHPLUS® on the content of reducing sugars in roots and hypocotyls of *P. vulgaris* L. cv. Tomeguín. Different letters indicate statistical differences among the treatments according to the Tukey's test ($P<0.05$). T1: Control (immersion in distilled water for 6 h); T2: 2% IHPLUS® - immersion for 2 h; T3: 2% IHPLUS® - immersion for 4 h; T4: 2% IHPLUS® - immersion for 6 h; T5: 4% IHPLUS® - immersion for 2 h; T6: 4% IHPLUS® - immersion for 4 h; T7: 4% IHPLUS® - immersion for 6 h; T8: 6% IHPLUS® - immersion for 2 h; T9: 6% IHPLUS® - immersion for 4 h, and T10: 6% IHPLUS® - immersion for 6 h. Data are the mean of four replicates and vertical bars represent the standard error ($P<0.05$).

in the IHPLUS® medium. Bioactive GAs induce the α-amylase gene expression and the synthesis of α-amylase that are essential to mobilize the stored starch, releasing glucose that can be used by the embryo to produce metabolic energy during respiration (Liu *et al*., 2018). However, other studies have reported a concentration-dependent effect of IAA on seed germination attributes and α-amylase activity (Tabatabaei *et al*., 2016) that suggests that those hormones, along with cytokinins, should act in concert to regulate germination.

Content of reducing sugars

The concentration of reducing sugars in roots significantly increased in T2 and T3 when compared to the untreated control (Fig. 3). The rest of the treatments showed non-significant differences between them and the control. IHPLUS® also enhanced the content of those compounds in the hypocotyl. The highest concentrations were shown in T2 and T3 that were 2.7-fold and 2.3-fold higher, respectively, compared to the control. The treatments T9, T10, T7 and T5 also increased the content of reducing sugars compared to the control seedlings.

Such an increment in reducing sugars might be attributed to the increase in α-amylase activity observed in the tested seedlings. The molecules of glucose released by the catalytic activity of α-amylase are essential to obtain metabolic energy through the respiration process and represent a source of carbon skeletons to form new molecules and cellular structures in growing tissues and organs (Taiz & Zeiger, 2010).

The results agree with those reported by Angeles-Núñez *et al*. (2015) in plantlets of *P. vulgaris* L. var. Flor de Mayo, treated with *Ramlibacter* sp., *Sinorhizobium* sp., *Sinorhizobium fredii* and *Bradyrhizobium japonicum*. In that study, an increase in reducing sugar content (glucose and fructose) in roots was reported as compared to the control. However, only *Ramlibacter* sp. was able to increase the reducing sugars in shoots, demonstrating the importance of the plant-microorganism interaction on the plant physiological response. Those authors also explained the change in soluble sugars due to an increase in α-amylase activity associated with raising bioactive GAs.

Total soluble proteins

The application of IHPLUS® to seeds of common bean increased protein contents in the root and hypocotyl of 7-d-old seedlings (Fig. 4). The highest protein content in the root was observed in seedlings treated with 2% IHPLUS® and 4 h of immersion (T3), followed by T2, T5, and T8, with non-significant differences among them, but significantly higher values compared to the control. The protein content in the hypocotyl increased in all treatments with IHPLUS®, except for T6, T9 and T10 that showed similar values to the control seedlings. The highest contents were recorded in seedlings treated with T3 and T2, with 5.3-fold and 3.4-fold increases, respectively, compared to the

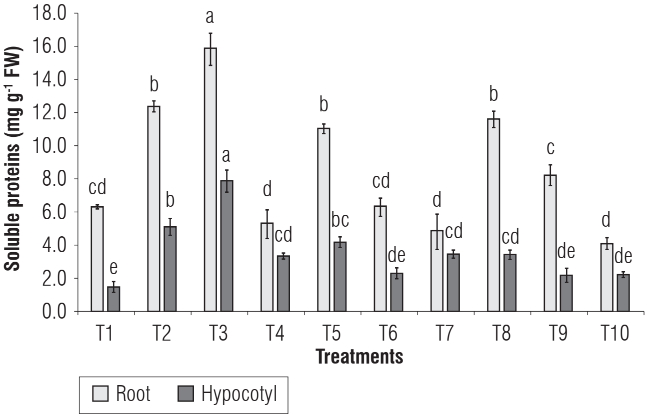


FIGURE 4. Effect of IHPLUS® on the content of soluble proteins in seedlings of *P. vulgaris* L. cv. Tomeguín. Different letters indicate statistical differences among the treatments according to the Tukey's test (P<0.05). T1: Control (immersion in distilled water for 6 h); T2: 2% IHPLUS® - immersion for 2 h; T3: 2% IHPLUS® - immersion for 4 h; T4: 2% IHPLUS® - immersion for 6 h; T5: 4% IHPLUS® - immersion for 2 h; T6: 4% IHPLUS® - immersion for 4 h; T7: 4% IHPLUS® - immersion for 6 h; T8: 6% IHPLUS® - immersion for 2 h; T9: 6% IHPLUS® - immersion for 4 h, and T10: 6% IHPLUS® - immersion for 6 h. Data are the mean of four replicates and vertical bars represent the standard error (P<0.05).

control. Similar results were reported by Prathibha and Siddalingeshwara (2013) in *Sorghum bicolor* L. varieties CSH-14 and Proagro, who found an increase in total protein content after the seed inoculation with *Pseudomonas fluorescens* and *Bacillus subtilis*.

The stimulating effect of IHPLUS® on protein metabolism may be partially associated with the availability of reducing sugars found in the seedling tissues. Reducing sugars may be used not only for obtaining metabolic energy during cellular respiration, but also for supplying carbon skeletons to form amino acids and other molecules after their oxidation during glycolysis and the tricarboxylic acid cycle (Taiz & Zeiger, 2010). Thus, the growth-promoting effect of IHPLUS® might enhance the protein biosynthesis that is essential to support the functioning of the new growing seedling tissues and organs.

The results obtained here demonstrate the capability of IHPLUS® to enhance *P. vulgaris* germination, seedling growth, and metabolism that is essential for latter seedling establishment and crop yield (Channaoui *et al*., 2017; Ha-Tran *et al*., 2021). Therefore, IHPLUS® might be considered an eco-friendly alternative to hazardous chemical fertilizers to promote seed germination and seedling growth of common beans.

In general, the immersion of *P. vulgaris* seeds into IHPLUS® enhanced their metabolism by increasing α-amylase activity; however, other enzymatic pathways different to this might be stimulated since plant growth promoting microorganisms may elicit distinct phytohormone-related pathways simultaneously, conferring additive responses that enhance seedling growth and development (Gholami *et al*., 2009).

The metabolic energy derived from the oxidation of reducing sugars is an essential factor for the active biosynthetic processes that take place in the embryo tissues during germination. It also supports the energy demand for cell division and elongation that is intense in the first stages of plant development and lead to vigorous seed germination (Yan *et al*., 2014).

Considering all the obtained results, it was possible to select those treatments with the highest impact on seed germination and seedling growth of *P. vulgaris*. Among them, T5 (4% IHPLUS® - immersion for 2 h) and T6 (4% IHPLUS® - immersion for 4 h) resulted in the best combinations of dose/immersion times. These treatments were able to increase the germination percentage in a short period of time, the germination rate, as well as root and hypocotyl length. Root growth promotion is particularly important to achieve a large root system as quickly as possible that allows the plant to explore soil more efficiently and find nutrients to sustain growth. Similarly, T8 (6% IHPLUS® - immersion for 2h) was shown to be effective for stimulating seed germination and seedling growth; however, from an economical point of view, T5 and T6 seem to be more suitable candidates.

Conclusions

The application of EM-based IHPLUS® to seeds of *P. vulgaris* induced biochemical changes in the seedlings that led to improvement in seed germination and seedling growth. IHPLUS® improved the germination percentage in the first days of the experiment and the germination rate that is important to obtain rapid germination and seedling growth uniformity, suitable qualities for achieving higher crop yield. IHPLUS® also increased root length that may ensure a rapid anchorage of the plantlet to soil and nutrient uptake. Among the assessed treatments, T5 and T6 showed the most advantageous results from the biological and economical points of view.

Acknowledgments

The authors want to thank Carolyn Amy Elizabeth Brown for editing the manuscript and her precise suggestions.

Conflict of interest statement

The authors declare that there is no conflict of interest regarding the publication of this article.

Author's contributions

MDS, LFA and YPH designed the experiments. MDS, LGS, MMT, and YPH carried out the laboratory experiments. LFA, and YPH contributed to the data analysis. YPH, MDS, and RLG wrote the article. All the authors reviewed the manuscript.

Literature cited

Abri, Kuswinanti, T., Sengin, E. L., & Sjahrir, R. (2015). Production of indole acetic acid (IAA) hormone from fungal isolates collected from rhizosphere of aromatic rice in Tana Toraja. *International Journal of Current Research in Biosciences and Plant Biology, 2*(6), 198–201.

Alori, E. T., Glick, B. R., & Babalola, O. O. (2017). Microbial phosphorus solubilization and its potential for use in sustainable agriculture. *Frontiers in Microbiology*, *8*, Article 971. https://doi.org/10.3389/fmicb.2017.00971

Angeles-Núñez, J. G., & Cruz-Acosta, T. (2015). Aislamiento, caracterización molecular y evaluación de cepas fijadoras de nitrógeno en la promoción del crecimiento de frijol. *Revista Mexicana de Ciencias Agrícolas*, *6*(5), 929–942.

Ayala-Villegas, M. J., Ayala-Garay, Ó. J., Aguilar-Rincón, V. H., & Corona-Torres, T. (2014). Evolución de la calidad de semillas de *Capsicum annuum* L. durante su desarrollo en el fruto. *Revista Fitotecnia Mexicana*, *37*(1), 79–87.

Blanco-Betancourt, D., Ojeda-García, F., Cepero-Casas, L., Estupiñán-Carrillo, L. J., Álvarez-Núñez, L. M., & Martín-Martín, G. J. (2017). Efecto del bioproducto IHplus® en los indicadores productivos y de salud de precebas porcinas. *Pastos y Forrajes*, *40*(3), 201–205.

Calero-Hurtado, A., Pérez-Díaz, Y., González-Pardo, Y., Olivera-Viciedo, D., Peña-Calzada, K., Castro-Lizazo, I., & Meléndrez-Rodríguez, J. F. (2020). Complementary application of two bioproducts increasing the productivity on common bean. *Cultivos Tropicales, 41*(3), Article e07.

Calero Hurtado, A., Pérez Díaz, Y., Olivera Viciedo, D., Quintero Rodríguez, E., Peña Calzada, K., Theodore Nedd, L. L., & Jiménez Hernández, J. (2019). Effect of different application forms of efficient microorganisms on the agricultural productive of two bean cultivars. *Revista Facultad Nacional de Agronomía Medellín*, *72*(3), 8927–8935. https://doi.org/10.15446/rfnam.v72n3.76272

Celmeli, T., Sari, H., Canci, H., Sari, D., Adak, A., Eker, T., & Toker, C. (2018). The nutritional content of common bean (*Phaseolus vulgaris* L.) landraces in comparison to modern varieties. *Agronomy*, *8*(9), Article 166. https://doi.org/10.3390/agronomy8090166

Channaoui, S., El Kahkahi, R., Charafi, J., Mazouz, H., El Fechtali, M., & Nabloussi, A. (2017). Germination and seedling growth of a set of rapeseed (*Brassica napus*) varieties under drought stress conditions. *International Journal of Environment, Agriculture and Biotechnology, 2*(1), 487–494. https://doi.org/10.22161/ijeab/2.1.61

Custodio, C. C., Araújo, F. F., Ribeiro, A. M., Souza Filho, N. V., & Machado-Neto, N. B. (2013). Seed treatment with *Bacillus subtilis* or indol butyric acid: germination and early development of bean seedlings. *Interciencia*, *38*(4), 273–279.

Díaz Solares, M., Pérez Hernández, Y., González Fuentes, J., Castro Cabrera, I., Fuentes Alfonso, L., Matos Trujillo, M., & Sosa del Castillo, M. (2019). Efecto del IHPLUS® sobre el proceso de germinación de *Sorghum bicolor* L. (Moench). *Pastos y Forrajes*, *42*(1), 30–38.

Djavanshir, K., & Pourbeik, H. (1976). Germination value - a new formula. *Silvae Genetica, 25*(2), 79–83.

Felestrino, É. B., Santiago, I. F., Freitas, L. S., Rosa, L. H., Ribeiro, S. P., & Moreira, L. M. (2017). Plant growth promoting bacteria associated with *Langsdorffia hypogaea*-rhizosphere-host biological interface: a neglected model of bacterial prospection. *Frontiers in Microbiology*, *8*, Article 172. https://doi.org/10.3389/fmicb.2017.00172

Finch-Savage, W. E., & Bassel, G. W. (2016). Seed vigour and crop establishment: extending performance beyond adaptation. *Journal of Experimental Botany*, *67*(3), 567–591. https://doi.org/10.1093/jxb/erv490

Gabre, V. V., Venancio, W. S., Moraes, B. A., Furmam, F. G., Galvão, C. W., Goncalves, D. R. P., & Etto, R. M. (2020). Multiple effect of different plant growth promoting microorganisms on beans (*Phaseolus vulgaris* L.) crop. *Brazilian Archives of Biology and Technology*, *63,* Article e20190493. https://doi.org/10.1590/1678-4324-solo-2020190493

Gholami, A., Shahsavani, S., & Nezarat, S. (2009). The effect of plant growth promoting rhizobacteria (PGPR) on germination, seedling growth and yield of maize. *International Journal of Agricultural and Biosystems Engineering, 3*(1), 9–14.

Ha-Tran, D. M., Nguyen, T. T. M., Hung, S. H., Huang, E., & Huang, C. C. (2021). Roles of plant growth-promoting rhizobacteria (PGPR) in stimulating salinity stress defense in plants: a review. *International Journal of Molecular Sciences*, *22*(6), Article 3154. https://doi.org/10.3390/ijms22063154

ISTA. (2014). *Rules proposals for the international rules for seed testing 2014 edition*. International Seed Testing Association.

Jochum, M. D., McWilliams, K. L., Borrego, E. J., Kolomiets, M. V., Niu, G., Pierson, E. A., & Jo, Y. K. (2019). Bioprospecting plant growth-promoting rhizobacteria that mitigate drought stress in grasses. *Frontiers in Microbiology*, *10*, Article 2106. https://doi.org/10.3389/fmicb.2019.02106

Kumar, P., Pandey, P., Dubey, R. C., & Maheshwari, D. K. (2016). Bacteria consortium optimization improves nutrient uptake, nodulation, disease suppression and growth of the common bean (*Phaseolus vulgaris*) in both pot and field studies. *Rhizosphere*, *2*, 13–23. https://doi.org/10.1016/j.rhisph.2016.09.002

Liu, L., Xia, W., Li, H., Zeng, H., Wei, B., Han, S., & Yin, C. (2018). Salinity inhibits rice seed germination by reducing α-amylase activity via decreased bioactive gibberellin content. *Frontiers in Plant Science, 9*, Article 275. https://doi.org/10.3389/fpls.2018.00275

Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein measurement with the folin phenol reagent. *Journal of Biological Chemistry*, *193*(1), 265–275. https://doi.org/10.1016/S0021-9258(19)52451-6

Maan, P. K., Garcha, S., & Walia, G. S. (2019). Prevalence of bacteriocinogenic *Rhizobium* spp. in mungbean (*Vigna radiata). Legume Research*, *42*(4), 557–564. https://doi.org/10.18805/LR-3884

Mahadevamurthy, M., Channappa, T. M., Sidappa, M., Raghupathi, M. S., & Nagaraj, A. K. (2016). Isolation of phosphate solubilizing fungi from rhizosphere soil and its effect on seed growth parameters of different crop plants. *Journal of Applied Biology and Biotechnology*, *4*(6), 22–26. https://doi.org/10.7324/JABB.2016.40604

Marquina, M. E., Ramírez, Y., & Castro, Y. (2018). Efecto de bacterias rizosféricas en la germinación y crecimiento del pimentón *Capsicum annuum* L. var. Cacique Gigante. *Bioagro*, *30*(1), 3–16.

Miller, G. L. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Analytical Chemistry*, *31*(3), 426–428. https://doi.org/10.1021/ac60147a030

Mohd Din, A. R. J., Hanapi, S. Z., Supari, N., Alam, S. A. Z., Javed, M. A., Tin, L. C., & Sarmidi, M. R. (2014). Germination, seedling growth, amylase and protease activities in Malaysian upland rice seed under microbial inoculation condition. *Journal of Pure and Applied Microbiology, 8*(4), 2627–2635.

Nushair, A. M., Saha, A. K., Mandal, A., Rahman, A., Mohanta, M. K., Hasan, A., & Haque, F. (2018). *Rhizobium* sp. CCNWYC119: a single strain highly effective as biofertilizer for three different peas (pigeon pea, sweet pea and chick pea). *Legume Research*, *41*(5), 771–777. https://doi.org/10.18805/LR-389

Olle, M., & Williams, I. (2015). The influence of effective microorganisms on the growth and nitrate content of vegetable transplants. *Journal of Advanced Agricultural Technologies*, *2*(1), 25–28. https://doi.org/10.12720/joaat.2.1.25-28

Pérez-Hernández, Y., Díaz-Solares, M., Rondón-Castillo, A. J., Fuentes-Alonso, L., González-Sierra, L., & Guzmán-Cedeño, A. M. (2020). Aislamiento de cepas de *Bacillus* spp. a partir del bioproducto IHPLUS® con potencialidades para el desarrollo agropecuario e industrial. *Pastos y Forrajes*, *43*(1), 56–65.

Prathibha, K. S., & Siddalingeshwara, K. G. (2013). Effect of plant growth promoting *Bacillus subtilis* and *Pseudomonas fluorescence* as rhizobacteria on seed quality of sorghum. *International Journal of Current Microbiology and Applied Sciences*, *2*(3), 11–18.

Remans, R., Beebe, S., Blair, M., Manrique, G., Tovar, E., Rao, I., Croonenborghs, A., Torres-Gutiérrez, R., El-Howeity, M., Michiels, J., & Vanderleyden, J. (2008). Physiological and genetic analysis of root responsiveness to auxin-producing plant growth-promoting bacteria in common bean (*Phaseolus vulgaris* L.). *Plant and Soil*, *302*, 149–161. https://doi.org/10.1007/s11104-007-9462-7

Rezende, A. A., Pacheco, M. T. B., Silva, V. S. N., & Ferreira, T. A. P. C. (2018). Nutritional and protein quality of dry Brazilian beans (*Phaseolus vulgaris* L.). *Food Science and Technology, 38*(3), 421–427. https://doi.org/10.1590/1678-457x.05917

Romero-García, V. E., García-Ortiz, V. R., Hernández-Escareño, J. J., & Sánchez-Yáñez, J. M. (2016). Respuesta de *Phaseolus vulgaris* a microorganismos promotores de crecimiento vegetal. *Scientia Agropecuaria*, *7*(3), 313–319. https://doi.org/10.17268/sci.agropecu.2016.03.20

Saxena, J., Geetika, R., & Pandey, M. (2013). Impact of addition of biochar along with *Bacillus* sp. on growth and yield of French beans. *Scientia Horticulturae*, *162*, 351–356. https://doi.org/10.1016/j.scienta.2013.08.002

Sözer Bahadir, P., Liaqat, F., & Eltem, R. (2018). Plant growth promoting properties of phosphate solubilizing *Bacillus* species isolated from the Aegean Region of Turkey. *Turkish Journal of Botany*, *42*, 183–196. https://doi.org/10.3906/bot-1706-51

Tabatabaei, S., Ehsanzadeh, P., Etesami, H., Alikhani, H. A., & Glick, B. R. (2016). Indole-3-acetic acid (IAA) producing *Pseudomonas* isolates inhibit seed germination and α-amylase activity in durum wheat (*Triticum turgidum* L.). *Spanish Journal of Agricultural Research*, *14*(1), Article e0802.

Taiwo, L. B., Ailenokhuoria, B. V., & Oyedele, A. O. (2017). Profiling rhizosphere microbes on the root of maize (*Zea mays*) planted in an alfisol for selection as plant growth promoting rhizobacteria (PGPR). *Microbiology Research Journal International*, *21*(5), 1–10.

Taiz, L., & Zeiger, E. (2010). *Plant Physiology* (5th ed.). Sinauer Associates Inc.

Talaat, N. B., Ghoniem, A. E., Abdelhamid, M. T., & Shawky, B. T. (2015). Effective microorganisms improve growth performance, alter nutrients acquisition and induce compatible solutes accumulation in common bean (*Phaseolus vulgaris* L.) plants subjected to salinity stress. *Plant Growth Regulation*, *75*, 281–295. https://doi.org/10.1007/s10725-014-9952-6

Tarekegn, M. M., Salilih, F. Z., & Ishetu, A. I. (2020). Microbes used as a tool for bioremediation of heavy metal from the environment. *Cogent Food and Agriculture*, *6*(1), Article 1783174. https://doi.org/10.1080/23311932.2020.1783174

Tellez-Soria, T., & Orberá-Ratón, T. (2018). Efecto estimulador del crecimiento de dos biopreparados biotecnológicos en cultivos de remolacha (*Beta vulgaris* L.). *Revista Cubana de Química*, *30*(3), 483–494.

Thakur, D., Kaur, M., & Mishra, A. (2017). Isolation and screening of plant growth promoting *Bacillus* spp. and *Pseudomonas* spp. and their effect on growth, rhizospheric population and phosphorous concentration of *Aloe vera*. *Journal of Medicinal Plants Studies*, *5*(1), 187–192.

Ugochi, K., Etienne, C., & Egbadon, E. (2016). Enhancement potential of plant growth-promoting rhizobacteria on white beans (*Phaseolus vulgaris*) seedlings. *International Letters of Natural Sciences*, *57*, 11–17. https://doi.org/10.18052/www.scipress.com/ILNS.57.11

Vasallo Cristia, D. C., Montejo Viamontes, J. L., López Labarta, P., Morgado, A. I., Robinson Pérez, M., & Piñeiro Esquivel, D. (2018). Efficient microorganisms as biostimulators to enhance yields of *Phaseolus vulgaris* L. cultivar Delicia Rojo 364. *Agrisost*, *24*(3), 152–159.

Wangdi, U., Ngawang, Yangden, T., Phuentsho, T., & Kencho. (2020). Effect of effective microorganism (EM) application and mulching on the yield of Japanese pole bean (*Phaseolus vulgaris*). *Bhutanese Journal of Agriculture*, *2*(1), 138–147.

Yadav, S. K., Dave, A., Sarkar, A., Singh, H. B., & Sarma, B. K. (2013). Co-inoculated biopriming with *Trichoderma*, *Pseudomonas* and *Rhizobium* improves crop growth in *Cicer arietinum* and *Phaseolus vulgaris*. *International Journal of Agriculture, Environment & Biotechnology*, *6*(2), 255–259.

Yan, D., Duermeyer, L., Leoveanu, C., & Nambara, E. (2014). The functions of the endosperm during seed germination. *Plant & Cell Physiology*, *55*(9), 1521–1533. https://doi.org/10.1093/pcp/pcu089

Yaxley, J. R., Ross, J. J., Sherriff, L. J., & Reid, J. B. (2001). Gibberellin biosynthesis mutations and root development in pea. *Plant Physiology*, *125*(2), 627–633. https://doi.org/10.1104/pp.125.2.627

Characterization of potassium solubilizing bacteria isolated from corn rhizoplane

Caracterización de bacterias solubilizadoras de potasio provenientes del rizoplano de maíz

Reneé Pérez-Pérez[1, 2]*, Ionel Hernández Forte[1], Yarixa Openda Sanabria Álvarez[3], Juan Carlos Salcedo Benítez[4], Daynet Sosa-del Castillo[5], and Simón Pérez-Martínez[6]

ABSTRACT

Potassium is one of the most important elements in plant growth and development. Most of the potassium reserves on Earth are in insoluble mineral form, which represents a limitation for the absorption of this nutrient by plants. Some microorganisms can solubilize the mineral forms of potassium. This study aimed to isolate and identify potassium solubilizing bacteria resident in corn rhizoplane. To do this, bacteria that formed a solubilization halo around the colony on solid Aleksandrov culture medium were selected. These bacteria were then characterized considering the appearance of the colonies and cell morphology and were identified by partial 16S rDNA sequencing. Their solubilizing and potassium-releasing capacity was determined under different conditions of temperature, pH, and salinity, using potassium feldspar and muscovite as insoluble sources of potassium. Eight strains identified within the genera *Paenibacillus, Lysinibacillus, Arthrobacter, Bacillus, Pseudomonas*, and *Stenotrophomonas* were obtained. The release of potassium from feldspar was favored at 28 and 30°C, pH 7.5 and a saline concentration of 4 g L^{-1}, while in the presence of muscovite the best conditions were 30 and 37°C, pH 5.5 and 7.5, and 4 g L^{-1} of NaCl. The most efficient strains were *Bacillus* sp. INCA-FRc7 and *Bacillus* sp. INCA-FRc19x with yields of up to 2.095 mg L^{-1}. These strains could become alternatives to the use of potassium fertilizers and contribute to the ecological sanitation of the agroecosystems.

Key words: K solubilizing capacity, K release capacity, K-feldspar, muscovite.

RESUMEN

El potasio es uno de los elementos más importantes en el crecimiento y desarrollo vegetal. La mayor parte de las reservas potásicas en la Tierra se encuentra en forma de mineral insoluble, lo cual representa una limitante para la absorción del nutriente por las plantas. Algunos microorganismos tienen la capacidad de solubilizar este mineral en formas minerales de potasio. Este estudio tuvo como objetivo aislar e identificar bacterias residentes en el rizoplano del maíz con capacidad para solubilizar potasio. Para esto, se seleccionaron las bacterias que formaron un halo de solubilización alrededor de la colonia en el medio de cultivo Aleksandrov sólido. Estas bacterias se caracterizaron posteriormente teniendo en cuenta la apariencia de las colonias y la morfología celular, y se identificaron por secuenciación parcial del ADNr 16S. Se determinó su capacidad solubilizadora y liberadora de potasio bajo diferentes condiciones de temperatura, pH y salinidad, empleando feldespato de potasio y moscovita como fuentes insolubles de potasio. Se obtuvieron ocho cepas identificadas dentro de los géneros *Paenibacillus, Lysinibacillus, Arthrobacter, Bacillus, Pseudomonas* y *Stenotrophomonas*. La liberación de potasio a partir de feldespato se vio favorecida a los 28 y 30°C, pH 7.5 y una concentración salina de 4 g L^{-1}, mientras que en presencia de moscovita las mejores condiciones fueron de 30 y 37°C, pH 5.5 y 7.5 y 4 g L^{-1} de NaCl. Las cepas más eficientes fueron *Bacillus* sp. INCA-FRc7 y *Bacillus* sp. INCA-FRc19x con rendimientos de hasta 2.095 mg L^{-1}. Estas cepas podrían convertirse en alternativas al uso de fertilizantes potásicos y contribuir al saneamiento ecológico de los agroecosistemas.

Palabras clave: capacidad solubilizadora de K, capacidad liberadora de K, feldespato-K, moscovita.

Introduction

Potassium (K) is important for cellular processes, such as cell turgor and elongation, and quality factors such as organoleptic and aesthetic properties of the plants; however, these factors do not represent direct contributions to crop yields (Sardans & Peñuelas, 2015). For this reason, the importance of the soil as a supplier of K is less in extensive

Received for publication: 17 September, 2021. Accepted for publication: 30 December, 2021. Doi: 10.15446/agron.colomb.v39n3.98522

[1] Departamento de Fisiología y Bioquímica Vegetal, Instituto Nacional de Ciencias Agrícolas (INCA), Mayabeque (Cuba).
[2] Institute of Biology, Biotechnology and Environmental Protection, University of Silesia in Katowice, Katowice (Poland).
[3] Departamento de Inspección y Ensayo, Laboratorios MedSol, La Habana (Cuba).
[4] Instituto de Investigaciones de Pastos y Forrajes (IIPF), La Habana (Cuba).
[5] Centro de Investigaciones Biotecnológicas del Ecuador (CIBE), Escuela Superior Politécnica del Litoral (ESPOL), Guayaquil (Ecuador).
[6] Facultad de Ciencias de la Ingeniería, Universidad Estatal de Milagros (UNEMI), Milagros (Ecuador).
* Corresponding author: rene.perez-perez@us.edu.pl

field crops, such as cotton or cereals, which are not paid for quality but for quantity. In these cases, many farmers neglect K fertilization generally in favor of nitrogen (N) causing a nutritional imbalance, leading to a decrease in K reserves in the soil, low yields and increased production costs (Shirale *et al*., 2019; Kour, Rana, Kaur, *et al*., 2020).

Potassium in soil represents 2.6% of the weight of the Earth's crust and can be found in two fundamental forms, as free ions in the soil solution and in diverse forms in different mineral and organic compounds. Despite this abundance, 90-98% of K is fixed in forms not available to plants (Sun *et al*., 2020) within the minerals of magmatic origin such as potassium feldspars and micas such as muscovite and biotite (Shirale *et al*., 2019). The low amounts of available K in the soil tend to deplete quickly during cultivation due to high demand by some plants. Therefore, the constant renewal of easily removable K sources is required, such as from mineral fertilizers that represent around 95% of the K exploited in the world (Shirale *et al*., 2019).

The release of K from minerals occurs slowly through weathering or by the action of a diverse group of microorganisms including fungi and bacteria (Sarikhani *et al*., 2018); bacteria play an essential role in the biogeochemical cycle of this element (Sattar *et al*., 2019). There is little information related to the microbial process of K solubilization at the molecular level. However, existing reports indicate that the K release mechanisms used by these microbes include: a) a direct solubilization pathway (strong organic acid production) (Basak *et al*., 2017; Sattar *et al*., 2019); b) an indirect solubilization pathway (chelation of cations bound to K silicate, exchange or complexation reactions, and metal complexing ligands) (Basak *et al*., 2017), c) secretion of polysaccharides (capsular exopolysaccharides) (Sattar *et al*., 2019), and d) biofilm formation on mineral surfaces (Nagaraju *et al*., 2017). The exudation of organic acids, especially oxalic, tartaric, and citric (Rajawat *et al*., 2016), may be the most important mechanism of solubilization of K minerals (Etesami *et al*., 2017), mainly muscovite, feldspar and biotite (Basak *et al*., 2017).

Previous studies have focused on potassium solubilizing bacteria (KSB), especially those that inhabit the rhizospheric zone, where genera such as *Pseudomonas, Pantoea, Rahnella, Bacillus,* and *Paenibacillus* have been found (Xiao *et al*., 2017; Khanghahi *et al*., 2018; Dong *et al*., 2019). Inoculation of KSB in crops of agricultural interest like tea, rice, wheat, barley, tomato, lettuce, cotton and corn has shown good results in growth variables such as plant height, length and dry weight of leaves and roots, shoot growth, biomass yield, nutrient uptake, and fruit weight and yield (Bagyalakshmi *et al*., 2017; Khanghahi *et al*., 2018; Sarikhani *et al*., 2018; Sattar *et al*., 2019), in addition to a notable increase in the available forms of K in the rhizosphere and in the K uptake by plants. However, most of these studies use bacteria isolated from ecosystems or crops different from those where they are inoculated. In this sense, the use of native strains resident in the rhizosphere of the crop may represent an advantage compared to the use of allochthonous strains. The autochthonous strains exhibit better capacity to interact positively with the resident edaphic microbiota, greater adaptability to local climatic and agroecological conditions, and the promotion of sustainable ecological management of agroecosystems (Reinhold-Hurek *et al*., 2015; Koskey *et al*., 2017; Berger *et al*., 2018; Tchakounté *et al*., 2018).

Corn is an exhaustive crop whose nutritional needs include N and K to a greater extent (Feng *et al*., 2019); sometimes, the K requirement is higher than the N requirement depending on the chemical characteristics of the soil (Wang *et al*., 2018). This leads to the use of fertilizers as a common and mandatory practice to obtain acceptable yields in this crop. However, the long-term indiscriminate use of agrochemicals has a negative impact on agricultural productivity (Wang *et al*., 2018; Kour, Rana, Kaur, *et al*., 2020). Furthermore, it constitutes the main cause of loss of soil fertility (Berger *et al*., 2018) and generates groundwater contamination (Sun *et al*., 2020), the displacement of microbial communities or the loss of some of their ecological functions, and, in some cases, affectations of human health (Vejan *et al*., 2016). In Cuba, the land area destined to the cultivation of corn is greater compared to other crops of agricultural interest, with a total of 128,604 ha in 2020 (ONEI, 2021). However, the crop yield is remarkably low (2 t ha^{-1}) and does not meet the nutritional demands of the population or the livestock sector (ONEI, 2021). Fundora *et al*. (2010) state that this is due to insufficiencies in the production chain, fundamentally to the shortage of chemical fertilizers (N and K fertilizers), which represents the highest cost of the process. Additionally, nutrient deficiencies and the deterioration of the structure of Cuban soils as a result of long years of monoculture and intensive cultivation threaten the agricultural sustainability and yield of this crop. For this reason, the aim of this research was to identify and characterize KSB from corn rhizoplane for future use as inoculants for this crop.

Materials and methods

Sampling

Sampling was carried out at the ¨El Mulato¨ farm (23°00'26.8" N; 82°08'12.6" W), Mayabeque province, Cuba. Thirty-day old (vegetative development) corn plants grown in leached Red Ferralitic soil were sampled. Five random sampling points were established, and two plants were selected from each of them. A 20 cm^3 region was excavated around the root system, which was completely extracted. A sample of non-rhizospheric soil and rhizospheric soil was collected at each of the sampling points for chemical characterization in the Chemical Analysis Laboratory of the National Institute of Agricultural Sciences. The root samples were placed in polyethylene bags and kept at 4°C until their processing in the Microbiology Laboratory of the same center.

Chemical characterization of the soil

The pH of the rhizospheric and non-rhizospheric soil samples was measured by potentiometry in a 1:2.5 (1N) soil/KCl suspension. Moisture content was determined gravimetrically by drying a sample of wet field soil in an oven at 105°C for 24 h. The organic matter content was determined by the wet combustion method with potassium dichromate ($K_2Cr_2O_7$) (1N) (Paneque Pérez *et al*., 2010). The total K was extracted from the air-dried soil by digestion in a hot concentrated mixture of 7 ml of HNO_3 and 1 ml of $HClO_4$, held for 3 h and then adjusted to 100 ml after cooling, while the available K was extracted from 1 M NH_4OAc (Sun *et al*., 2020). Both extraction solutions were analyzed by flame emission spectrophotometry (λ = 766 nm) (410 Classic Flame Photometer, Sherwood Scientific Ltd, UK).

Isolation of potassium solubilizing bacteria

The isolation of potassium solubilizing bacteria was carried out from the rhizoplane of the corn plants on solid Aleksandrov culture medium (5 g L^{-1} glucose, 0.005 g L^{-1} $MgSO_4.7H_2O$, 0.1 g L^{-1} $FeCl_3$, 2.0 g L^{-1} $CaCO_3$, 3.0 g L^{-1} K-feldspar powder, 2.0 g L^{-1} $Ca_3(PO_4)_2$, 20 g L^{-1} agar, pH 7.5) (Vincent, 1970). First, the roots were superficially washed with sterile water until most of the attached rhizospheric soil was removed. Clean roots were then cut into 1 cm long portions and placed in 50 ml Erlenmeyer flasks with 10 ml of sterile distilled water. The flasks were continuously stirred for 2 h at 150 rpm. With the suspensions obtained, serial decimal dilutions from 10^{-1} to 10^{-6} were made, of which the 10^{-4}, 10^{-5} and 10^{-6} dilutions were cultured by dissemination on solid Aleksandrov medium. Potassium feldspar powder (K-feldspar) was used as the insoluble mineral. The cultures were incubated at 29 ± 1°C for 7 d. The colonies that formed a solubilization halo around themselves were selected and re-isolated on Aleksandrov medium under the same culture conditions by successive passages. Once the axenic cultures were obtained, they were stored at 4°C for future use.

Morphological and colony characterization

For the colony characterization, the coloration, opacity, shape, elevation, edges, and mucus of the colonies on Aleksandrov solid medium were considered. A Gram stain was performed to determine morphology, staining, and sporulation.

Identification and phylogenetic analysis

The molecular identification of KSB was carried out by partial sequencing of the 16S rDNA. The extraction of the genetic material was carried out by alkaline lysis of bacterial colonies in Eppendorf tubes containing 40 µL of NaOH (0.2 M). The suspension was heated in a microwave oven at 10% power for 1 min and then immediately cooled on ice for 5 min. Subsequently, the suspension was centrifuged for 10 min at 10,000 *g* (Eppendorf 5702R, Merck KGaA, Germany) to remove cell debris and the supernatant was stored at room temperature.

Amplification of 16S rDNA was carried out by the PCR technique, using a 25 µL mixture containing 1 µL (10 µM) of universal primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3 ') and 1492R (5'-TACGGTTACCTTGTTACGACTT -3 '), 1 µL of rDNA, and 23 µL of GoTaq® Green Master Mix (Promega Corporation, USA). The process was carried out in an MS mini thermal cycler (Major Science, USA) with the following parameters: initial denaturation 95°C for 10 min and additional denaturation 92°C for 1 min; 35 cycles at 72°C each for 2 min. The amplification products were observed by 1% (m/v) agarose gel electrophoresis (1 g of agarose in 150 ml of 1X TAE buffer) at 80 V for 45 min. Sequencing was performed using the Sanger method at the Macrogen® company (Republic of Korea, http://www.macrogen.com/en/main/index.php).

Subsequently, the obtained sequences were indexed and compared with the National Center for Biotechnology Information (NCBI) database using the BLASTn tool (basic local alignment search tool) and aligned using the ClustalW multiple sequence alignment tool in the MEGA-X software (version 10.0.4) (Kumar *et al*., 2018). Additionally, a phylogenetic tree was made from the sequences obtained and the closest type sequences using the Neighbor-joining method in the same software.

K solubilizing capacity

Bacterial suspensions of each isolate were prepared in sterile distilled water and the optical density was adjusted to 0.5 (λ = 600 nm) by spectrophotometry (Thermo GENESYS™ 10UV UV-Vis, Thermo Fisher Scientific, USA).

The solubilizing capacity was determined by calculating the solubilization index (SI) on solid Aleksandrov medium (30°C, pH 7) using two separate insoluble sources of K, K-feldspar (3 g L^{-1}) and muscovite (3 g L^{-1}). Ten µL of the bacterial suspensions were inoculated for a total of four isolates per plate, and six replicates were established per isolate. After 7 d of incubation, the SI was calculated using the formula:

$$SI = \frac{DH}{DC} \quad (1)$$

where DH is the diameter of the solubilization halo (cm) and DC is the diameter of the bacterial colony (cm).

The dissolution rate was characterized as "fast" when the solubilization halo appeared before the third day of culture and "slow" if it did so later. The solubilizing capacity was characterized as "low" (SI<2.00), "intermediate" (2.00≤SI≤4.00) and "high" (SI≥4.00) according to Saha *et al*. (2016).

K releasing capacity under different conditions of temperature, pH, and salinity

One hundred milliliter Erlenmeyer flasks were prepared with 20 ml of the liquid Aleksandrov medium independently supplemented with K-feldspar (3 g L^{-1}) and muscovite (3 g L^{-1}). To evaluate the effect of pH, the medium was adjusted to values of 5.5, 7.5, and 9.0 by adding hydrochloric acid (HCl) or sodium hydroxide (NaOH). Four ranges of salinity were established by applying 4 g L^{-1}, 8 g L^{-1}, 12 g L^{-1}, and 16 g L^{-1} of sodium chloride (NaCl) and the effect of temperature was verified at 28°C, 30°C, 37°C, and 40°C. In all cases, the Erlenmeyer flasks were continuously stirred at 150 rpm for 7 d. The cultures were subsequently centrifuged at 3000 *g* (Eppendorf 58XX with rotor F-34-6-38, USA) for 15 min and the amount of K released in the supernatant was determined by flame spectrophotometry (BWB SFP Flame Photometer, BWB Technology, UK). The standard curve was prepared with a potassium chloride (KCl) solution (Saha *et al*., 2016). Six replicates were established per treatment.

Statistical analysis

The data from the determinations of the solubilizing and releasing capacity of K from K-feldspar and muscovite were subjected to the normality test (Bartlett's test) and homogeneity of variance (Kolmogorov-Smirnov's test). Simple classification analysis of variance (ANOVA) was applied, using the Tukey's mean comparison test at $P<0.05$. Version 21 of the statistical package for the social sciences program (SPSS®, IBM Corporation, USA) was used for the statistical processing of the data, and Excel 2016 to create the graphs.

Results

Chemical characterization of the soil

The pH at the rhizosphere level was slightly more acidic compared to the non-rhizospheric soil, which was found closer to neutral. The water content indicated good moisture retention, while the organic matter levels were low. The total K content determined in the non-rhizospheric soil was higher compared to the rhizospheric soil, although both values were considered relatively high according to John-Louis *et al*. (2017). However, the available K values for the plants were considered low in non-rhizospheric soil and medium in rhizospheric soil (Tab. 1) (John-Louis *et al*., 2017).

Isolation and morphological characterization

A total of eight bacterial isolates with the presence of a solubilization halo around the colonies were obtained on the solid Aleksandrov culture medium. The cultural and morphological characterization showed diversity among the selected isolates (Tab. 2). Five of these were characterized as Gram-positive and three as Gram-negative. The INCA-FRr15 and INCA-FRr5 isolates showed identical morphological and colony characteristics.

Identification and phylogenetic analysis

The partial sequencing of the 16S rDNA of the eight isolates allowed the identification of six genera (Tab. 3) corresponding to the phyla Actinobacteria, Firmicutes and Proteobacteria (Fig. 1).

TABLE 1. Physico-chemical analysis of the corn non-rhizospheric and rhizospheric soil.

Soil type	Depth (cm)	Location	pH	Water content (%)	Organic matter (%)	Potassium content	
						Total K (cmol kg^{-1})	Available K (cmol kg^{-1})
Typical Eutric leached Red Ferralitic	10-20	Non-rhizospheric soil	6.7	17.1	2.2	0.53 ^{high}	0.30 ^{low}
		Rhizospheric soil	5.6	19.3	1.8	0.48 ^{high}	0.37 ^{medium}

TABLE 2. Colony and morpho-staining characteristics of potassium solubilizing bacteria (KSB) from corn rhizoplane.

Isolates	Colony characteristics							Morpho-staining characteristics	
	Diameter (cm)	Color	Opacity	Shape	Elevation	Edges	Mucus	Gram stain	Sporulation
INCA-FRc17	0.2	Beige	Opaque	Circular	Elevated	Smooth	Dry	G+	No
INCA-FRc7	1.2	Beige	Sparkly	Circular	Elevated	Rough	Dry	G+	Yes
INCA-FRc19x	1.2	Grey	Sparkly	Irregular	Elevated	Smooth	Mucous	G+	Yes
INCA-FRc27	1.5	Whitish	Sparkly	Irregular	Flat	Wavy	Dry	G+	Yes
INCA-FRc9	0.5	Whitish	Opaque	Circular	Convex	Smooth	Dry	G+	Yes
INCA-FRr15	0.4	Beige	Sparkly	Circular	Convex	Smooth	Mucous	G-	No
INCA-FRr5	0.4	Beige	Sparkly	Circular	Convex	Smooth	Mucous	G-	No
INCA-FRr1	0.6	Whitish	Opaque	Circular	Convex	Wavy	Mucous	G-	No

G+: Gram-positive; G-: Gram-negative.

TABLE 3. Molecular identification of potassium solubilizing bacteria (KSB) from corn rhizoplane, from the partial sequencing of 16S rDNA.

Isolates	Identity	Accession	Closest identification	Accession	% Id
INCA-FRc17	*Arthrobacter* sp.	MT793084	*Arthrobacter pokkalii* P3B162	NR _ 149802.1	100
INCA-FRc7	*Bacillus* sp.	MT793079	*Bacillus aryabhattai* B8W22	NR _ 118442.1	100
INCA-FRc19x	*Bacillus* sp.	MT793087	*Bacillus safensis* IHBB14387	KM888116	100
INCA-FRc27	*Lysinibacillus* sp.	MT793090	*Lysinibacillus boronitolerans* NBRC 103108	NR _ 114207.1	100
INCA-FRc9	*Paenibacillus* sp.	MT793081	*Paenibacillus pocheonensis* Gsoil 1138	NR _ 112565.1	100
INCA-FRr15	*Pseudomonas* sp.	MT793106	*Pseudomonas hibiscicola* HPG72	JQ291604.1	100
INCA-FRr5	*Pseudomonas* sp.	MT793096	*Pseudomonas brenneri* CFML 97-391	NR _ 025103	98
INCA-FRr1	*Stenotrophomonas* sp.	MT793092	*Stenotrophomonas rhizophila* e-p10	NR _ 121739.1	99

% Id - Percentage of identity.

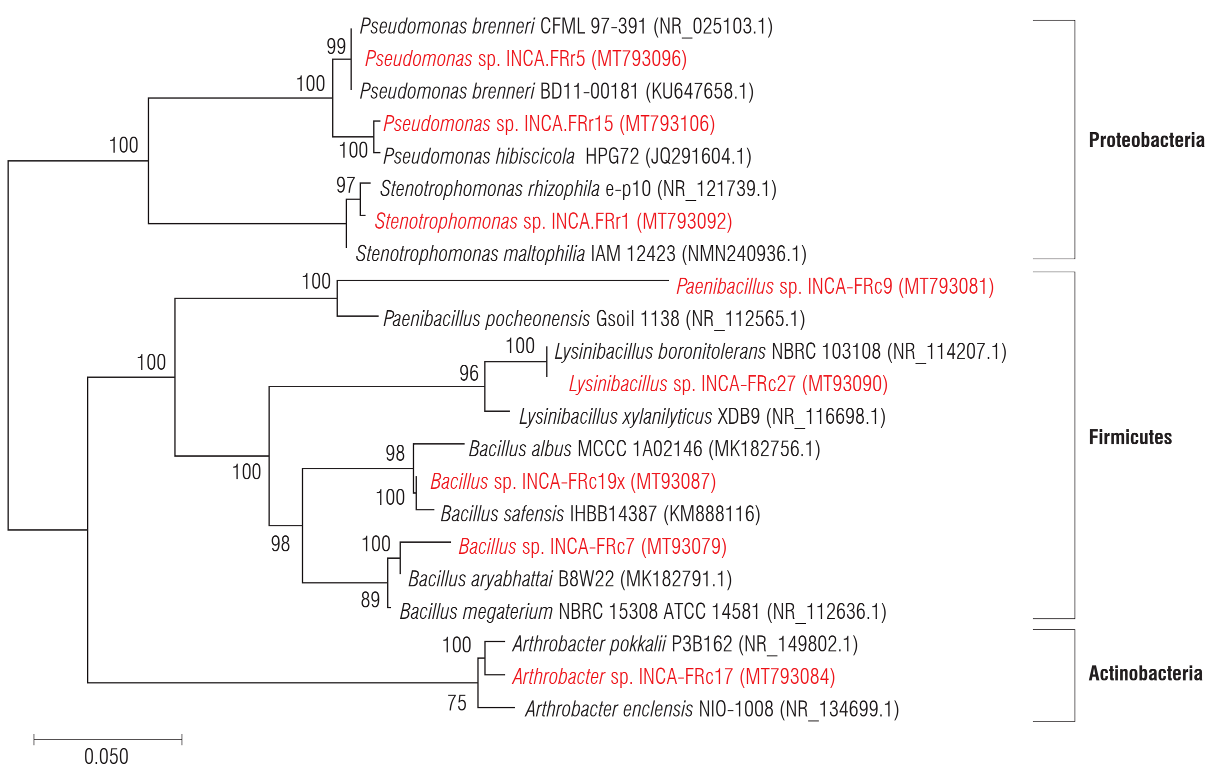


FIGURE 1. Phylogenetic tree obtained by the Neighbor-joining method with 1000 bootstrap replicas using partial 16S rDNA sequences belonging to 22 strains. The numbers in each branch correspond to the percentage of replicated trees that are associated with the taxon in question and the evolutionary distances were determined by the maximum likelihood method. The potassium solubilizing bacteria (KSB) strains identified in this study are shown in red.

K solubilizing capacity

One hundred percent of the strains developed a solubilization halo both on the medium supplemented with K-feldspar and on the medium supplemented with muscovite. Additionally, the appearance of the halo occurred within the first 48 h of the culture, so the dissolution rate was classified as fast.

The solubilization indices remained within the ranges of 0.73 - 5.36 for K-feldspar and 1.07 - 3.39 for muscovite. The solubilizing capacity did not vary from one mineral to another except for the strains *Bacillus* sp. INCA-FRc7 and *Bacillus* sp. INCA-FRc19x, which showed contrasting solubilizing capacities, high for K-feldspar mineral and low for muscovite. In the case of K solubilization from muscovite, the most prominent strain was *Arthrobacter* sp. INCA-FRc17, with an SI of 3.39 for an intermediate solubilizing capacity (Tab. 4).

K releasing capacity under different conditions of temperature, pH, and salinity

In general, the Gram-positive strains performed better under the different cultivation conditions evaluated, except for *Lysinibacillus* sp. INCA-FRc27 and *Paenibacillus* sp. INCA-FRc9, which showed the lowest values in all determinations. Similarly, the amounts of K released from muscovite were higher compared to those obtained from K-feldspar (Figs. 2-4).

The temperature that most favored the release of K in both sources was 30°C (Fig. 2). The most efficient strains were *Bacillus* sp. INCA-FRc19x with 1.982 mg L^{-1} of K released from K-feldspar at 28°C and 2.096 mg L^{-1} from muscovite at 30°C, and *Bacillus* sp. INCA-FRc7 with 1.873 mg L^{-1} of K released from K-feldspar at 30°C and 2.051 mg L^{-1} from muscovite at 37°C. The Gram-negative strains obtained similar amounts of K released in each source of 1.162 - 1.201

TABLE 4. Determination of the solubilization index (SI) and solubilizing capacity (SC) of K-feldspar and muscovite on solid Aleksandrov medium (30ºC, pH 7).

Strains	K-feldspar powder		Muscovite	
	SI	SC	SI	SC
Arthrobacter sp. INCA-FRc17	3.65 ± 0.21[b]	Intermediate	3.39 ± 0.56[a]	Intermediate
Bacillus sp. INCA-FRc7	4.64 ± 0.40[a]	High	1.80 ± 0.08[cd]	Low
Bacillus sp. INCA-FRc19x	5.36 ± 0.24[a]	High	1.97 ± 0.11[c]	Low
Lysinibacillus sp. INCA-FRc27	0.73 ± 0.37[d]	Low	1.07 ± 0.00[d]	Low
Paenibacillus sp. INCA-FRc9	1.82 ± 0.10[c]	Low	1.85 ± 0.18[cd]	Low
Pseudomonas sp. INCA-FRr15	3.40 ± 0.31[b]	Intermediate	2.86 ± 0.32[ab]	Intermediate
Pseudomonas sp. INCA-FRr5	2.31 ± 0.50[c]	Intermediate	2.40 ± 0.10[bc]	Intermediate
Stenotrophomonas sp. INCA-FRr1	2.05 ± 0.05[c]	Intermediate	3.02 ± 0.31[ab]	Intermediate

Tukey's mean comparison test ($P<0.05$; n = 6). Values represent means ± standard error. Different letters in the same column indicate statistical differences.

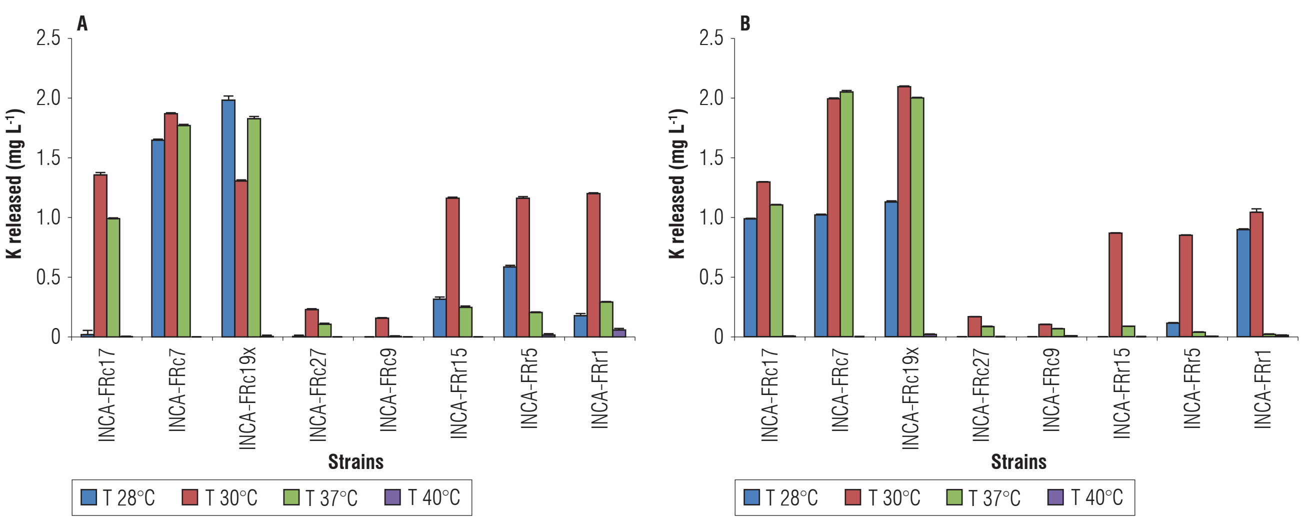


FIGURE 2. K released from A) K-feldspar powder and B) muscovite in liquid Aleksandrov medium under different conditions of temperature. Values represent means ± standard deviation (n = 5).

mg L^{-1} in K-feldspar (Fig. 2B) and 0.850 - 1.044 mg L^{-1} in muscovite at 30°C (Fig. 2B). The strain *Stenotrophomonas* sp. INCA-FRr1 was the only one to release detectable amounts of K from K-feldspar at all four temperatures (Fig. 2A).

K solubilizing activity was favored at pH 7.5, where all the strains, except for *Pseudomonas* sp. INCA-FRr5, released between 0.490 - 2.001 mg L^{-1} (Fig. 3). The highest values with this pH were obtained by the strains *Bacillus* sp. INCA-FRc19x and *Bacillus* sp. INCA-FRc7, which reached up to 1.798 mg L^{-1} from K-feldspar (Fig. 3A) and 1.986 mg L^{-1} from muscovite (Fig. 3B). However, the highest values of K released were obtained at pH 5.5 by these same strains. On the other hand, the alkaline pH was more successful in releasing K from K-feldspar, with the highest value for the strain *Bacillus* sp. INCA-FRc7 with 1.004 mg L^{-1}. Strains *Arthrobacter* sp. INCA-FRc17, *Bacillus* sp. INCA-FRc7, *Pseudomonas* sp. INCA-FRr15 and *Stenotrophomonas* sp. INCA-FRr1 released detectable amounts of K from K-feldspar under the three measured pH conditions (Fig. 3A), while *Bacillus* sp. INCA-FRc7 and *Stenotrophomonas* sp. INCA-FRr1 released it from muscovite (Fig. 3B).

Salinity affected the release of K, mainly at high concentrations of NaCl (Fig. 4). The K releasing activity was manifested in all strains at a concentration of 4 g L^{-1} of salt, with 0.036 - 1.306 mg L^{-1} released from K-feldspar and

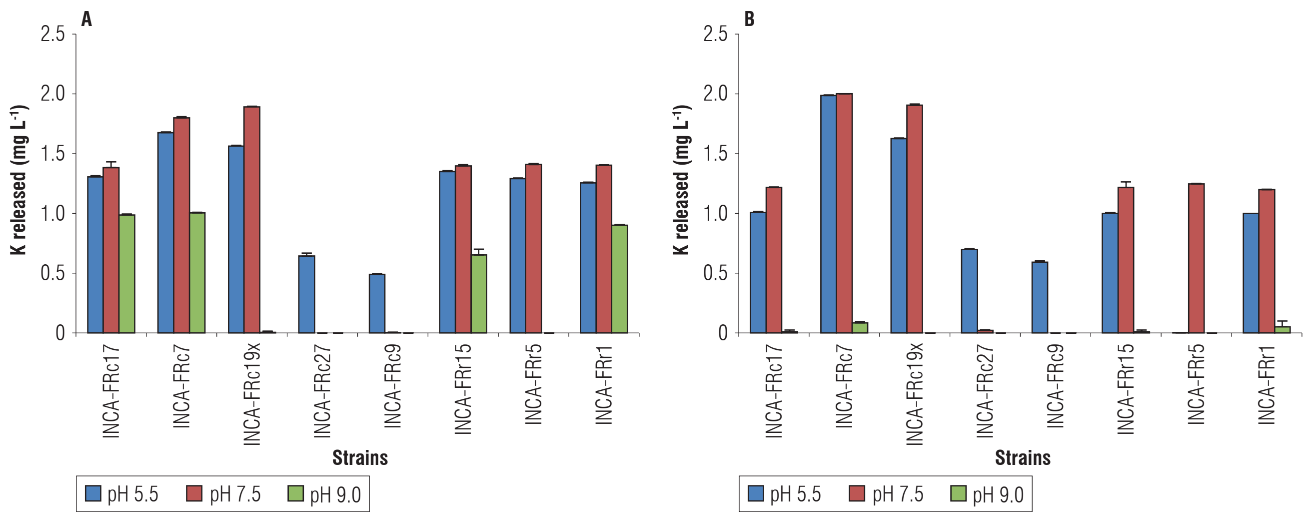


FIGURE 3. K released from A) K-feldspar powder and B) muscovite in liquid Aleksandrov medium under different conditions of pH. Values represent means ± standard deviation (n = 5).

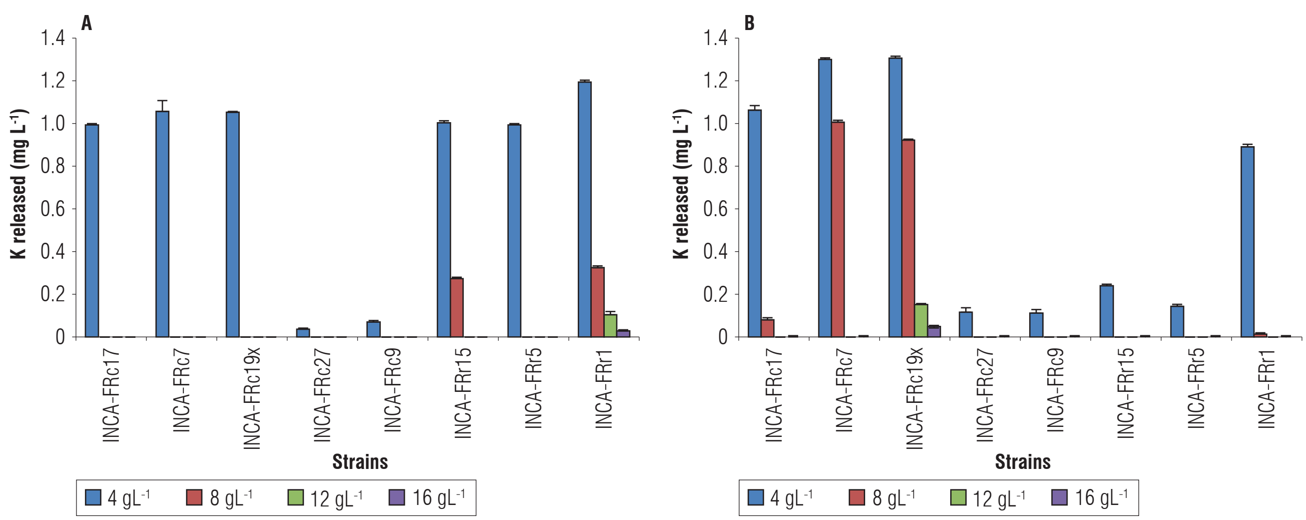


FIGURE 4. K released from A) K-feldspar powder and B) muscovite in liquid Aleksandrov medium under different concentrations of salinity. Values represent means ± standard deviation (n = 5).

muscovite. The highest values of K released were obtained at this concentration by the strain *Stenotrophomonas* sp. INCA-FRr1 from K-feldspar (Fig. 4A) and by strains *Bacillus* sp. INCA-FRc19x and *Bacillus* sp. INCA-FRc7 from muscovite (Fig. 4B). Only the strains *Stenotrophomonas* sp. INCA-FRr1 and *Bacillus* sp. INCA-FRc19x released K from K-feldspar and muscovite, respectively, under the four salinity concentrations tested.

Discussion

Potassium is part of the clay minerals that make up the mineral fraction of the soil; a soil rich in clay has a high content of K (Melgar & Castro, 2005). However, Red Ferralitic soils, considered clayey, are exceptions because the ferratilization process considerably reduces the total K content, as it does with the assimilable K content (Hernández Jiménez *et al*., 2010). As the K is depleted from the mineral interlayers of the soil, the release rate becomes progressively slower (Melgar & Castro, 2005), a process that increases with constant agricultural activity as is the case with the sampled soil. The use of K chemical fertilizers results in a strong adsorption of K to the minerals causing their contraction (potassium fixation) (Melgar & Castro, 2005; John-Louis *et al*., 2017) and making them inaccessible for the plants. This could explain the low amounts of assimilable K found in the sampled soil, especially in the soil furthest from the rhizosphere, which would allow the establishment of microbial populations with K solubilizing capacities. Atlas and Bartha (2005) state that the deficiency of a nutrient in the medium stimulates the microbial solubilization/fixation activity as a survival mechanism.

However, the content of assimilable K in the rhizosphere was slightly higher than in non-rhizospheric soil, which could indicate that at this level, the microbial activity of K solubilization has been stimulated, possibly influenced by radical exudates and plant-microorganism compatibility (Reinhold-Hurek *et al*., 2015). The microbial biodiversity of the rhizosphere is determined mainly by the composition of the radical exudates, which are specific to each plant species and decide the type and density of microbial populations associated with the plant (Verma *et al*., 2020) Additionally, the differences detected between the pH values in both soil zones indicate an acidification around the rhizosphere that would favor the solubilization of K minerals in this region (Verma *et al.,* 2020).

Studies on KSB from corn crops are scarce, and it is an interesting topic given the crop's high demand for K. Although KSB are the most important members of the microorganisms involved in the K cycle (Sattar *et al*., 2019), the few isolates obtained in this research highlight the exclusivity of this activity in the microbial world in general. The identified genera have previously been isolated from rhizospheric soil samples and roots of crops, such as soybean, rice, rape, apple, tea, Sudan grass, sorghum and wheat, and characterized as KSB by different authors (Verma *et al*., 2016; Egamberdieva *et al*., 2017; Verma *et al*., 2017; Kour, Rana, Yadav, *et al*., 2020; Rana *et al*., 2021). Considering that most of the KSB that are known correspond to the phylum Proteobacteria (Kour, Rana, Yadav, *et al*., 2020), the genus *Bacillus* (phylum Firmicutes) is one of the most studied and best yielding (Baghel *et al*., 2020; Kour, Rana, Yadav, *et al*., 2020; Sun *et al*., 2020). This supports the results obtained in this research where the strains INCA-FRc7 and INCA-FRc19x identified as *Bacillus* sp. showed the highest K solubilization index on solid Aleksandrov medium supplemented with K-feldspar. These strains also stood out in K release in liquid medium, showing the best yields under the different cultivation conditions, both with K-feldspar and with muscovite.

On the other hand, the K solubilizing capacity of the rest of the strains remained unchanged in the presence of both K mineral sources in solid medium, in accordance with the determinations of Nath *et al*. (2017), Nath *et al*. (2018), and Sun *et al*. (2020). The *Lysinibacillus* sp. INCA-FRc27 and *Paenibacillus* sp. INCA-FRc9 strains obtained the lowest K solubilization and K release indices in all the determinations; however, the values obtained did not correspond to those determined by Naureen *et al*. (2017) and Xiao *et al*. (2017) who characterize these genera as good K solubilizers.

The solubilization of K is mainly due to the action of organic acids (Ghadam Khani *et al*., 2019), both of microbial and plant origin (Verma *et al*., 2020). However, like all physiological processes, K solubilization is directly influenced by external factors such as temperature or pH that will determine the efficiency of the process (Verma *et al*., 2020). In this study, K release was most favored by a temperature of 30°C, a pH of 7.5 and the lowest saline concentration tested. These results are similar to those obtained by Sun *et al*. (2020) who obtained a maximum amount of K released from K-feldspar of 1.751 mg L^{-1}, while the K release efficiency by the strains in this research was 13% higher with the same K source.

Verma *et al*. (2020) identified *Bacillus, Paenibacillus* and *Pseudomonas* strains with the ability to solubilize K in temperatures up to 60°C. The growth and K solubilization at the different temperatures determined in this study was lower in comparison with those results. The critical temperature was 40°C for all isolates in the presence of both K sources, and 37°C for Gram-negative strains in the presence of muscovite. Only the *Stenotrophomonas* sp. strain INCA-FRr1 managed to develop and perform in all four temperature ranges. Additionally, the acidic and neutral pH generally favored more K release, while alkalinity conditions only stimulated the release of K by the *Arthrobacter* sp. INCA-FRc17, *Bacillus* sp. INCA-FRc7, *Pseudomonas* sp. INCA-FRr15 and *Stenotrophomonas* sp. INCA-FRr1, coinciding with the results of Verma *et al.* (2020). Finally, salt stress affected K solubilization in both mineral sources. The level of halotolerance of the strains was limited to the lowest concentrations of NaCl, while at 12 g L^{-1} and 16 g L^{-1} the solubilization was very low or there was practically no microbial growth. However, *Stenotrophomonas* sp. INCA-FRr1 and *Bacillus* sp. INCA-FRc19x showed good resistance to these conditions, which corresponds to the results of Alexander *et al.* (2020) and Silambarasan *et al.* (2020), who characterized halotolerant strains of *Stenotrophomonas* with potential for promoting *Lactuca sativa* L. and *Arachis hypogaea* L. growth, respectively, and to Hamid *et al.* (2021) who obtained a K solubilizing *Bacillus* strain under salt stress conditions.

Yields of K release from both mineral sources showed important differences. Like the results of Verma *et al.* (2020) and Ghanbari and Safari Sinegani (2021), K release from muscovite was more efficient compared to K-feldspar in the same period of time; this result is interesting since muscovite is structurally more complex and less abundant in nature than feldspar minerals (Alling, 1923; Sattar *et al*., 2019). Many strains such as KSB identified by other authors generally exhibit other mechanisms for promoting plant growth such as biological N fixation, solubilization of other nutrients, or phytostimulation (Vasanthi *et al*., 2018; Verma *et al.,* 2020), so the role of these strains as agricultural inoculants would not be limited only to the contribution of K but would act in a wide range of ecological niches.

Conclusions

In the rhizoplane of corn plants established in leached Red Ferralitic soil, KSB populations corresponding to the genera *Bacillus, Arthrobacter, Paenibacillus, Lysinibacillus, Pseudomonas,* and *Stenotrophomonas* coexist. The yields in the K release differed between strains and varied depending on the established abiotic conditions. The strains corresponding to the genera *Lysinibacillus* and *Paenibacillus* showed less efficiency as solubilizers, while strains *Bacillus* sp. INCA-FRc7 and *Bacillus* sp. INCA-FRc19x stood out for their performance in all determinations. These strains may be part of future biofertilizers for the corn crop, and this work constitutes the preliminary steps to achieve this.

Conflict of interest statement

The authors declare that there is no conflict of interest regarding the publication of this article.

Author's contributions

RPP formulated the research objectives, designed the work plan, performed the phylogenetic analysis, and supervised the experimental part. IHF performed the statistical analysis and its interpretation. YOS and JCSB carried out the lab work. DSC carried out the molecular identification and supervised the phylogenetic analysis. SPM performed the final review of the article. All authors contributed to the writing of the document.

Literature cited

Alexander, A., Singh, V. K., & Mishra, A. (2020). Halotolerant PGPR *Stenotrophomonas maltophilia* BJ01 induces salt tolerance by modulating physiology and biochemical activities of *Arachis hypogaea*. *Frontiers in Microbiology*, *11*, Article 568289. https://doi.org/10.3389/fmicb.2020.568289

Alling, H. L. (1923). The mineralography of the feldspars part II. *The Journal of Geology*, *31*(4), 282–305.

Atlas, R. M., & Bartha, R. (2005). *Ecología microbiana y microbiología ambiental* (4th ed.). Pearson Addison Wesley.

Baghel, V., Thakur, J. K., Yadav, S. S., Manna, M. C., Mandal, A., Shirale, A. O., Sharma, P., Sinha, N. K., Mohanty, M., Singh, A. B., & Patra, A. K. (2020). Phosphorus and potassium solubilization from rock minerals by endophytic *Burkholderia* sp. strain FDN2-1 in soil and shift in diversity of bacterial endophytes of corn root tissue with crop growth stage. *Geomicrobiology Journal*, *37*(6), 550–563. https://doi.org/10.1080/01490451.2020.1734691

Bagyalakshmi, B., Ponmurugan, P., & Balamurugan, A. (2017). Potassium solubilization, plant growth promoting substances by potassium solubilizing bacteria (KSB) from southern Indian tea plantation soil. *Biocatalysis and Agricultural Biotechnology*, *12*, 116–124. https://doi.org/10.1016/j.bcab.2017.09.011

Basak, B. B., Sarkar, B., Biswas, D. R., Sarkar, S., Sanderson, P., & Naidu, R. (2017). Bio-intervention of naturally occurring silicate minerals for alternative source of potassium: challenges and opportunities. In D. L. Sparks (Ed.), *Advances in Agronomy* (pp. 115–145). Academic Press. https://doi.org/10.1016/bs.agron.2016.10.016

Berger, B., Patz, S., Ruppel, S., Dietel, K., Faetke, S., Junge, H., & Becker, M. (2018). Successful formulation and application of plant growth-promoting *Kosakonia radicincitans* in maize cultivation. *BioMed Research International*, *2018*, Article 6439481. https://doi.org/10.1155/2018/6439481

Dong, X., Lv, L., Wang, W., Liu, Y., Yin, C., Xu, Q., Yan, H., Fu, J., & Liu, X. (2019). Differences in distribution of potassium-solubilizing bacteria in forest and plantation soils in

Myanmar. *International Journal of Environmental Research and Public Health*, *16*(5), Article 700. https://doi.org/10.3390/ijerph16050700

Egamberdieva, D., Wirth, S., Jabborova, D., Räsänen, L. A., & Liao, H. (2017). Coordination between *Bradyrhizobium* and *Pseudomonas* alleviates salt stress in soybean through altering root system architecture. *Journal of Plant Interactions*, *12*(1), 100–107. https://doi.org/10.1080/17429145.2017.1294212

Etesami, H., Emami, S., & Alikhani, H. A. (2017). Potassium solubilizing bacteria (KSB): mechanisms, promotion of plant growth, and future prospects - a review. *Journal of Soil Science and Plant Nutrition*, *17*(4), 897–911. https://doi.org/10.4067/S0718-95162017000400005

Feng, K., Cai, Z., Ding, T., Yan, H., Liu, X., & Zhang, Z. (2019). Effects of potassium-solubulizing and photosynthetic bacteria on tolerance to salt stress in maize. *Journal of Applied Microbiology*, *126*(5), 1530–1540. https://doi.org/10.1111/jam.14220

Fundora, Z. M., Castiñeiras, L., Shagarodsky, T., Barrios, O., Fernández, L., León, N., Moreno, V., Cristóbal, R., García, M., Giraudy, C., Hernández, F., Arzola, D., de Armas, D., García, R., & Fuentes, V. (2010). Destinos de la producción de diferentes cultivos en fincas rurales de dos provincias de Cuba. *Cultivos Tropicales*, *31*(3).

Ghadam Khani, A., Enayatizamir, N., & Norouzi Masir, M. (2019). Impact of plant growth promoting rhizobacteria on different forms of soil potassium under wheat cultivation. *Letters in Applied Microbiology*, *68*(6), 514–521. https://doi.org/10.1111/lam.13132

Ghanbari, M., & Safari Sinegani, A. A. (2021). Isolation and identification of 10 strains of potassium, phosphorus and iron solubilizing bacteria from the soil around the potato roots in Hamedan province. *Electronic Journal of Soil Management and Sustainable Production*, *10*(4), 55–76. https://doi.org/10.22069/ejsms.2021.18031.1957

Hamid, S., Ahmad, I., Akhtar, M. J., Iqbal, M. N., Shakir, M., Tahir, M., Rasool, A., Sattar, A., Khalid, M., Ditta, A., & Zhu, B. (2021). *Bacillus subtilis* Y16 and biogas slurry enhanced potassium to sodium ratio and physiology of sunflower (*Helianthus annuus* L.) to mitigate salt stress. *Environmental Science and Pollution Research*, *28*, 38637–38647. https://doi.org/10.1007/s11356-021-13419-2

Hernández Jiménez, A., Bojórquez Serrano, J. I., Morell Planes, F., Cabrera Rodríguez, A., Ascanio García, M. O., García Paredes, J. D., & Madueño Molina, A. (2010). *Fundamentos de la estructura de suelos tropicales* (1st ed.). Universidad Autónoma de Nayarit, Instituto Nacional de Ciencias Agrícolas.

John-Louis, C. M., Vantour-Causse, A., & Tamayo-Sierra, A. A. (2017). Estado de la fertilidad química de los suelos ferralíticos rojos de la granja Los Pinos. *Revista Ingeniería Agrícola*, *7*(3), 17–22.

Khanghahi, M. Y., Pirdashti, H., Rahimian, H., Nematzadeh, G., & Sepanlou, M. G. (2018). Potassium solubilising bacteria (KSB) isolated from rice paddy soil: from isolation, identification to K use efficiency. *Symbiosis*, *76*, 13–23. https://doi.org/10.1007/s13199-017-0533-0

Koskey, G., Mburu, S. W., Njeru, E. M., Kimiti, J. M., Ombori, O., & Maingi, J. M. (2017). Potential of native rhizobia in enhancing nitrogen fixation and yields of climbing beans (*Phaseolus vulgaris* L.) in contrasting environments of eastern Kenya. *Frontiers in Plant Science*, *8*, Article 443. https://doi.org/10.3389/fpls.2017.00443

Kour, D., Rana, K. L., Kaur, T., Yadav, N., Halder, S. K., Yadav, A. N., Sachan, S. G., & Saxena, A. K. (2020). Potassium solubilizing and mobilizing microbes: biodiversity, mechanisms of solubilization, and biotechnological implication for alleviations of abiotic stress. In A. A. Rastegari, A. N. Yadav, & N. Yadav (Eds.), *New and future developments in microbial biotechnology and bioengineering - trends of microbial biotechnology for sustainable agriculture and biomedicine systems: diversity and functional perspectives* (pp. 177–202). Elsevier. https://doi.org/10.1016/B978-0-12-820526-6.00012-9

Kour, D., Rana, K. L., Yadav, A. N., Yadav, N., Kumar, M., Kumar, V., Vyas, P., Dhaliwal, H. S., & Saxena, A. K. (2020). Microbial biofertilizers: bioresources and eco-friendly technologies for agricultural and environmental sustainability. *Biocatalysis and Agricultural Biotechnology*, *23*, Article 101487. https://doi.org/10.1016/j.bcab.2019.101487

Kumar, S., Stecher, G., Li, M., Knyaz, C., & Tamura, K. (2018). MEGA X: molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution*, *35*(6), 1547–1549. https://doi.org/10.1093/molbev/msy096

Melgar, R. J., & Castro, L. N. (2005). Potasio. In H. Nielson, & R. Sarudiansky (Eds.), *Minerales para la agricultura en Latinoamérica* (pp. 254–266). Centro de Estudios para la Sustentabilidad.

Nagaraju, Y., Triveni, S., Subhashreddy, R., & Jhansi, P. (2017). Biofilm formation of zinc solubilizing, potassium releasing bacteria on the surface of fungi. *International Journal of Current Microbiology and Applied Sciences*, *6*(4), 2037–2047. https://doi.org/10.20546/ijcmas.2017.604.241

Nath, D., Maurya, B. R., & Khan, S. (2018). *In-vitro* solubilization of waste minerals and morphological characteristics of potassium and phosphorus solubilizing bacteria. *Journal of Pharmacognosy and Phytochemistry*, *7*(2), 1626–1632.

Nath, D., Maurya, B. R., & Meena, V. S. (2017). Documentation of five potassium- and phosphorus-solubilizing bacteria for their K and P-solubilization ability from various minerals. *Biocatalysis and Agricultural Biotechnology*, *10*, 174–181. https://doi.org/10.1016/j.bcab.2017.03.007

Naureen, Z., Rehman, N. U., Hussain, H., Hussain, J., Gilani, S. A., Al Housni, S. K., Mabood, F., Khan, A. L., Farooq, S., Abbas, G., & Harrasi, A. A. (2017). Exploring the potentials of *Lysinibacillus sphaericus* ZA9 for plant growth promotion and biocontrol activities against phytopathogenic fungi. *Frontiers in Microbiology*, *8*, Article 1477. https://doi.org/10.3389/fmicb.2017.01477

ONEI. (2021). *Series estadísticas agricultura enero-diciembre 2020*. Oficina Nacional de Estadistica e Informacióon. http://www.onei.gob.cu/node/15778

Paneque Pérez, V. M., Calaña Naranjo, J. M., Calderón Valdés, M., Borges Benítez, Y., Hernández García, T. C., & Caruncho Contreras, M. (2010). *Manual de técnicas analíticas para análisis de suelo, foliar, abonos orgánicos y fertilizantes químicos*. Ediciones INCA.

Rajawat, M. V. S., Singh, S., Tyagi, S. P., & Saxena, A. K. (2016). A modified plate assay for rapid screening of potassium-solubilizing bacteria. *Pedosphere*, *26*(5), 768–773. https://doi.org/10.1016/S1002-0160(15)60080-7

Rana, K. L., Kour, D., Kaur, T., Devi, R., Yadav, A., & Yadav, A. N. (2021). Bioprospecting of endophytic bacteria from the Indian Himalayas and their role in plant growth promotion of maize (*Zea mays* L.). *Journal of Applied Biology & Biotechnology*, *9*(3), 41–50. https://doi.org/10.7324/JABB.2021.9306

Reinhold-Hurek, B., Bünger, W., Burbano, C. S., Sabale, M., & Hurek, T. (2015). Roots shaping their microbiome: global hotspots for microbial activity. *Annual Review of Phytopathology, 53*, 403–424. https://doi.org/10.1146/annurev-phyto-082712-102342

Saha, M., Maurya, B. R., Meena, V. S., Bahadur, I., & Kumar, A. (2016). Identification and characterization of potassium solubilizing bacteria (KSB) from Indo-Gangetic plains of India. *Biocatalysis and Agricultural Biotechnology*, *7*, 202–209. https://doi.org/10.1016/j.bcab.2016.06.007

Sardans, J., & Peñuelas, J. (2015). Potassium: a neglected nutrient in global change. *Global Ecology and Biogeography, 24*(3), 261–275. https://doi.org/10.1111/geb.12259

Sarikhani, M. R., Oustan, S., Ebrahimi, M., & Aliasgharzad, N. (2018). Isolation and identification of potassium-releasing bacteria in soil and assessment of their ability to release potassium for plants. *European Journal of Soil Science*, *69*(6), 1078–1086. https://doi.org/10.1111/ejss.12708

Sattar, A., Naveed, M., Ali, M., Zahir, Z. A., Nadeem, S. M., Yaseen, M., Meena, V. S., Farooq, M., Singh, R., Rahman, M., & Meena, H. N. (2019). Perspectives of potassium solubilizing microbes in sustainable food production system: a review. *Applied Soil Ecology*, *133*, 146–159. https://doi.org/10.1016/j.apsoil.2018.09.012

Shirale, A. O., Meena, B. P., Gurav, P. P., Srivastava, S., Biswas, A. K., Thakur, J. K., Somasundaram, J., Patra, A. K., & Rao, A. S. (2019). Prospects and challenges in utilization of indigenous rocks and minerals as source of potassium in farming. *Journal of Plant Nutrition*, *42*(19), 2682–2701. https://doi.org/10.1080/01904167.2019.1659353

Silambarasan, S., Logeswari, P., Ruiz, A., Cornejo, P., & Kannan, V. R. (2020). Influence of plant beneficial *Stenotrophomonas rhizophila* strain CASB3 on the degradation of diuron-contaminated saline soil and improvement of *Lactuca sativa* growth. *Environmental Science and Pollution Research*, *27*, 35195–35207. https://doi.org/10.1007/s11356-020-09722-z

Sun, F., Ou, Q., Wang, N., Guo, Z. X., Ou, Y., Li, N., & Peng, C. (2020). Isolation and identification of potassium-solubilizing bacteria from *Mikania micrantha* rhizospheric soil and their effect on *M. micrantha* plants. *Global Ecology and Conservation*, *23*, Article e01141. https://doi.org/10.1016/j.gecco.2020.e01141

Tchakounté, G. V. T., Berger, B., Patz, S., Fankem, H., & Ruppel, S. (2018). Data on molecular identification, phylogeny and *in vitro* characterization of bacteria isolated from maize rhizosphere in Cameroon. *Data in Brief*, *19*, 1410–1417. https://doi.org/10.1016/j.dib.2018.06.003

Vasanthi, N., Saleena, L. M., & Raj, S. A. (2018). Silica solubilization potential of certain bacterial species in the presence of different silicate minerals. *Silicon*, *10*, 267–275. https://doi.org/10.1007/s12633-016-9438-4

Vejan, P., Abdullah, R., Khadiran, T., Ismail, S., & Nasrulhaq Boyce, A. (2016). Role of plant growth promoting rhizobacteria in agricultural sustainability - a review. *Molecules*, *21*(5), Article 573. https://doi.org/10.3390/molecules21050573

Verma, P., Yadav, A. N., Khannam, K. S., Kumar, S., Saxena, A. K., & Suman, A. (2016). Molecular diversity and multifarious plant growth promoting attributes of Bacilli associated with wheat (*Triticum aestivum* L.) rhizosphere from six diverse agro-ecological zones of India. *Journal of Basic Microbiology*, *56*(1), 44–58. https://doi.org/10.1002/jobm.201500459

Verma, P., Yadav, A. N., Khannam, K. S., Kumar, S., Saxena, A. K., & Suman, A. (2020). Molecular diversity and functional annotation of potassium solubilizing bacteria associated with wheat (*Triticum aestivum* L.) from six diverse agro-ecological zones of India. *Research Journal of Biotechnology*, *15*(10), 41–56.

Verma, P., Yadav, A. N., Khannam, K. S., Saxena, A. K., & Suman, A. (2017). Potassium-solubilizing microbes: diversity, distribution, and role in plant growth promotion. In D. Panpatte, Y. Jhala, R. Vyas, & H. Shelat (Eds.), *Microorganisms for green revolution. Microorganisms for sustainability* (pp. 125–149). Springer. https://doi.org/10.1007/978-981-10-6241-4_7

Vincent, J. M. (1970). *A manual for the practical study of the root-nodule bacteria. IBP Handbook 15.* Blackwell Scientific Publishers.

Wang, Q., Jiang, X., Guan, D., Wei, D., Zhao, B., Ma, M., Chen, S., Li, L., Cao, F., & Li, J. (2018). Long-term fertilization changes bacterial diversity and bacterial communities in the maize rhizosphere of Chinese Mollisols. *Applied Soil Ecology*, *125*, 88–96. https://doi.org/10.1016/j.apsoil.2017.12.007

Xiao, Y., Wang, X., Chen, W., & Huang, Q. (2017). Isolation and identification of three potassium-solubilizing bacteria from rape rhizospheric soil and their effects on Ryegrass. *Geomicrobiology Journal*, *34*(10), 873–880. https://doi.org/10.1080/01490451.2017.1286416

Plant spacing assessment in cocoa (*Theobroma cacao* L.) agroforestry systems in the Colombian Pacific region

Evaluación de densidades de siembra en sistemas agroforestales con cacao (*Theobroma cacao* L.) en la región Pacífica colombiana

José Ives Pérez-Zuñiga[1], Jairo Rojas-Molina[2*], and Andrés Felipe Zabala-Perilla[3]

ABSTRACT

Cocoa beans produced in the municipality of Tumaco (Colombia) are very attractive for chocolate producers and consumers because of their fine and aromatic characteristics. However, optimal plant spacing that could improve income for cocoa farmers is uncertain. To address the problem, this study was carried out from 2016 to 2017 taking into account two phases. In the first phase, a characterization of the cocoa production system in the Colombian Pacific region was conducted. This characterization included plant spacing, cocoa-associated species, production costs, financial sources, and system yields. In the second phase, an analysis of optimal plant spacing was conducted using the results from the characterization phase. Thus, a linear programming model was developed that utilizes the three plant densities most used by producers (3x3 m, 3.5x3.5 m, and 4x4 m). Labor force, monthly bank fees, and minimum farmer income were considered as constraints of the model. Profitability was established as the objective function. This model was optimized with GAMS software, using the CPlex solver. Plant spacing of 4x4 m (625 plants ha^{-1}) was found to be the optimal solution that maximized profitability. The minimum cocoa bean price required for this solution was 5,000 Colombian pesos kg^{-1} and the rural capitalization incentive (RCI) needed to be greater than or equal to 40%.

Key words: linear programming, profitability, incentives, plant density.

RESUMEN

Los granos de cacao producidos en el municipio de Tumaco (Colombia) son muy atractivos para los productores y consumidores de chocolate por sus características finas y aromáticas. Sin embargo, la distancia de siembra óptima que podría mejorar los ingresos de los cacaoteros es incierta. Para abordar este problema, este estudio se realizó entre 2016 y 2017 en dos fases. En la primera fase se realizó una caracterización del sistema de producción de cacao en el Pacífico colombiano que incluyó distancias de siembra, especies asociadas al cacao, costos de producción, fuentes de financiación, y rendimientos del sistema. En la segunda fase se realizó un análisis del espaciamiento óptimo de siembra con los resultados de la fase de caracterización. Así, se desarrolló un modelo de programación lineal que utiliza las tres densidades más usadas por los productores (3x3 m, 3.5x3.5 m y 4x4 m). La mano de obra, las cuotas bancarias mensuales y un ingreso mínimo del agricultor se consideraron como las restricciones del modelo. La rentabilidad se estableció como la función objetivo. Este modelo se optimizó con el software GAMS, utilizando el solucionador CPlex. Se identificó que el espaciamiento de 4x4 m (625 plantas ha^{-1}) era la solución óptima que maximizaba la rentabilidad. El precio mínimo del cacao requerido para esta solución era de 5000 pesos colombianos kg^{-1} y el incentivo a la capitalización rural (ICR) debía ser mayor o igual al 40%.

Palabras clave: programación lineal, rentabilidad, incentivos, densidad de plantación.

Introduction

In Colombia, cocoa production has high socio-economic relevance. Twenty-five thousand families depend on this crop, and more than 7.5 million labor days are generated annually. The reported planted area during 2017 reached 175,000 ha with a dry bean yield of 65,535 t (Baquero López, 2018). According to official data, cocoa is planted in 29 of the 32 departments of Colombia (Zambrano & Chávez, 2018). Because of weather and soil variability in these areas, cocoa beans produced in Colombia have favorable organoleptic characteristics for the national and international markets. Thus, 95% of Colombian cocoa beans are classified as "fine aroma cocoa", a valuable distinction given to only 5% of the grains in the world (Ballesteros *et al*., 2015; FEDECACAO, 2019).

Received for publication: 27 August, 2020. Accepted for publication: 17 November, 2021. Doi: 10.15446/agron.colomb.v39n3.90131

[1] Corporación Colombiana de Investigación Agropecuaria (AGROSAVIA), Centro de Investigación El Mira, Tumaco, Nariño (Colombia).
[2] Corporación Colombiana de Investigación Agropecuaria (AGROSAVIA), Centro de Investigación La Suiza, Rionegro, Santander (Colombia).
[3] Purdue University, West Lafayette, Indiana (USA).
[*] Corresponding author: jrojas@agrosavia.co

As a result of external market conditions, the global cocoa bean market is expected to grow, and both demand and prices are expected to increase. Based on these market circumstances, the Colombian government defined cocoa as “the peace crop”, in a strategy that seeks the expansion of crops in the post-conflict areas of the country, as an option for economic development (USDA, 2018). The department of Nariño in the Colombian Pacific region stands out within the post-conflict areas as the sixth largest dry bean producer nationwide with 2871 t per year (FEDECACAO, 2019).

The municipality of Tumaco is responsible for 70% of the total cocoa production in the department. Several cocoa samples from Tumaco were internationally awarded the “Foreign Excellence” at the “Salon du Chocolat” in Paris (Ballesteros *et al*., 2015; Montoya-Restrepo *et al*., 2015).

Plant density is an important variable for crop productivity. Some previous reports have shown the variability in plant density (200 to 1,000 trees ha^{-1}) and cocoa production is frequently seen as a low-tech production system (Montoya-Restrepo *et al*., 2015). At the technical and production levels, variability in cocoa plant densities faces two main criteria. On the one hand, the government and chocolate factories (*e.g*., Casa Luker and Compañía Nacional de Chocolates) recommend cultivating 1,000 cocoa trees ha^{-1}, used as a reference density for granting agricultural loans and subsidies (*e.g*., rural capitalization incentive - RCI). The government only grants incentives to plantations with densities of 1,000 trees ha^{-1}. The percentage of government incentives can be as high as 40% (DNP, CVC, & UNICEF, 1983; Pinzón Useche *et al*., 2012). The criteria of some farmers in the Pacific coast of Colombia for planting cocoa are based on environmental conditions, such as high levels of relative humidity, high rainfall, and low luminosity that lead to planting at densities from 625 to 800 trees ha^{-1}. Since the densities differ from those recommended by the government, the RCI on agricultural loans is generally not granted to these producers.

Access to financial services, especially loans, enhances the development of the agricultural sector, mainly for small producers who do not have enough capital (Echavarría Soto *et al*., 2018). Perfetti *et al*. (2013) suggested that this practice has a negative impact on the aggregate consumption and, consequently, on the generation of new investment opportunities.

Formal loans are regularly offered to rural producers in Colombia through the Fund for the financing of the agricultural sector (FINAGRO), mainly by the Banco Agrario (Agricultural Bank of Colombia). These programs usually involve incentives, such as the RCI, that are granted as an additional resource that relieves the net cash flow of producers (FINAGRO, 2017).

Currently, there are no studies about the post-conflict of the Colombian Pacific zone that show that farmer incomes decrease when the planting density is reduced to less than 1,000 trees ha^{-1}. In this regard, some authors suggest that the plantations are easier to manage at slightly greater distances (Pinzón Useche *et al*., 2012). When cocoa is produced under agroforestry systems, shading plays an important role in the correct management of the crop and, consequently, in the generation of income for producers (Álvarez-Carrillo *et al*., 2013).

The main purpose of the algebraic linear modelling applied to agriculture is to identify the optimal outcome (maximum or minimum) from a mathematical model, including parameters, equations, and restrictions (Aliev *et al.,* 2015). This method of optimization has been followed by Colombian and Latin American research institutions. For example, the Agricultural Research Corporation of Colombia (AGROSAVIA) and CIAT performed algebraic linear modelling in the models of adaptation and agroclimatic prevention (MAPA) initiative. By doing this, they identified technologies that maximize farmer’s incomes in the country. Several regions and 18 crops, including cocoa, were evaluated. The results of the MAPA initiative allowed researchers and farmers to define the optimal method of introducing irrigation systems and fertilization plans to the crops (Ministerio de Agricultura, Fondo Adaptación, & CORPOICA, 2017).

This study aimed to assess the optimal plant spacing that would maximize the monetary income of cocoa producers in the Colombian Pacific, allowing the repayment of bank loans and the improvement of living standards.

Materials and methods

Study area

This research was carried out in the municipality of Tumaco (Fig. 1) located in the southeast of Colombia (2°48’24” N, 78°45’53” W) on the Pacific coast of the department of Nariño. It has a warm humid climate, with an average temperature of 28°C, average annual relative humidity of 83.86%, and annual precipitation of 2,531 mm. The municipality has an area of 3,760 km^2 and most of its territory is flat or slightly slopped (Angulo Guevara, 2016).

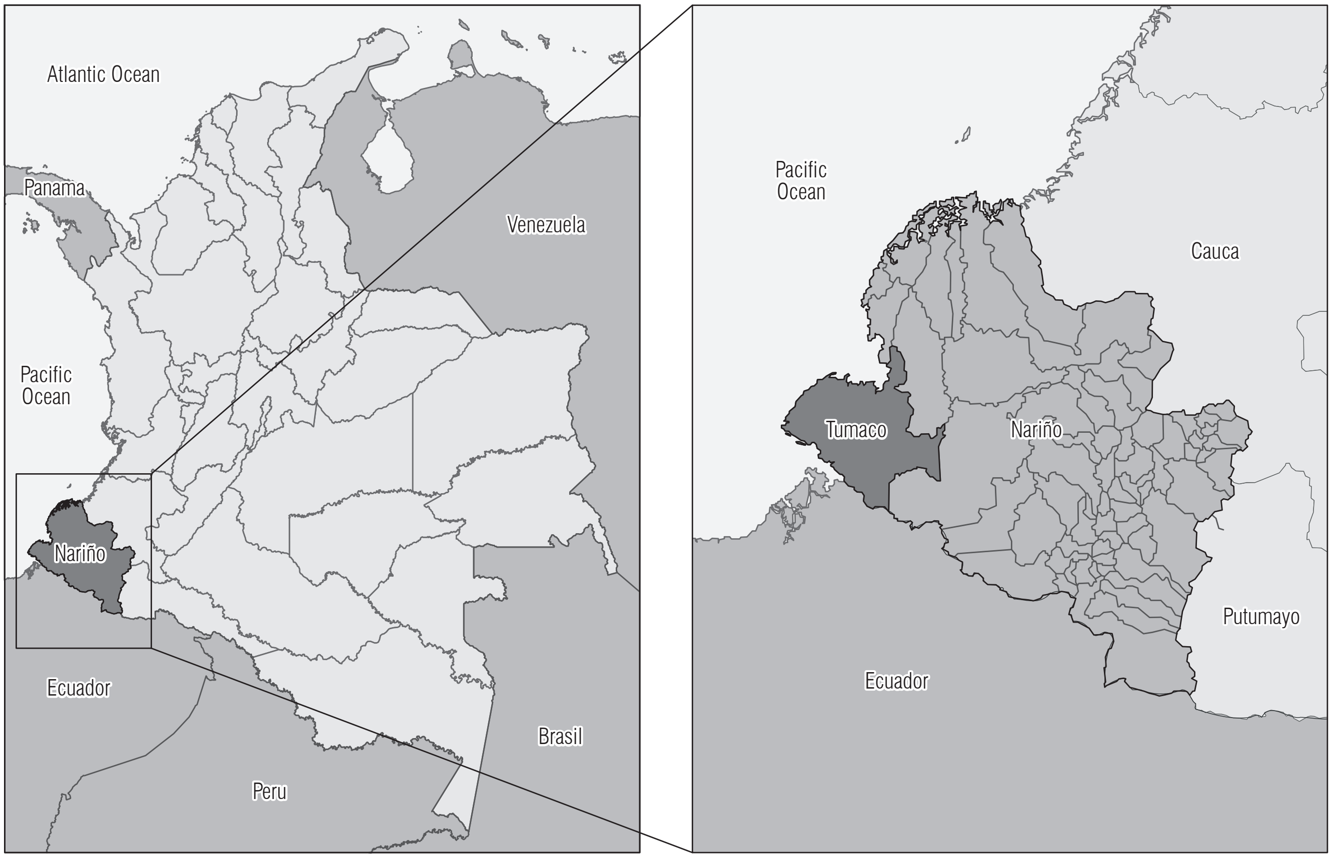


FIGURE 1. Location of the municipality of Tumaco in Colombia.

The municipality of Tumaco, with 134,992 ha suitable for agriculture (UPRA, 2020), has a vocation and productive commitment mainly based on traditional agriculture, fishing, forestry and, to a lesser extent, tourism. The agricultural sector is historically based on production systems such as cocoa, coconut, oil palm, and rice, and some specialty crops such as tabasco pepper (Bitácora & Territorio, 2017). However, illegal crops have affected traditional agricultural activities (UNODC, 2019). Cocoa crops are a productive alternative that is being promoted by the government for the substitution of illicit crops in the region (Barbosa, 2019).

Selection of cocoa farms and data collection in the municipality of Tumaco

Before visiting and/or selecting the farms to collect data, we carried out a review of reports and research studies focused on cocoa in the Pacific area of Nariño. By doing this, we were able to analyze the biophysical and socio-economic aspects of cocoa farmers in this region. To select the farms, we consulted cocoa corporations, community councils, producer associations, and agricultural technical assistance service providers (EPSAGROS). We then classified the study farms considering the variables crop age, planting distance, and type of clone. Villages were selected using the criteria of five or more cocoa producers in the area, accessibility, and a planted area greater than 1 ha. The number of farms selected for each density was defined by stratified probability sampling, in which 10 farms were required for each density for sampling (Barrantes *et al.,* 2018).

Characterization of the farms and identification of cocoa tree spacing

Cocoa corporations and organizations provided a database of 926 producers, of which 95% had plant densities below 1,000 trees ha^{-1} (Compañía Nacional de Chocolates, 2012; Pinzón Useche *et al*., 2012). A total of 483 producers were surveyed in the townships of the municipality of Tumaco: Alto Mira (n = 45), Las Varas (n = 71), Medio Mira (n = 110), Rio Chagui (n = 87), Rio Tablon Dulce (n = 29), veredas adicionales (n = 8), zona carretera (n = 86), Rio Rosario (n = 47). Based on this data, 358 producers were not included, 335 because they were in areas affected by a social conflict (*e.g.,* presence of illegal armed groups), 15 because they were located in distal regions where there were no more than five cocoa farmers, and 8 because they had less than 1 ha of cocoa crop.

The information collected through the surveys and the structure (Pássaro, 2014) and function of traditional cocoa production systems were systematized using Excel software. A descriptive statistical analysis was performed to characterize the cocoa farms in the municipality of Tumaco. As a result, three planting densities were defined for the region, and these were used to model the optimal planting density.

Determining the optimum plant spacing

To determine tree spacing that allows cocoa farmers to maximize their profits, the linear programming method was used.

An algebraic model was developed based on costs and yields of each tree spacing (1,111, 816, or 625 trees ha^{-1}) along with parameters of typical prices and financial costs of the cocoa market in the municipality of Tumaco. The model was optimized with the Cplex solver, using the general algebraic modelling system (GAMS). Following linear programming, the model was used to identify the planting density that maximizes the gross margin (income minus costs) and allows local farmers to pay their annual obligations of a bank loan (offered by the Banco Agrario). Land and capital restrictions were included, stating that the start-up budget required was 15 million Colombian pesos (COP) ha^{-1}, adding the loan value and the farmer's own contribution. Given the purpose of the model, cash flow and optimal planting area outputs were used to determine the optimal condition for maximizing the producer's profit. Once the optimal planting density was obtained, simulations of price scenarios (3,500, 4,000, 4,500, 5,000, and 5,500 COP kg^{-1} of dry cocoa beans) and loan subvention levels (RCI = 20%, 40%, and no incentive = 0%) were carried out.

These simulations helped to determine the levels of prices and subventions that generate feasible and non-feasible outputs at a given density. In addition, a financial analysis was carried out using the net present value (NPV) approach. The NPV is an absolute metric for measuring economic profitability. This parameter expresses rates of return that allow investors to identify the financial efficiency of a project (*e.g.*, a cocoa crop) (Magni & Marchioni, 2020). Although other financial parameters could be used (*e.g.*, internal rate of return), because of the purpose of the study, only the NPV was calculated. This parameter allowed us to identify the cocoa plant spacing that maximizes financial income.

The NPV is calculated as follows:

$$NPV = \frac{Rt}{(1+i)^t} \quad (1)$$

where NPV is the net present value (COP), Rt is the net cash flow at time t (COP), i is the discount rate (percentage), and t is the time of the cash flow (years).

Description of the model used

Sets:

D: Densities (*ha*); ($d = \{1,\ldots..m\}$)

T: Time periods (*year*); ($t=\{1,\ldots.p\}$)

Objective function:

$$\sum_{(d\in D)} \sum_{t\in T} \left((Y_t\, N_t\, P) - (C_t\, N_t)\right)$$

Parameters:

A: Farm size (*ha*)

P: Price (*COP* kg^{-1})

Y_t: *Yield per planting density per period* (*kg* ha^{-1})

C_t: *Costs per planting density per period* (*COP* ha^{-1})

I_t: *Minimum family income* (*COP*)

K_t: *start-up budget* (*COP*)

Variables:

N_t: *planted area per density per period* (*ha per year*)

CF_t: *cashflow* (*COP per year*)

Constraints:

Land:

$$\sum_{(d\in D)} N_t \leq A \quad \forall\, t \in T, \quad \forall\, d \in D$$

Cash flow:

$$\sum_{(d\in D)} \left((Y_t\, N_t\, P) - (C_t\, N_t)\right) + CF_t\,(t-1) + K_t - I_t = CF_t,\ \forall\, t \in T$$

Results

Characterization of farms and identification of local planting densities

Among the 483 producers interviewed, 95% were currently using densities lower than 1,000 plants ha^{-1}. The most used densities by producers were: 816 trees ha^{-1} (87%), 943 trees ha^{-1} (7%), 1,100 trees ha^{-1} (5%), and 625 trees ha^{-1} (1%).

Regarding land ownership, 97% of the farmers owned the land they used for the cocoa crop and most of crops were on lands situated within the territories of community councils (ethnic entities that manage a territory collectively). Therefore, these lands were assigned as work areas for the families that were members of the community council, according to decree 1745 of 1995 (Ministerio de Agricultura, Fondo Adaptación, & CORPOICA, 2017), where 2% was leased, and 1% was under another form of land ownership.

Moreover, 72% of the producers had less than 3.3 ha planted with cocoa (Fig. 2) that showed that cocoa cultivation was a small-scale activity in this region. In many cases, producers complemented their labors in cocoa with other activities.

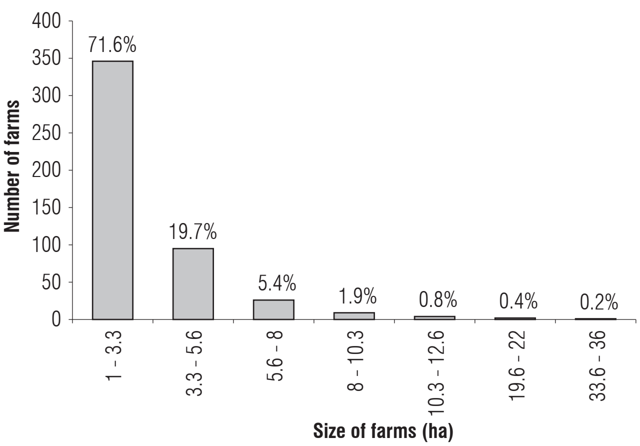


FIGURE 2. Size of areas established with cocoa crop in the municipality of Tumaco.

Cocoa accompanying species

On the Pacific coast of Nariño, cocoa is planted in agroforestry systems that were not included in the monoculture study. Although cocoa crops were already in the production stage, different species were still present in the crop (Fig. 3), predominantly timber, fruit, and plantain. These species are important for food security and generate income in the short, medium, and long terms.

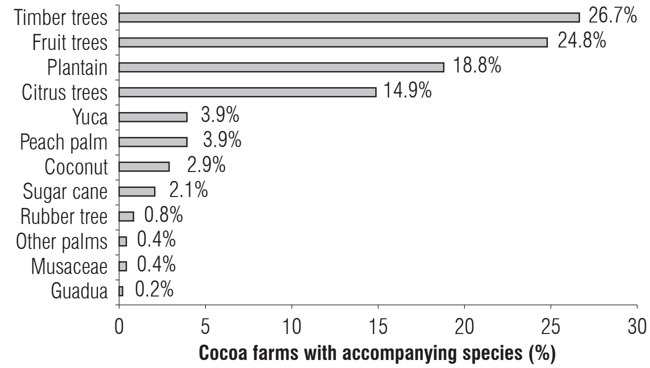


FIGURE 3. Cocoa farms with accompanying species in the municipality of Tumaco.

Financing the establishment and management of cocoa farming

Among the producers surveyed, 76.3% stated that the area they currently own and maintain was established with their own resources, and only 19.6% did so with the help of a loan (Fig. 4). However, when producers were asked why they did not expand their cocoa growing area, 63% of them indicated that the lack of resources through loan lines was one of the major causes for not planting new areas of cocoa (Fig. 5). Because of a lack of guarantees, cocoa farmers have difficulties for accessing bank loans. Most producers have not defined the legal situation of their land, so they do not have deeds to support the loan credit application since these are collective territories. Moreover, they cannot obtain the loan scores needed because they were not able to meet their obligations when loans were granted to them. This situation is sometimes due to a lack of banking assistance (CORDEAGROPAZ, 2011).

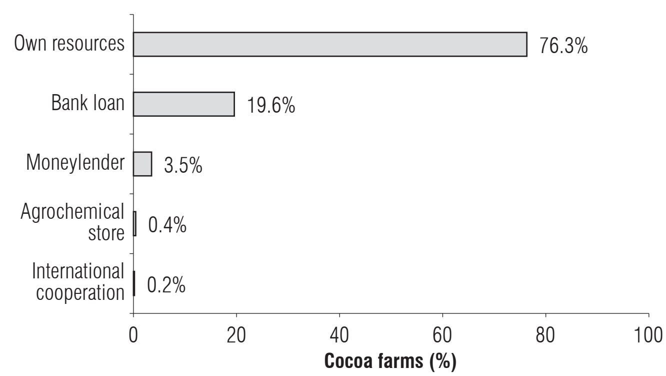


FIGURE 4. Percentage of farms with the financing sources used for the establishment and maintenance of cocoa cultivation in the municipality of Tumaco.

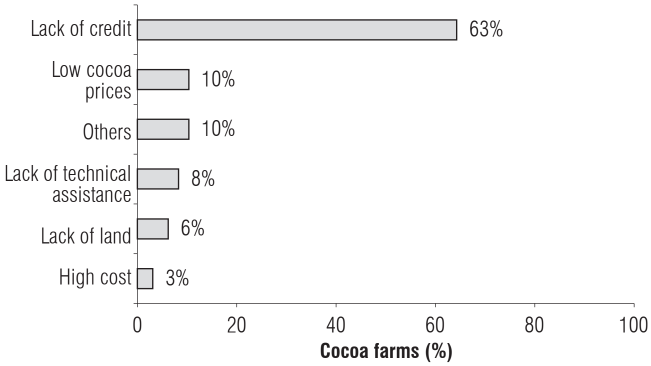


FIGURE 5. Percentage of farms with causes for not planting cocoa in new areas in the municipality of Tumaco.

Cocoa marketing

According to the results obtained in the diagnostics, there is a high level of intermediation; 65% of cocoa beans was sold to middlemen, suggesting that the price paid to the producer does not generate a surplus, making production non-profitable, 17% was sold to producer organizations, 17% was sold to the industry, and 1% was sold to cooperatives. Although cocoa farmers could sell the beans at higher prices at the market center without intermediation, the costs associated with the transportation from the farm to the market center are higher than the benefit of selling without intermediation. Therefore, they prefer to sell their products to an intermediate buyer. This situation was specially aggravated during seasons in which cocoa trees do not produce enough quantities of pods (April to October).

Determination of the optimal plant density in the municipality of Tumaco

Sixty-five farms were pre-selected using the data from the survey and the criteria areas planted with cocoa (greater than 1 ha) and sowing density. These farms were then visited by the research team to verify the information provided by the producer at field level. Among these farms, 16 did not meet the above-mentioned criteria and, therefore, were discarded. Also, several farms were not selected because they showed the same density of cocoa planting. This allowed, in some cases, selecting up to 10 farms per planting density. Finally, the data revealed that the most common densities used by cocoa farmers with technified crops in Tumaco are 625, 816, and 1,111 trees ha^{-1}. However, for the last density it was not possible to obtain the number of representative farms. This left a total of 22 farms in different parts of the municipality of Tumaco to be analyzed by the model.

A specific economic survey was applied to the producers of the 22 selected farms. In addition, an area of 40 x 40 m was delineated in the farms, and, within this area, 20 cocoa trees were selected to monitor production and incidence of diseases. This process allowed the consolidation of information for quantifying the costs and income generated by the activity of cocoa producers in the region of Tumaco. With the organized information, it was possible to determine the activities and infer the costs (considering prices in 2016) incurred in the cocoa activity in the region of Tumaco (Tab. 1).

The analysis of start-up and production costs (Tab. 1) allowed inferring that the farmers did not invest in drainage construction during any year, despite the high levels of rainfall and constant crop flooding. The number of daily labors used in the first year of cultivation was 194, 182, and 84 for planting densities of 816, 1,111, and 625 trees ha^{-1}, respectively. Moreover, the greatest demand for labor was observed in the planting density of 816 trees ha^{-1} (1072 labor days). This was mainly because this density requires more crop management tasks than other densities. Indeed, under a density of 1,111 trees ha^{-1} 668 labor days are required, and under a density of 625 trees ha^{-1} 493 labor days are required (Fig. 6).

Regarding start-up and general management activities, more than 20% of labor days corresponded to weed control. This proportion is higher in the 625 trees ha^{-1} scheme, probably because of more free space in which weeds can grow.

The yield ha^{-1} in the main components of the agroforestry system with cocoa also varied (Tab. 2). For this specific analysis, the harvest of other crops (different from cocoa and plantain) was not included (*i.e.*, some other crops were harvested to be consumed within the farm). Likewise, within the evaluation period, the yield of timber products

TABLE 1. Estimated annual costs ha^{-1} for the cocoa plant densities in the municipality of Tumaco.

Plant density (trees ha^{-1})	Years of establishment of the plantation				
	1	2	3	4	5-10
1,111	8,928,400	6,406,000	3,905,800	2,789,500	3,327,000
816	9,683,600	12,552,800	8,430,400	4,162,600	4,392,600
625	4,553,300	4,386,600	3,394,000	2,288,00	2,533,000

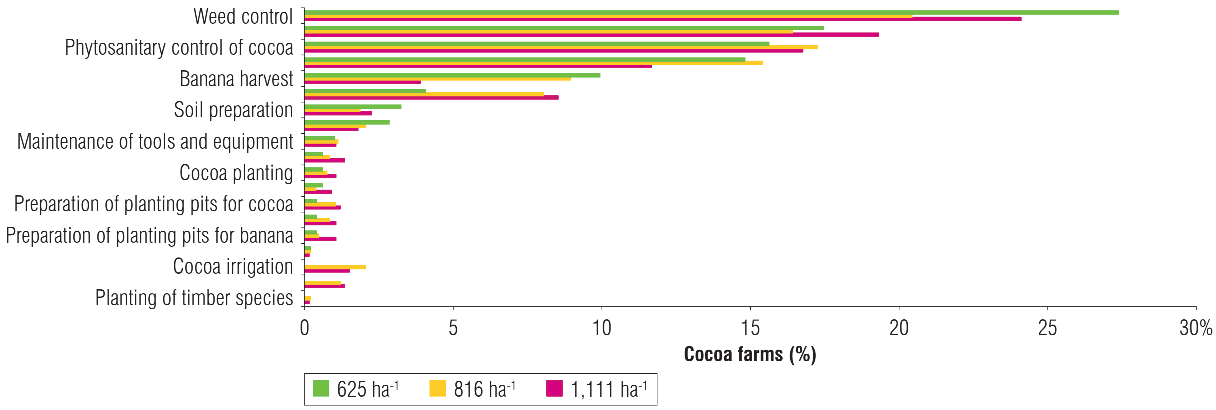


FIGURE 6. Percentage of farms with activities reporting the greatest use of wages by cocoa crop in the first 5 years of cultivation in the municipality of Tumaco.

TABLE 2. Yield (ha^{-1}) of the components of the cocoa agroforestry system by planting density.

Planting density (plants ha^{-1})	Yield (ha^{-1})	Year				
		1	2	3	4	5-11
1,111	Cocoa dry beans (kg)	0	561	514	510	455
	Plantain (hundred)	44	46	29	0	0
816	Cocoa dry beans (kg)	0	620	944	711	733
	Plantain (hundred)	107	88	64	x	x
625	Cocoa dry beans (kg)	0	630	772	701	864
	Plantain (hundred)	73	90	43	20	x

Hundred: 100 fingers of bananas, which is the way bananas are marketed in the municipality of Tumaco.

was not included because the harvesting period was longer than 12 years. In the short term (years 2 to 4), the best yield was obtained at a density of 816 trees ha^{-1} and in the medium term (years 6 to 11) the highest yield was recorded with a density of 625 trees ha^{-1}. This is probably related to cocoa tree growth since as the crop matures, the rows become more closed and, if there is no adequate pruning and disease control, this affects the production of the crop (Tab. 2).

Optimal planting area

The data shown in Table 3 (sets, scalars, and parameters) was used as an input for the model studied here. After compiling these data, iterations under linear programming assumptions rendered financial viability results only in the 4x4 m plant density. For this density, the model compilation found an optimal solution, in which the profit variable (revenues minus costs) was maximized, and land and cash flow constraints were met.

According to the results obtained, the solution that maximizes the profit and complies with all the restrictions was the planting of the 4x4 m density, in the totality of the available area (1 ha) (Fig. 7), during the deadline for the payment of the loan (11 years).

This result indicated that, for the area studied, the 4x4 m density maximized the income of the cocoa producers and allowed farmers to cover the obligations of the agricultural loan offered by the Banco Agrario.

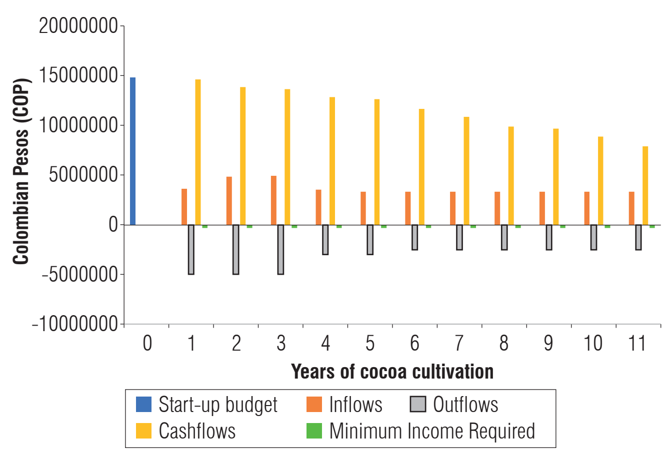


FIGURE 7. Cocoa system cash flow under a 4x4 m plant density in the municipality of Tumaco.

TABLE 3. Input from the microeconomic optimization model for three densities of cocoa planting in the municipality of Tumaco.

Type of data (input)	Data description	Data
Set	Year	1-11
Set	Densities (m)	3x3 3.5x3.5 4x4
Scalar	Crop area (ha)	1
Scalar	Price (COP kg^{-1})	5,500
Parameter	Minimum family income (COP) per year	500,000
Parameter	Start-up budget (COP)	15,000,000
Parameter	RCI (%)	40
Parameter	Annual loan payment (COP)	Years 1-3 = 1,552,000 Year 4 = 2,882,000 Year 5 = 2,688,000 Year 6 = 2,494,000 Year 7 = 2,300,000 Year 8 = 2,106,000 Year 9 = 1,912,000 Year 10 = 1,718,000 Year 11 = 1,524,000
Parameter	Gross margin (COP)	= incomes – total costs

RCI - Rural capitalization incentive.

Cash flow

The output of this component (Fig. 7) was made up of total income, expenses, and initial capital, corresponding to the value of the loan granted by the Banco Agrario and the amount contributed by the farmer. It should be noted that the annual income included the minimum income value for the family, in this case $500,000 COP per year ha^{-1} (above this value, the model does not find an optimal solution).

The cashflow values for all periods were positive, which guarantees that the farmer had the necessary financial resources to carry out the cultivation labors. Given the value of the initial capital, the farmer could cover the expenses from year 1 to year 4, the time range in which cocoa maintains low production and relatively low income. From year 4 onwards, expenses are stabilized and income increases, generating a considerable increment in capital flow. This increase can be considered as the surplus between total income and the established minimum family income.

Behavior of the net present value (NPV)

Given the planting system cash flow for the 4x4 m density in the municipality of Tumaco, NPV behavior was evaluated with a 10% discount rate to determine, under this parameter, the financial viability of the investment. This discount rate is between the values mentioned by Piraquive Galeano *et al.* (2018). The exception is that this cash flow does not include the leverage that the farmer carries out with the bank loan; therefore, neither the initial capital nor the annual loan obligations shown in Figure 8 were considered.

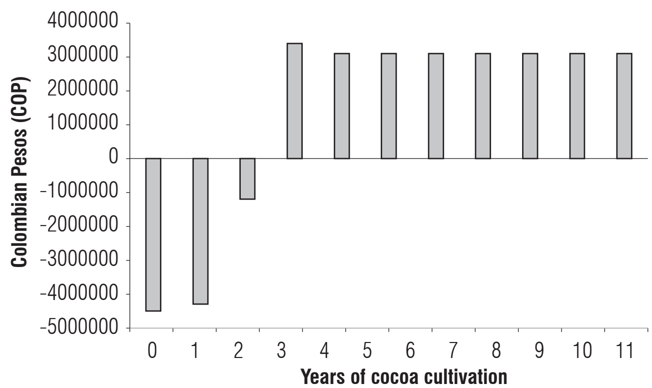


FIGURE 8. Discounted cash flow of the cocoa production system under a plant density of 4x4 m.

The cash flow behavior (income and expenses) of the density that maximized the profit for the municipality (4x4 m) showed a positive NPV (4,390,003 COP) during the evaluated period (11 years) (Fig. 8). Under this modeling scenario, based on this parameter, the planting system showed financial viability and the data are consistent with the results of the previously proposed model.

RCI and price scenarios

To determine the effect of the variation on both the RCI and the cocoa bean price in Tumaco, modeling was carried out and the viability of the cocoa production system was observed at a density of 4x4 m in nine combined scenarios (three price scenarios and three RCI scenarios) (Tab. 4). The modeling "base" was selected subjectively, assuming a cocoa price at the lowest level in the current period (4,500 COP kg^{-1}) and a bank loan without subsidy or incentive (RCI = 0%). The other values, 20% and 40% for the RCI, were set assuming the actual rate for the area (40%) and its average value (20%). The price values of the remaining scenarios were set considering the average of historical cocoa prices over the last 7 years (5,500 COP kg^{-1}) and the price at the time of modelling (4,700 COP kg^{-1}).

TABLE 4. Feasibility of rural capitalization incentive (RCI) scenarios and prices for a 4x4 m plant density in the cocoa system in the municipality of Tumaco.

Price (COP)	RCI (%)		
	40	20	0
5,500	Feasible	Not feasible	Not feasible
5,000	Feasible	Not feasible	Not feasible
4,500	Not feasible	Not feasible	Not feasible

According to the RCI scenarios and prices proposed in the model, only under conditions of RCI 40% with prices above 5,000 COP kg^{-1}, the model showed viable results (Tab. 3).

Discussion

The information from the survey of 483 producers revealed that most of them planted cocoa with a density of less than 1,000 trees ha^{-1}, disagreeing with the recommendations for cocoa cultivation in the country (Compañía Nacional de Chocolates, 2012; Pinzón Useche *et al*., 2012). These recommendations have not been implemented due to several factors such as low support from governmental and/or private institutions, absence of bank loan programs focused on cocoa crop establishment and development, proliferation of illicit crops and/or crop switching (*e.g.*, establishment of oil palm), and insufficient knowledge about the technical management of the crop (CORDEAGROPAZ, 2011).

Regarding the area planted with cocoa, 72% of the producers have less than 3.3 ha planted with this species (Fig. 2) that is the minimum recommended economic area for the crop (Moreno Rozo & López Villalobos, 2013). This result is consistent with a characterization performed in a community council in Tumaco where the size of the production unit of 55.4% of the producers included areas between 2 and 3 ha. Preciado *et al*. (2011), in a study of the traditional system of cocoa in the region, concluded that 53% of producers have areas of 1 to 5 ha. These assessments state that the size and low yields of areas cultivated with cocoa do not generate capital accumulation because income and expenditure are equivalent, making producers engage in other productive activities. In Tumaco and other frontier regions of Colombia, the infrastructure for transporting the product is less developed and there are difficulties for moving the cocoa beans to purchasing centers. Therefore, marketing costs are higher, and the prices and income received by the producer are low (Abbott *et al*., 2018).

Espinosa-Álzate and Ríos-Osorio (2016) classified the cocoa production systems in Tumaco into two groups: complex local systems with greater biodiversity and commercial exploitation systems with less biodiversity and a greater number of cocoa trees (plant density greater than 1,000 trees ha^{-1}). In this study, there was no evidence for the presence of crops with commercial exploitation systems, also identified by several authors. In these crops the predominance of timber and fruit species and banana stands out, with the latter being important for food security and, in the case of timber, for generating income in the short, medium and long terms (Aguiño, 2010; Laing, 2011; Preciado *et al*., 2011).

Other studies have shown that cocoa production profitability depends on four factors: 1) the plant material used, 2) the plant densities, 3) the type of system adopted (*e.g.*, agroforestry), and 4) the crop technological management. Moreover, agroforestry systems with yields above 1,000 kg ha^{-1} and cocoa prices above 4,800 COP kg^{-1} of bean dry weight are economically viable options for farmers (Pinzón Useche *et al*., 2012; Yambure *et al*., 2014). For profitability indicators to be positive, annual production should be between 1,500 and 2,000 kg of dry beans ha^{-1}, with the minimum production unit being 3 ha (ERS - ABC USAID/MIDAS Crops, 2009). In this way, it is possible to obtain the internal rate of return (IRR that is an interest rate that equates the current worth of a cash flow stream to zero) values of 46.7% and 55.5%, respectively.

In Mexico, cocoa cultivation is profitable for the humid tropics when it produces more than 770 kg dry beans ha^{-1} even with variations in price and increased costs of fertilizers (Espinosa-García *et al*., 2015). A net profit for cocoa cultivation is obtained when the crop is properly managed with 1,000 trees ha^{-1} under the conditions of Colombia, with a yield of 2,000 kg ha^{-1} and a price of 7,000 COP kg^{-1} of dry cocoa in 2016 (Barón Urquijo, 2016). For the Caquetá region, a feasibility study of cocoa cultivation in agroforestry arrangement results in positive financial indicators with an IRR of 5.23%, a benefit-cost ratio of 6.45, and a planting density for cocoa of 1,000 trees ha^{-1} (Yañez Agudelo, 2009).

In Tumaco, cocoa is traditionally cultivated with low planting densities from 200 to 250 trees ha^{-1}. However, new crops are being planted at densities such as 800 trees ha^{-1}, especially by using loans to finance them. This is the determining factor for access to financial services used to boost economic growth because of its positive impact on aggregate consumption and the generation of investment opportunities (Perfetti *et al.,* 2013). However, due to the difficulties for accessing loans in the region, this factor was not identified as a strategy for improving or increasing cocoa areas according to Montoya-Restrepo *et al.* (2015). Espinosa-Álzate and Ríos-Osorio (2016) identified the reduction of product sales to intermediaries in the chain as a priority action for strengthening organizational processes to achieve economies of scale. However, this is a complex action since the producers only harvest small volumes in very heterogeneous conditions of presentation and quality, making it very difficult for farmers to meet the market demands (DNP, 2014).

FEDECACAO currently recommends planting approximately 1,000 trees ha^{-1}, which is a reference for FINAGRO to apply for 40% RCI. This is a complex issue for the Pacific region, that has high cloudiness, rainfall, and temperature. These conditions favor a rapid development of plants with exuberant foliage, but they also allow the occurrence of diseases as well as increased production costs due to a higher demand for labor such as pruning and phytosanitary management. In this study, the number of labor days used in the first year of cultivation was 194, 182, and 84 for planting densities of 816, 1,111, and 625 trees ha^{-1}, respectively. For the planting density of 1,111 trees ha^{-1}, this value is lower than that reported by Pinzón Useche *et al.* (2012) for the same density.

The economic analysis carried out in this study showed that RCI is required for the producer to maintain a positive cash flow. Therefore, the cocoa farmers might ask FINAGRO and the Banco Agrario to recognize the incentive for new crops with planting densities used in the municipality of around 625 to 800 trees ha^{-1}.

The linear programming method used helps to improve resource allocation and optimize profit. For example, Adewumi *et al.* (2020) used linear programming to allocate the best land use efficiency in farms in Nigeria and found that the best solution for increasing yield was to employ mixed cropping instead of monocropping. Indeed, these data showed that the mixed cropping strategy increased the net income return to farmers and optimized the land use efficiency. Shirshahi *et al.* (2020) also used linear programming to optimize crop areas and water allocation in Iran, with profit maximization as the objective function. In that study, higher revenue was obtained using less area and water, by selecting species that are well acclimated to those environmental conditions.

Conclusions

Cocoa farmers in Tumaco have, on average, less than 1,000 trees ha^{-1} planted and a total yield of 400 kg of dry cocoa ha^{-1}. Consequently, to make cocoa crops viable in the Colombian Pacific zone, a rural capitalization incentive (RCI) of 40% is required. Additionally, we recommend to cocoa farmers to use the 4x4 m planting density when establishing their crops. These conditions, with prices above 5,500 COP kg^{-1}, allow cocoa farmers not only to pay the bank loan fees but also to obtain an income from the cocoa production.

Acknowledgments

This study was carried out as part of the project "Technical recommendations for alternative intensive and traditional cocoa production systems with emphasis on agroforestry arrangements (SAF)" implemented by Corporación Colombiana de Investigación Agropecuaria (the Colombian Agricultural Research Corporation) AGROSAVIA and financed by the Ministerio de Agricultura y Desarrollo Rural (MADR). We thank the research support professionals Jaydy Segura Estupiñan, Beto Alonso Moreno, and Allende Pesca Moreno for their assistance in collecting information during the development of the field work.

Conflict of interest statement

The authors declare that there is no conflict of interest regarding the publication of this article.

Author's contributions

JIPZ and JRM designed the experiments; JIPZ conducted the experiments; JIPZ, JRM and AFZP wrote the article; JIPZ, JRM and AFZP contributed to the data analysis. All authors improved and approved the article.

Literature cited

Abbott, P. C., Benjamin, T. J., Burniske, G. R., Croft, M. M., Fenton, M. C., Kelly, C. R., Lundy, M. M., Rodriguez Camayo, F., & Wilcox Jr., M. D. (2018). *An analysis of the supply chain of cacao in Colombia*. Purdue University, International Center for Tropical Agriculture (CIAT). https://cgspace.cgiar.org/bitstream/handle/10568/96636/Cacao_Final_Report.pdf?sequence=5&isAllowed=y

Adewumi, A., Tanko, L., Ibrahim, F. D., & Yisa, E. S. (2020). Net returns maximizing combination of arable crops among smallholder farmers in Kaiama agricultural zone of Kwara State, Nigeria. *Asian Journal of Economics, Business and Accounting*, *15*(4), 66–74.

Aguiño, J. E. (2010). *La perspectiva de desarrollo agroindustrial y empresarial de los productores de cacao del municipio de Tumaco departamento de Nariño*. [Unpublished master's thesis]. Universidad de Nariño.

Aliev, R. A., Alizadeh, A. V., Huseynov, O. H., & Jabbarova, K. I. (2015). Z-number-based linear programming. *International Journal of Intelligent Systems*, *30*(5), 563–589. https://doi.org/10.1002/int.21709

Álvarez-Carrillo, F., Rojas-Molina, J., & Suarez-Salazar, J. C. (2013). Simulation arrangements cocoa agroforestry as a diagnosis and planning strategy for producers. *Ciencia y Tecnología Agropecuaria, 13*(2), 145–150. https://doi.org/10.21930/rcta.vol13_num2_art:249

Angulo Guevara, M. E. (2016). *Plan de desarrollo municipal 2016 - 2019: Tumaco nuestra PAZión*. Alcaldía municipal de Tumaco. https://cpd.blob.core.windows.net/test1/52835planDesarrollo.pdf

Ballesteros, W., Lagos, T. C., & Ferney, H. (2015). Morphological characterization of elite cacao trees (*Theobroma cacao* L.) in Tumaco, Nariño, Colombia. *Revista Colombiana de Ciencias Hortícolas*, *9*(2), 313–328.

Baquero López, E. (2018, November 23–24). *Federación Nacional de Cacaoteros, Fondo Nacional del cacao* [Conference presentation]. Quinto seminario internacional de saberes y sabores de cacao: aromas de paz, Bogotá, Colombia. https://drive.google.com/file/d/1s5molI7fsrauRD8AAYX1-fvy4XwvcWNr/view

Barbosa, F. (2019, July 21). Plátano, coco y cacao remplazan la coca en Tumaco. *Semana*. https://www.semana.com/medio-ambiente/articulo/cultivos-tradicionales-reemplazan-la-coca-en-tumaco/45085/.

Barón Urquijo, J. D. (2016). *El cultivo del cacao; un negocio rentable, competitivo y ambientalmente sostenible en Colombia*. Fondo Nacional del Cacao.

Barrantes, C., Siura, S., Castillo, E., Huayarca, M., & Rado, J. (2018). *Manual para el análisis de la sostenibilidad de sistemas de producción de la agricultura familiar*. Instituto Interamericano de Cooperación para la Agricultura. http://repositorio.iica.int/bitstream/handle/11324/7035/BVE18040193e.pdf?sequence=1

Bitácora & Territorio. (2017). *Lectura territorial de San Andrés de Tumaco*. Fondo Internacional de Desarrollo Agrícola, Centro Latinoamericano para el Desarrollo Rural. https://www.rimisp.org/wp-content/files_mf/1514388162Producto2_LecturaterritorialTumaco_GRANTFIDA1.pdf

Compañía Nacional de Chocolates. (2012, January 24). *El cultivo de cacao: paquete tecnológico*. http://infocafes.com/portal/wp-content/uploads/2016/12/paquete_tecnologico_cacao_cnch_enero_2012.pdf

CORDEAGROPAZ. (2011). *Acuerdo regional de competitividad de cacao de Nariño*. CORDEAGROPAZ, PNUD, Gobernación de Nariño, Agencia de Desarrollo Local Nariño.

DNP. (2014). *Documento insumo para la Misión para la Transformación del Campo - propuesta para desarrollar un modelo eficiente de comercialización y distribución de productos*. Centro Regional de Estudios Regionales Cafeteros y Empresariales. https://colaboracion.dnp.gov.co/CDT/Agriculturapecuarioforestal%20y%20pesca/Comercializaci%C3%B3n.pdf

DNP, CVC, & UNICEF. (1983). *Plan de desarrollo integral para la Costa Pacífica colombiana*. Corporación Autónoma Regional del Cauca, Departamento Nacional de Planeación, UNICEF. https://ecopedia.cvc.gov.co/mas/costa-pacifica/plan-de-desarrollo-integral-para-la-costa-pacifica-colombiana-pladeicop-sector

Echavarría Soto, J. J., Villamizar-Villegas, M., Restrepo-Tamayo, S., & Hernández-Leal, J. D. (2018). Impacto del crédito sobre el agro en Colombia: evidencia del nuevo Censo Nacional Agropecuario. In J. J. Echavarría Soto, M. Villamizar Villegas, & S. Restrepo Tamayo (Eds.), *Superando barreras: el impacto del crédito en el sector agrario en Colombia* (pp. 41–72). Banco Interamericano de Desarrollo (BID). https://babel.banrepcultural.org/digital/collection/p17054coll18/id/452

Espinosa-Álzate, J. A., & Ríos-Osorio, L. A. (2016). Caracterización de sistemas agroecológicos para el establecimiento de cacao (*Theobroma cacao* L.), en comunidades afrodescendientes del Pacífico Colombiano (Tumaco - Nariño, Colombia). *Acta Agronómica*, *65*(3), 211–217. https://doi.org/10.15446/acag.v65n3.50714

Espinosa-García, J. A., Uresti-Gil, J., Vélez-Izquierdo, A., Moctezuma-López, G., Inurreta-Aguirre, H. D., & Góngora-González, S. F. (2015). Productividad y rentabilidad potencial del cacao (*Theobroma cacao* L.) en el trópico mexicano. *Revista Mexicana de Ciencias Agrícolas, 6*(5), 1051–1063. https://doi.org/10.29312/remexca.v6i5.598

ERS - ABC USAID/MIDAS Crops. (2009). Situación actual y perspectivas del mercado de cacao en grano colombiano. In C. F. Espinal, L. Narváez, J. Naranjo, O. Pantoja, & M. A. Urueña (Eds.), *La producción nacional frente a las tendencias de los mercados nacional e internacional de cacao en grano* (pp. 79–96). USAID.

FEDECACAO. (2019). *Economía nacional*. Federación Nacional de cacaoteros, Fondo Nacional del Cacao.

FINAGRO. (2017). *ICR programa general*. Ministerio de Agricultura y Desarrollo Rural. https://www.finagro.com.co/productos-y-servicios/icr-programa-general

Laing, D. R. (2011). Análisis de actividades agrícolas potenciales para incrementar los ingresos de los afrocolombianos, con énfasis en la región pacífica. *Revista Agricultura Tropical*, *34*(3–4), 18–50.

MADR. (2017). *Decreto 1745 de 1995*. Ministerio de Agricultura y Desarrollo Rural. https://www.minagricultura.gov.co/Normatividad/Decretos/Decreto%20No.%201745%20de1995.pdf

Magni, C. A., & Marchioni, A. (2020). Average rates of return, working capital, and NPV-consistency in project appraisal: a sensitivity analysis approach. *International Journal of Production*

Economics, *229*, Article 107769. https://doi.org/10.1016/j.ijpe.2020.107769

Ministerio de Agricultura, Fondo Adaptación, & CORPOICA. (2017). *Modelos de prevención y adaptación agroclimática - MAPA. Boletín 19*. http://hdl.handle.net/20.500.12324/34920

Montoya-Restrepo, I. A., Montoya-Restrepo, L. A., & Lowy-Ceron, P. D. (2015). Oportunidades para la actividad cacaotera en el municipio de Tumaco, Nariño, Colombia. *Entramado, 11*(1), 48–59. https://doi.org/10.18041/entramado.2015v11n1.21107

Moreno Rozo, F., & López Villalobos, A. (2013). Nuevos proyectos de cacao en 2013. *Colombia Cacaotera, 6*(15), 4–5.

Pássaro, C. (2014). *Informe final de producto. Oferta tecnológica para el proceso de beneficio de cacao en Colombia: caracterización, análisis crítico y selección de sistemas a evaluar*. CORPOICA.

Perfetti, J. J., Balcázar, Á., Hernández, A., & Leibovich, J. (2013). *Políticas para el desarrollo de la agricultura en Colombia*. FEDESARROLLO, Sociedad de Agricultores de Colombia, INCODER, FINAGRO, Banco Agrario. https://www.repository.fedesarrollo.org.co/bitstream/handle/11445/61/LIB_2013_Pol%c3%adticas%20para%20el%20desarrollo%20de%20la%20agricultura_Completo.pdf?sequence=1&isAllowed=y

Pinzón Useche, J. O., Rojas Ardila, J., Rojas, F., Ramírez, O. D., Moreno, F., & Castro, G. A. (2012). *Guía técnica para el cultivo del cacao* (5th ed.). FEDECACAO.

Piraquive Galeano, G., Matamoros Cárdenas, M., Céspedes Rangel, E., & Rodríguez Chacón, J. (2018). *Actualización de la tasa de rendimiento del capital en Colombia bajo la metodología de Harberger*. Dirección de Estudios Económicos, Departamento Nacional de Planeación. https://colaboracion.dnp.gov.co/CDT/Estudios%20Econmicos/487.pdf

Preciado, O., Ocampo, C. I., & Ballesteros Possú, W. (2011). Caracterización del sistema tradicional de producción de cacao (*Theobroma cacao* L.), en seis núcleos productivos del municipio de Tumaco, Nariño. *Revista de Ciencias Agrícolas*, *18*(2), 58–69.

Shirshahi, F., Babazadeh, H., Ebrahimi Pak, N. A., & Khaledian, M. R. (2020). Determining optimum major crops cultivation areas in different levels of deficit irrigation in Qazvin irrigation and drainage district. *Water and Soil Science*, *30*(1), 83–95.

UNODC. (2019, August). *Colombia, monitoreo de territorios afectados por cultivos ilícitos 2018*. Oficina de las Naciones Unidas contra la Droga y el Delito. https://www.unodc.org/documents/colombia/2019/Agosto/Informe_de_Monitoreo_de_Territorios_Afectador_por_Cultivos_Ilicitos_en_Colombia_2018_.pdf

UPRA. (2020). *Planificación nacional*. Unidad de Planificación Rural Agropecuaria. https://sipra.upra.gov.co/

USDA. (2018). *Colombia: cacao for peace update: firing on all cylinders*. United States Department of Agriculture. https://www.fas.usda.gov/data/colombia-cacao-peace-update-firing-all-cylinders

Yambure, O., Fernández, A. C., & Maldonado, R. (2014). *Proyecto de emprendimiento de cultivo de cacao bajo sombreado permanente* [Undergraduate thesis, Colegio de Estudios Superiores de Administración]. Repositorio Institucional CESA. https://repository.cesa.edu.co/handle/10726/1294

Yañez Agudelo, M. G. (2009). *La estrategia del cacao para la recuperación económica de familias campesinas desplazadas por la violencia del departamento de Caquetá* [Undergraduate thesis, Universidad de La Salle]. Ciencia Unisalle repositorio institucional. https://ciencia.lasalle.edu.co/cgi/viewcontent.cgi?article=1059&context=administracion_agronegocios

Zambrano, J. L., & Chávez, E. F. (2018). *Diagnóstico del estado del arte de la cadena de valor del cacao en América Latina y el Caribe*. Instituto Nacional Autónomo de Investigaciones Agropecuarias de Ecuador, FONTAGRO, Banco Interamericano de Desarrollo. https://www.fontagro.org/wp-content/uploads/2019/03/2018-CacaoDocFinal.pdf

Segmentation of Colombian organic food consumers focused on the consumption of the Andean blackberry

Segmentación de consumidores colombianos de alimentos orgánicos, con énfasis en el consumo de la mora andina

Silvana Builes Gaitán[1*] and María Clara Hurtado Pérez[2]

ABSTRACT

As one of the most traditional Colombian fruits, the Andean blackberry is consumed either fresh or as juice or marmalade. However, recent research findings indicate that farmer and consumer's health may be at risk owing to elevated doses of agrochemicals applied to produce the crop. Aiming to identify potential market opportunities for organic Andean blackberry, 164 organic consumers were surveyed using the "Gower's distance" clustering technique for the assessment of 86 consumer response variables. These included consumer preferences associated with the Andean blackberry, the price they were paying for the non-organic product, their willingness to pay for its organic version, and the information they provided on environmental attitudes, perceptions about organic products, lifestyle, demographics, and socioeconomics. Of the three segments obtained from the cluster analysis, namely premium, medium and budget, medium consumers were the most knowledgeable about the benefits of the fruit, whereas those belonging to the budget segment attributed a higher value to money. In turn, premium consumers were willing to pay more for the organic version of the fruit. Regarding organics consumption across the three groups, the budget segment contained the highest number of consumers buying organics every week. The medium segment stood out for their recognition of the Colombian organic certification for ecological foods.

Key words: consumer segmentation, Colombia, Gower's distance, clustering, certifications.

RESUMEN

La mora andina es una de las frutas colombianas más tradicionales, consumida en fresco, en jugo o en mermeladas. Sin embargo, los resultados de investigaciones recientes indican que la salud del productor y del consumidor de mora puede estar en riesgo debido a las elevadas dosis de agroquímicos aplicados en la producción del cultivo. Con el objetivo de identificar oportunidades potenciales de mercado para la mora andina orgánica, se aplicó una encuesta a 164 consumidores de orgánicos, usando la técnica de agrupamiento de "distancia de Gower" para evaluar 86 variables de respuesta de los consumidores. Estas incluyeron las preferencias de los consumidores asociadas a la mora andina, el precio que pagaban por la fruta no orgánica, su disposición para pagar por su versión orgánica y la información que brindaron sobre actitudes ambientales, percepciones sobre los productos orgánicos, los estilos de vida, la demografía y la información socioeconómica. De los tres segmentos resultantes del análisis de agrupamiento, premium, medio y presupuesto, los consumidores del segmento medio fueron los más conocedores de los beneficios de la fruta, mientras que los consumidores del segmento de presupuesto atribuyeron un mayor valor al dinero. A su vez, los consumidores premium estuvieron dispuestos a pagar más por la versión orgánica de la fruta. Con respecto al consumo de productos orgánicos en los tres grupos, el segmento de presupuesto contenía el mayor número de consumidores que compraban productos orgánicos cada semana. El segmento medio se destacó por su reconocimiento de la certificación orgánica colombiana para alimentos ecológicos.

Palabras clave: segmentación de consumidores, Colombia, distancia de Gower, agrupamiento, certificaciones.

Introduction

The Andean blackberry (*Rubus glaucus* Benth), also known as Castilla blackberry, is a fruit belonging to the group of berries, many of which have been found to offer multiple benefits such as high fiber (Howarth *et al*., 2001; Chutkan *et al*., 2012) and antioxidant contents (Mazza *et al*., 2002; Burton-Freeman *et al*., 2016) and cholesterol (Jenkins *et al*., 2008; Jeong *et al*., 2014), sugar (Martineau *et al*., 2006; Törrönen *et al*., 2012) and insulin (Törrönen *et al*., 2013) regulation properties. Additionally, this fruit represents an important source of polyphenols, carotenes, and vitamin C (Alarcón-Barrera *et al*., 2018).

Received for publication: May 24, 2021. Accepted for publication: December 20, 2021.

Doi: 10.15446/agron.colomb.v39n3.96034

[1] Corporación Colombiana de Investigación Agropecuaria Agrosavia, Centro de Investigación La Selva, Rionegro, Antioquia (Colombia).
[2] Independent researcher.
[*] Corresponding author: sbuiles@agrosavia.co

Marketed in Colombia through both agroindustry and fresh market channels, the Andean blackberry is consumed mainly in the form of juice, pulp, jam, preserves, sweets, and colorants (Cámara de Comercio de Bogotá, 2015). Despite its acceptance in the market, fruit safety problems related to the excessive use of agrochemicals for the crop production have become notorious in recent years, potentially putting farmer and consumer health at risk. Naranjo Marin (2011) has observed the Andean blackberry production in Colombia to be highly dependent on organophosphate insecticides for pest control. This author found these products to be applied outside the technical parameters recommended for their use and management, increasing the presence and concentration of their active ingredients in the fruit and, therefore, exceeding the corresponding maximum residual limits (MRLs). Furthermore, the FAO/WHO alliance on pesticide residues (JMPR) determined that some of the pesticides commonly applied to the Andean blackberry should not be permitted and their use represented a risk for human health (Naranjo Marin, 2011).

Farmer's actions to migrate towards a cleaner and organic production of the Andean blackberry have been gradually taking place in the departments of Cundinamarca, Antioquia, and Santander. Although not significant, this cleaner production is being marketed through specialized stores and agroecological markets but has not yet reached supermarkets, which are the main trading channel for organic foods in Colombia. By 2013, agroindustry, fresh market, and exportation channels respectively accounted for 60%, 38% and 2% of the total production of the non-organic Andean blackberry in the country (MADR, 2013). This setting offers an opportunity to develop an internal market for a cleaner and organic Andean blackberry.

Little is known about the size of the Colombian organic market and the actual areas destined for this mode of production. In addition to not being regularly updated, the figures about these areas are substantially different from the corresponding international data (Martínez Bernal *et al*., 2012). Furthermore, neither the Ministry of Agriculture nor the certification companies, the organisms in charge of creating the normative framework and issuing the certifications, have made the figures on certified organic areas publicly available. Of the total area of 31,621 ha cultivated organically by 2017 (Willer & Lernoud, 2019), 5.52% was represented by tropical and subtropical fruits such as banana, mango, strawberry, guava, pineapple, and plantain (Sánchez Castañeda, 2017; Willer & Lernoud, 2019). According to Willer and Lernoud (2019), the area destined to organic agriculture in Colombia has had an unsteady dynamic, with ups and downs throughout the 2010-2018 period. Additionally, the Colombian organic market is still in its infancy. Despite the sales growth during the last years, more than 90% of the national organic production is exported (Becerra Elejalde, 2018). Domestic consumption is limited by factors such as high prices associated with organic fruits and vegetables, little available information on their production and benefits, and low added value (Martínez Bernal *et al*., 2012).

Some of the existing literature on organic consumer segmentation has drawn attention to the importance of designing sound marketing strategies and public policies that consider the specific needs and profiles of consumers (Gil *et al*., 2000; Chinnici *et al*., 2002; Nie & Zepeda, 2011; Maciel *et al*., 2015). Studies on organic consumer segmentation have provided valuable information on the differences among groups within this market niche, which are mainly related to product availability and information and pricing strategies (Nie & Zepeda, 2011). However, these results should be considered in light of some of the points made by Claycamp and Massy (1968), such as the difficulty in finding mutually exclusive segments and the existence of logistic constraints to target specific groups. When reviewing the literature, two segmentation types for consumer can be found, namely those within the organic niche and those resulting from mass market assessments.

Reviews on this topic by Hughner *et al*. (2007) and Pearson *et al*. (2011) have pointed out that, despite the many studies to determine standard segmentation criteria for organic consumers, a clear profile remains elusive due to the multiple factors and complex decisions involved in organic food purchasing (Zepeda *et al*., 2006). Segmentation has resorted to multiple consumer classification criteria such as by socioeconomics and demographics (Chinnici *et al*., 2002; Maciel *et al*., 2015), food and non-food related lifestyles (Gil *et al*., 2000; Mora González *et al*., 2010; Nie & Zepeda, 2011), values (Chryssohoidis & Krystallis, 2005; Salgado Beltrán, 2019), behaviors (Chinnici *et al*., 2002; Nie & Zepeda, 2011), attitudes and perceptions (Chinnici *et al*., 2002; Mora González *et al*., 2010; Higuchi & Avadi, 2015; Maciel *et al*., 2015; Salgado Beltrán, 2019), purchase frequency (Chinnici *et al*., 2002; Krystallis *et al*., 2006), and level of awareness (Krystallis *et al*., 2006).

Explaining that individual lifestyles are more likely to influence the willingness to pay (WTP) for organic products, Gil *et al*. (2000) proposed a market segmentation for Spanish food shoppers based on consumer lifestyle rather than socioeconomic variables. By clustering individuals according to diet, exercise, and private and personal life habits, they identified three groups: actual organic food consumers, likely and unlikely organic food consumers. Mora González *et al*. (2010) also found that lifestyle and attitudes can provide a more accurate explanation on organic wine consumption in Chile. They found the consumer segments to be mainly marked by consumption habits, leisure activities and food-lifestyle, as well as perceptions on the contribution of organic production to the environment and the actual taste of organics. These criteria allowed identifying three groups, indifferent and positive consumers towards organic wine, plus actual organic wine consumers. These groups were differentiated mainly by organic wine frequency consumption and general food preferences (Mora González *et al*., 2010).

Higuchi and Avadi (2015) segmented organic consumers in the metropolitan area of Lima, Peru, by focusing on consumer's attitudes towards organics, their perceptions about their attributes, the resulting ecological welfare, health concerns and food safety and convenience. These authors used the segmentation framework of the Hartman Group (2020) that categorizes organic buyers into three groups, core, mid-level, and periphery. The core consumer buys organics for self-interest and welfare reasons, the periphery consumer buys them for convenience (proximity and novelty), and the mid-level consumer has a more integral approach by also considering environmental issues. Similarly, by considering consumer's attitudes and perceptions about organics, Nie and Zepeda (2011) found three US food shopper segments, adventurous, careless, and conservative uninvolved consumers. They further stated that the factors they addressed probably reflect psychological profiles and, as such, may provide information about the motivations influencing the purchase of organics.

In a more value-centered segmentation, Chryssohoidis and Krystallis (2005) proposed a Greek organic-consumer profile based on personal values that might motivate or hinder the consumption of organic food products. Their list of values was grouped around three factors: "belong" (*i.e.*, interpersonal relations), "self-respect" (personal values), and "fun" (non-personal values). The relative importance assigned by the consumers to these factors allowed differentiating four clusters: "explorers", featured by attributing high importance to all three factors; "loyal organic buyers", who gave average importance to self-respect and fun; "health-conscious organic buyers", who give least importance to fun and belonging, and "independent", who stood out for giving little importance to belonging values.

To provide valuable information for farmers and marketers willing to commercialize organic Andean blackberry, this study presents a market segmentation for organics consumers, with emphasis on blackberry consumers. This assessment is based on the Andean blackberry preferences of this particular target group, the price they currently pay for the non-organic Andean blackberry, their WTP for an organic version of the fruit, and their data on demographics, socioeconomics, lifestyle, environmental attitudes, and perceptions about organics. The results of this study will support not only the development of communication and marketing strategies by the Andean blackberry farmers and marketers, but also the design of public policies aimed at benefiting all the agents of this supply chain, including the consumers.

Materials and methods

Data and survey design

The study was conducted in the cities of Bogota and Medellin, the two largest cities and organic product markets of Colombia. As there was no pre-existing database or list of organic consumers, a sample size of 164 participants to be interviewed was defined through a tailored formula for unknown populations, at an 80% confidence level and a 5% margin error. Stratified random sampling was used, considering the marketing channels as strata and assuming differences between the organic consumers of each channel in terms of lifestyle, trust in organic foods, and attitudes towards environmental and social issues. The sales percentages of the different marketing channels, as estimated from the information provided by organic food marketers in both cities, were used to estimate the proportion of consumers to be interviewed in each channel. These marketing channels corresponded to retail stores, health-food stores, sale points of farmer organizations and agro-ecological markets.

A questionnaire was used for data collection consisting of eight sections which evaluated different consumer features: i) socio-demographic features, ii) lifestyle, iii) environmental attitude, iv) criteria when buying the Andean blackberry, v) attitude toward organic fruits and vegetables, vi) confidence in organic marketers and certifications, vii) organics consumption habits, and viii) perception of barriers to increasing organic food consumption (Supplementary material 1). Most of the responses were scored using a Likert scale and a few were defined as yes or no questions. Due to difficulties in obtaining income related information through the survey, socioeconomic strata were used as a proxy income level variable. This socioeconomic stratification of residential properties, which is the basis of the public utility billing strategy, determines that those who have higher economic capacity pay more for their public utilities, whereas the opposite occurs for the lower strata (Congreso de Colombia, 1994). To encourage participation in the survey, an incentive in the form of organic fruits or vegetables was given to the consumers.

Data collection took place from July to September 2019. To take advantage of high peak consumer shopping, specialized stores were visited on those days they received fresh product, while retail markets were mainly visited on weekends and fruit-and-vegetable discount days. Consumers were approached while in the vicinity of the organics section at the specific market channels. The criteria used to decide the consumers to be included in the sample were those who: i) actually consumed organic fruits and vegetables, as reflected in the purchase of these products; ii) were aware of the term "organic" as chemical-free, and (iii) consumed Andean blackberry.

Statistical analysis: market segmentation

To identify market segments within the target population (*i.e.*, organics and the Andean blackberry consumers), a clustering was run on a multiple dimension database containing the consumer's information on socio-demographics, lifestyle, environmental attitudes, preferences related to the Andean blackberry attributes and consumption, and perceptions and knowledge about organic food (fruits and vegetables). No hypotheses were specified before the data were collected as the analysis was data-driven.

The cluster analysis was implemented in a Gower's dissimilarity matrix (Gower, 1971) used to compute the distance between the different individuals in the dataset. Information on the 164 consumers was contained in 86 variables of continuous and categorical nature. Gower's distance between consumers, resulting from integrally computing all the variables, yielded the dissimilarity matrix, which was subsequently used to run a cluster analysis. After trying different numbers of groups, a clear separation among three consumer segments was evident, mainly marked by the relation between WTP for the organic Andean blackberry and the current price paid for the non-organic version of the fruits (Fig. 1). Mathematically speaking, the three resulting segments exist in an 86-dimensional space, corresponding to the number of variables on which the clustering was based. Most of the statistically significant variables across the segments were identified using ANOVA and Fisher's tests.

Results and discussion

Although it was the cluster analysis (as obtained by computing the 86 variables under study) that allowed identifying the three segments of organics consumers, these actually derived their names from the plotting of the above-mentioned price-related variables ("WTP for the organic Andean blackberry" and "current price paid for the non-organic version of the fruit"). This two-dimensional display resulted in three price bands in which the participants of the survey were paying (and willing to pay): relatively low, medium and high prices, respectively corresponding to the "Budget", "Medium", and "Premium" consumer segments. Thus, an intuitive and more natural understanding of the clustering results was provided, as shown in Figure 1. Tables 1, 2, 3 and 4 describe the groups through these and other significant variables.

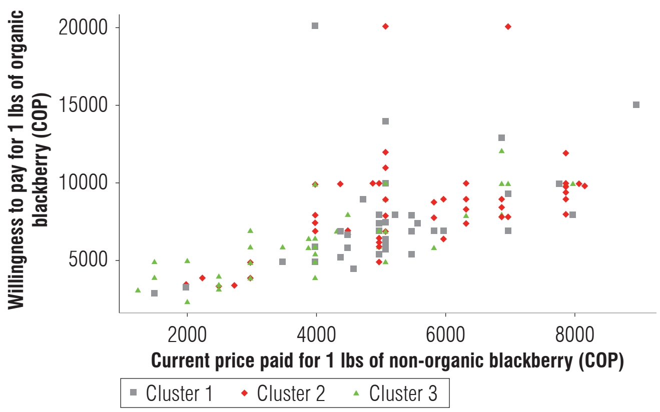


FIGURE 1. Willingness to pay for the organic Andean blackberry vs. current price paid for the non-organic Andean blackberry. Distribution across segments.

The findings suggest that the three consumer segments in question were mainly shaped by their preferences on the Andean blackberry and perceptions about organics. As mentioned by Chryssohoidis and Krystallis (2005), organic consumer groups share many features, explained by the similar nature of the overall sample of respondents. Similarities were mainly found in perceptions and beliefs surrounding organic food, considerations about consumption increase barriers, and environmental and health awareness.

Middle-aged women were found to be the main purchasers of organics across the three identified segments (Tab. 1). However, this does not necessarily imply that they are more interested in organics than men, but simply that they usually do the food shopping for the household, which is consistent with multiple studies on organics (Davies *et* *al*., 1995; Roddy *et al*., 1996; Schifferstein & Ophuis, 1998; Cicia *et al*., 2002). As shown in Table 1, significant differences among educational levels showed that the budget segment had the most educated consumers, with almost half of them holding a postgraduate degree. Regardless of the statistically insignificant socioeconomic stratum differences across segments, half of the consumers of the Budget group did not live in the highest strata.

Across the three segments (Tab. 1), most consumers were active in healthy practices, confirming the association between healthy lifestyle and consumption of organic foods (Gil *et al*., 2000; Mora González *et al*., 2010; Nie & Zepeda, 2011). In this regard, premium consumers were the strictest, as shown by their permanent exercise routines, very frequent consumption of fruits and vegetables, low salt and sugar intake, and involvement in mental and spiritual therapies. This finding relates to that of "core consumers" in Higuchi and Avadi (2015).

Regarding the Andean blackberry preferences and attributes (Tab. 2), the medium segment contained the highest

TABLE 1. Socioeconomic and lifestyle profiles of consumer segments.

	Middle segment (n = 45)			Premium segment (n = 68)			Budget segment (n = 52)		
	Mean	**Median**	**SD**	**Mean**	**Median**	**SD**	**Mean**	**Median**	**SD**
Age	47.67	45.00	14.70	51.24	54.00	15.37	45.77	43.00	13.86
Household members*	2.91	3.00	1.34	2.47	2.00	1.28	3.11	3.00	1.54
Female (%)		78			78			84	
Socioeconomic strata									
2 (%)		2.22			0.00			1.92	
3 (%)		8.88			14.70			9.61	
4 (%)		15.55			17.64			34.61	
5 (%)		28.88			32.35			28.84	
6 (%)		44.44			35.29			25.00	
Educational level**									
Secondary school (%)		2.22			9			11	
Technical school (%)		8.88			11			8	
Bachelor's degree (%)		68.88			62			33	
Postgraduate (%)		20.00			18			48	
Physical/mental/spiritual therapy*									
Always (%)		15.56			33.82			25.00	
Very often (%)		15.56			23.53			15.38	
Sometimes (%)		11.11			5.88			1.92	
Rarely (%)		13.33			1.47			7.69	
Never (%)		44.44			35.29			50.00	
Vegetarian									
Yes (%)		8.88			8.82			7.69	
Diseased family member**									
Yes (%)		31.11			61.76			63.46	
7-8 h sleep***									
Always (%)		26.66			61.76			51.92	
Very often (%)		28.88			25.00			11.53	
Sometimes (%)		26.66			1.47			11.53	
Rarely (%)		17.77			7.35			21.15	
Never (%)		0.00			4.41			3.84	

SD - Standard deviation. Significance levels of 5%, 1%, and 0.1% are indicated by *, **, and ***, respectively. The significance levels of continuous and categorical variables were estimated using ANOVA and Fisher's test, respectively.

TABLE 2. Andean blackberry preference profiles across consumer segments.

	Middle segment (n = 45)			Premium segment (n = 68)			Budget segment (n = 52)		
	Mean	**Median**	**SD**	**Mean**	**Median**	**SD**	**Mean**	**Median**	**SD**
Price paid for Andean blackberry (COP)*	5076	5100	1398	5589	5100	1643	3985	4000	1672
Where do you buy Andean blackberry? (main place of purchase)*									
Supermarkets (%)		75.56			67.65			36.54	
Farmer markets (%)		2.22			5.88			5.77	
Agroecological markets (%)		2.22			0.00			3.85	
Neighborhood shops (%)		2.22			5.88			3.85	
Particular supplier (%)		8.89			4.41			13.46	
Specialized stores (%)		2.22			1.47			3.85	
Market places (%)		4.44			14.71			32.69	
Other (%)		2.22			0.00			0.00	
Do you know the nutritional benefits of Andean blackberry?*									
Some of them (%)		73.33			50.00			76.92	
All of them (%)		20.00			50.00			23.08	
None of them (%)		6.67			0.00			0.00	
Is the Andean blackberry´s place of origin (where it has been grown) important at the time of purchase?*									
Very important (%)		15.56			5.88			17.31	
Important (%)		20.00			11.76			11.54	
Indifferent (%)		28.89			2.94			11.54	
Not Important (%)		20.00			57.35			42.31	
Not important at all (%)		15.56			22.06			17.31	
Is the price of Andean blackberry important at the time of purchase?*									
Very important (%)		4.44			2.94			13.46	
Important (%)		33.33			27.94			40.38	
Indifferent (%)		24.44			10.29			15.38	
Not important (%)		24.44			47.06			25.00	
Not important at all (%)		13.33			11.76			5.77	
Is the color of Andean blackberry important at the time of purchase?*									
Very important (%)		35.56			77.94			80.77	
Important (%)		55.56			14.71			15.38	
Indifferent (%)		6.67			1.47			1.92	
Not important (%)		2.22			4.41			1.92	
Not important at all (%)		0.00			1.47			0.00	
Is knowing that Andean blackberry contains antioxidants important at the time of purchase?*									
Very important (%)		35.56			14.71			28.85	
Important (%)		28.89			7.35			13.46	
Indifferent (%)		4.44			2.94			9.62	
Not important (%)		0.00			1.47			1.92	
Did not know about it (%)		31.11			73.53			46.15	
Is the degree of ripeness of Andean blackberry important at the time of purchase?*									
Very important (%)		31.11			66.18			76.92	
Important (%)		55.56			23.53			17.31	
Indifferent (%)		4.44			4.41			3.85	
Not important (%)		6.67			5.88			1.92	
Not important at all (%)		2.22			0.00			0.00	
Is the environmental impact resulting from Andean blackberry production important at the time of purchase?*									
Very important (%)		20.00			11.76			26.92	
Important (%)		31.11			8.82			15.38	
Indifferent (%)		26.67			8.82			7.69	
Not important (%)		20.00			50.00			32.69	
Not important at all (%)		2.22			20.59			17.31	

SD - Standard deviation. Significance levels of 5%, 1%, and 0.1% are indicated by *, **, and ***, respectively. The significance levels of continuous and categorical variables were estimated using ANOVA and Fisher's test, respectively.

proportion of consumers who considered both the multiple benefits of the fruit and the packaging label (*e.g.*, vitamin C, calcium, phosphorus, and antioxidant contents) as very important or important. As can be seen, this is the segment most knowledgeable about the fruit. Given the willingness of middle consumers to be informed, they could rapidly develop an interest in the organic Andean blackberry if they were provided with information about the current use of agrochemicals on the non-organic Andean blackberry crops and the benefits of organic production. Additionally, most middle consumers also gave great importance to the origin of the fruit and the likely environmental impact of its production, which suggests that communication strategies emphasizing local consumption of the organic Andean blackberry could be effective with them.

Most budget consumers considered price, color, appearance, ripeness stage and place of purchase as "very important" criteria when deciding to buy the Andean blackberry fruits. This indicates they gave the highest value to money and that a marketing strategy combining affordable prices, good quality and an *ad hoc* approach to different distribution channels (*e.g.*, supermarkets, marketplaces, and particular suppliers) could awaken their interest in organic Andean blackberry. The foregoing is consistent with the current Andean blackberry price paid by budget consumers and their WTP for the organic version, which are the lowest within the three groups.

In terms of these prices, premium consumers were willing to pay 40% more than budget consumers and 10% more than medium consumers, despite the fact that this last group had a higher frequency of purchase. One likely reason explaining why premium and medium consumers were paying (and willing to pay) more for the fruit (and its organic version), could be their higher socioeconomic strata, used in this study as a proxy for income. This coincides with previous findings of several studies (Nandi *et al.*, 2017; Vapa-Tankosić *et al.,* 2018; Bhattarai, 2019) associating higher income with higher willingness to pay for organics. Nonetheless, the assumption that consumer's public utility expenses can be extrapolated to estimate their food budget assignation is certainly an ambitious one and, as such, needs to be interpreted with caution. These findings suggest that there may be a potential market for organic Andean blackberry beyond the highest socioeconomic strata, which could positively respond to competitive price strategies and be the target of future consumer-support policies.

Results on consumption of organics are shown in Table 3. More than half of the participants interviewed in all segments were buying organics for all the members of the family on a weekly basis. A slightly higher proportion of these consumers belonged to the budget segment. Furthermore, 50% of the middle consumers recognized the Colombian ecological foods certification, while 7% and 20% of the premium and budget consumers, respectively, did so. This shows that, at least for the medium segment, even though the organic certification intends to guarantee that a food product is truly free of chemicals, consumers do not always consider this as a purchase-defining criterion. Despite this, more than half of the premium and budget consumers regarded certification of the organic product as important, whereas half of the medium consumers did not. This result can be interpreted considering what Hughner *et al.* (2007) have stated on consumer's likely distrust and skepticism with regards to certification authorities and agencies and organic food credentials. Interestingly, more than 60% of the consumers in the three groups expressed trust in the (non-certified) "organic" label as well as in the marketers of organic products. Thus, the distrust in certification can be overcome by the mentioned trust in organic producers and marketers (Veldstra *et al.*, 2014).

Regarding important criteria at the time of buying organics (Tab. 3), most of the premium consumers gave more importance to the brand and packaging of these products than did the middle and budget consumers, while the latter considered label, origin, price and nutritional value to be more important. In terms of perceptions and beliefs surrounding organic food, the segments coincided on several criteria and barriers that may hinder the expansion of these products: participants from the three segments believed that the high price of organics is the main barrier to increasing their consumption, agreeing with Nandi *et al.* (2017) and differing with Chryssohoidis and Krystallis (2005), who found that price is not as important as the organic's limited availability. Other factors hindering organics consumption were lack of knowledge about organic certifications and the plastic packaging of these products, considered by some consumers as a contradiction of what these products environmentally represent. Such packaging has been a requirement of marketers such as supermarkets to differentiate organics from conventional products, and even if some organics marketers have started using materials other than plastic, there is still some non-acceptance from consumers.

Table 4 shows that more than 80% of the consumers in all segments agreed or strongly agreed that organic food is superior in quality and helps prevent diseases. Likewise, 90% of them considered organics healthier and more expensive than non-organics, similar to the findings of Higuchi and Avadi (2015).

TABLE 3. Attitudes towards organics across consumer segments.

	Middle segment (n = 45)			Premium segment (n = 68)			Budget segment (n = 52)		
	Mean	**Median**	**SD**	**Mean**	**Median**	**SD**	**Mean**	**Median**	**SD**
Willingness to pay for organic Andean blackberry (COP)*	7583	7000	3014	8312	8000	2896	6505	6000	2382
Where do you buy organic fruits and vegetables? (Main place of purchase)									
Supermarkets (%)		77.77			82.35			69.23	
Farmer markets (%)		2.22			0.00			1.92	
Agroecological markets (%)		4.44			1.47			5.76	
Specialized stores (%)		11.11			13.23			19.23	
Particular supplier (%)		2.22			2.94			1.92	
Other (%)		2.22			0.00			1.92	
How often do you buy organic fruits and vegetables?									
Every week (%)		55.55			64.70			65.38	
Several times a month (%)		20.00			26.47			30.76	
Once a month (%)		20.00			8.82			3.84	
Every 2/3 months (%)		4.44			0.00			0.00	
Do you know organic certifications?* **									
Yes (%)		51.11			7.35			21.15	
Who do you buy organic fruits and vegetables for?* **									
For me (%)		13.33			48.88			5.76	
For all the family (%)		86.66			67.64			94.23	
Is the brand of organics important at the time of purchase?* **									
Very important (%)		0.00			13.23			5.76	
Important (%)		13.33			29.41			13.46	
Indifferent (%)		48.88			10.29			5.76	
Not Important (%)		24.44			36.76			55.76	
Not important at all (%)		13.33			10.29			19.23	
Is the price of organics important at the time of purchase?* **									
Very important (%)		6.66			11.76			19.23	
Important (%)		48.88			30.88			53.84	
Indifferent (%)		17.77			16.17			17.30	
Not Important (%)		11.11			35.29			9.61	
Not important at all (%)		15.55			5.88			0.00	
Is the origin of organics important at the time of purchase?* **									
Very important (%)		13.33			4.41			15.38	
Important (%)		24.44			8.82			21.15	
Indifferent (%)		33.33			2.94			3.85	
Not Important (%)		17.78			57.35			46.15	
Not important at all (%)		11.11			26.47			13.46	
Is the packaging of organics important at the time of purchase?* **									
Very important (%)		15.56			14.71			11.54	
Important (%)		46.67			64.71			46.15	
Indifferent (%)		26.67			5.88			11.54	
Not Important (%)		8.89			14.71			25.00	
Not important at all (%)		2.22			0.00			5.77	
Is the nutritional value of organics important at the time of purchase?* **									
Very important (%)		24.44			64.71			69.23	
Important (%)		53.33			27.94			25.00	
Indifferent (%)		13.33			2.94			1.92	
Not Important (%)		8.89			2.94			1.92	
Not important at all (%)		0.00			1.47			1.92	

SD - Standard deviation. Significance levels of 5%, 1%, and 0.1% are indicated by *, **, and ***, respectively. The significance levels of continuous and categorical variables were estimated using ANOVA and Fisher's test, respectively.

TABLE 4. Perceptions about organics across consumer segments.

	Middle segment (n = 45)	Premium segment (n = 68)	Budget segment (n = 52)
Do you think organic food is superior?*			
Strongly agree (%)	40.00	72.06	75.00
Agree (%)	44.44	16.18	15.38
Uncertain (%)	13.33	8.82	7.69
Disagree (%)	2.22	1.47	1.92
Strongly disagree (%)	0.00	1.47	0.00
Do you think organic food is healthier?*			
Strongly agree (%)	53.33	77.94	78.85
Agree (%)	44.44	20.59	17.31
Uncertain (%)	0.00	0.00	3.85
Disagree (%)	2.22	1.47	0.00
Do you think organic food is more expensive?***			
Strongly agree (%)	46.67	77.94	78.85
Agree (%)	44.44	14.71	17.31
Uncertain (%)	6.67	1.47	0.00
Disagree (%)	2.22	5.88	3.85
Do you think organic food helps to prevent diseases?			
Strongly agree (%)	37.78	63.24	69.23
Agree (%)	44.44	26.47	21.15
Uncertain (%)	17.78	10.29	5.77
Disagree (%)	0.00	0.00	3.85

Significance levels of 5%, 1%, and 0.1% are indicated by *, **, and ***, respectively. The significance levels of continuous and categorical variables were estimated using an ANOVA and Fisher's test, respectively.

These results indicate that potential farmers and marketers of Andean blackberry should target consumers in the high yielding segments (*i.e.*, premium and medium) in order to profit from their higher WTP. Although most consumers who know organic certifications (51%) are in the medium segment, almost as many in this group do not give much importance to such certification. This contrasts with the case of premium and budget consumers who, despite not having prior knowledge about this credentials system, consider it important for future purchases. Therefore, medium consumers could be targeted as potential buyers of non-certified organic Andean blackberry, whereas the certification could be more significant for the other two segments. This is particularly important considering that many small farmers struggle to get and maintain certifications due to multiple reasons such as the required transition time to become organic, high infrastructure investments, extensive paperwork, and harmful contamination from non-organic neighbor farmers.

As to the implementation of the current results, individually targeting consumer segments in the present context is troublesome due to the existence of common features among them, such as the main place for buying organics, which makes it virtually impossible to address a specific segment through a factor like price. Similar problems have already been reported by Claycamp and Massy (1968). The fact that some groups purchase the product in different shop types (*i.e.,* supermarkets, marketplaces and online shops, the latter mainly used by budget and premium consumers) could be exploited by better targeting consumers. Commercial strategies attempting to reach premium consumers should consider sales at specialized healthy food stores, supported by organic certification, brand promotion and specialized packaging for organic Andean-blackberry. Medium consumers, in turn, could be approached by using fair-trade certification along with information about the benefits of organic Andean blackberry consumption, its place of origin and the environmental benefits of organic production. An alternative certification to be used for medium consumers could be one offered by participatory guarantee systems, which is used by the agroecological markets network of Bogota. Finally, budget consumers could also be reached in more affordable organic product stores such as market places or agroecological markets.

Acknowledgments

This project was supported by the Colombian Agricultural Research Corporation - AGROSAVIA, with funds received from the Colombian Ministry of Agriculture through the TV-19 transfer. AGROSAVIA was only a sponsor for the current research project. The authors would like to thank all those who took part in the survey, including the marketers who gave us permission to work in their facilities, as well as John Martínez who supported data collection.

Conflict of interest statement

The authors declare that there is no conflict of interest regarding the publication of this article.

Authors' contributions

SBG formulated the overarching research goals and aims. SBG and MCH carried out activities to collect and filter data in the commercial channel for organics. SBG and MCH applied statistical, mathematical, computational, and other formal techniques to analyze and synthesize study data. SBG obtained the financial support for the project leading to this publication. SBG and MCH developed and designed the methodology. SBG and MCH implemented the computer code and supporting algorithms/software in R. SBG prepared, created, and presented the published work and oversaw its visualization/data presentation. SBG and MCH wrote the initial draft.

Literature cited

Alarcón-Barrera, K. S., Armijos-Montesinos, D. S., García-Tenesaca, M., Iturralde, G., Jaramilo-Vivanco, T., Granda-Albuja, M. G., Giampieri, F., & Alvarez-Suarez, J. M. (2018). Wild Andean blackberry (*Rubus glaucus* Benth) and Andean blueberry (*Vaccinium floribundum* Kunth) from the highlands of Ecuador: nutritional composition and protective effect on human dermal fibroblasts against cytotoxic oxidative damage. *Journal of Berry Research*, *8*(3), 223–236. https://doi.org/10.3233/JBR-180316

Becerra Elejalde, L. L. (2018, September 21). *"En Colombia exportamos 95% de la producción orgánica": presidente de Fedeorgánicos*. Agronegocios. https://www.agronegocios.co/agricultura/en-colombia-exportamos-95-de-la-produccion-organica-presidente-de-fedeorganicos-2773418

Bhattarai, K. (2019). Consumers' willingness to pay for organic vegetables: empirical evidence from Nepal. *Economics and Sociology*, *12*(3), 132–146. https://doi.org/10.14254/2071-789X.2019/12-3/9

Burton-Freeman, B. M., Sandhu, A. K., & Edirisinghe, I. (2016). Red raspberries and their bioactive polyphenols: cardiometabolic and neuronal health links. *Advances in Nutrition, 7*(1), 44–65. https://doi.org/10.3945/an.115.009639

Cámara de Comercio de Bogotá. (2015). *Manual Mora*. Programa de Apoyo Agrícola y Agroindustrial, Vicepresidencia de Fortalecimiento Empresarial, Cámara de Comercio de Bogotá. https://bibliotecadigital.ccb.org.co/bitstream/handle/11520/14319/Mora.pdf?sequence=1&isAllowed=y

Chinnici, G., D'Amico, M., & Pecorino, B. (2002). A multivariate statistical analysis on the consumers of organic products. *British Food Journal*, *104*(3–4–5), 187–199. https://doi.org/10.1108/00070700210425651

Chryssohoidis, G. M., & Krystallis, A. (2005). Organic consumers' personal values research: testing and validating the list of values (LOV) scale and implementing a value-based segmentation task. *Food Quality and Preference, 16*(7), 585–599. https://doi.org/10.1016/j.foodqual.2005.01.003

Chutkan, R., Fahey, G., Wright, W. L., & McRorie, J. (2012). Viscous versus nonviscous soluble fiber supplements: mechanisms and evidence for fiber-specific health benefits. *Journal of the American Academy of Nurse Practitioners*, *24*(8), 476–487. https://doi.org/10.1111/j.1745-7599.2012.00758.x

Cicia, G., Del Giudice, T., & Scarpa, R. (2002). Consumers' perception of quality in organic food: a random utility model under preference heterogeneity and choice correlation from rank-orderings. *British Food Journal*, *104*(3–4–5), 200–213. https://doi.org/10.1108/00070700210425660

Claycamp, H. J., & Massy, W. F. (1968). A theory of market segmentation. *Journal of Marketing Research, 5*(4), 388–394. https://doi.org/10.1177/002224376800500405

Congreso de Colombia. (1994). *Ley 142 de 1994. Por la cual se establece el régimen de los servicios públicos domiciliarios y se dictan otras disposiciones.* https://www.funcionpublica.gov.co/eva/gestornormativo/norma.php?i=2752

Davies, A., Titterington, A. J., & Cochrane, C. (1995). Who buys organic food? A profile of the purchasers of organic food in Northern Ireland. *British Food Journal, 97*(10), 17–23. https://doi.org/10.1108/00070709510104303

Gil, J. M., Gracia, A., & Sánchez, M. (2000). Market segmentation and willingness to pay for organic products in Spain. *The International Food and Agribusiness Management Review, 3*(2), 207–226. https://doi.org/10.1016/S1096-7508(01)00040-4

Gower, J. C. (1971). A general coefficient of similarity and some of its properties. *Biometrics, 27*(4), 857–871. https://doi.org/10.2307/2528823

Higuchi, A., & Avadi, A. (2015). Organic purchasing factors and consumer classification through their preferences in the metropolitan area of Lima, Peru. *Agronomía Colombiana, 33*(2), 271–279. https://doi.org/10.15446/agron.colomb.v33n2.50013

Howarth, N. C., Saltzman, E., & Roberts, S. B. (2001). Dietary fiber and weight regulation. *Nutrition Reviews*, *59*(5), 129–139. https://doi.org/10.1111/j.1753-4887.2001.tb07001.x

Hughner, R. S., McDonagh, P., Prothero, A., Shultz, C. J., & Stanton, J. (2007). Who are organic food consumers? A compilation and review of why people purchase organic food. *Journal of Consumer Behaviour*, *6*(2–3), 94–110. https://doi.org/10.1002/cb.210

Jenkins, D. J. A., Nguyen, T. H., Kendall, C. W. C., Faulkner, D. A., Bashyam, B., Kim, I. J., Ireland, C., Patel, D., Vidgen, E., Josse, A. R., Sesso, H. D., Burton-Freeman, B., Josse, R. G., Leiter, L. A., & Singer, W. (2008). The effect of strawberries in a cholesterol-lowering dietary portfolio. *Metabolism, 57*(12), 1636–1644. https://doi.org/10.1016/j.metabol.2008.07.018

Jeong, H. S., Hong, S. J., Lee, T. B., Kwon, J. W., Jeong, J. T., Joo, H. J., Park, J. H., Ahn, C. M., Yu, C. W., & Lim, D. S. (2014). Effects of black raspberry on lipid profiles and vascular endothelial function in patients with metabolic syndrome. *Phytotherapy Research*, *28*(10), 1492–1498. https://doi.org/10.1002/ptr.5154

Krystallis, A., Fotopoulos, C., & Zotos, Y. (2006). Organic consumers' profile and their willingness to pay (WTP) for selected organic food products in Greece. *Journal of International Consumer Marketing*, *19*(1), 81–106. https://doi.org/10.1300/J046v19n01_05

Maciel, W. R. E., Oliveira, D. M., Sanches, A. C., & Lima-Filho, D. O. (2015). Segmentação dos consumidores a respeito dos produtos orgânicos. *Revista Capital Científico, 13*(3), 76–92. https://doi.org/10.5935/2177-4153.20150023

MADR. (2013). *Acuerdo de competitividad para la cadena productiva de la mora en Colombia.* Ministerio de Agricultura y Desarrollo Rural. https://sioc.minagricultura.gov.co/Mora/Normatividad/D.C.%20-%20Acuerdo%20Competitividad.pdf

Martineau, L. C., Couture, A., Spoor, D., Bernhaddou-Andaloussi, A., Harris, C., Meddah, B., Leduc, C., Burt, A., Vuong, T., Mai Le, P., Prentki, M., Bennett, S. A., Arnason, J. T., & Haddad, P. S. (2006). Anti-diabetic properties of the Canadian lowbush blueberry *Vaccinium angustifolium* Ait. *Phytomedicine*, *13*(9–10), 612–623. https://doi.org/10.1016/j.phymed.2006.08.005

Martínez Bernal, L. F., Bello Rodríguez, P. L., & Castellanos Domínguez, Ó. F. (2012). *Sostenibilidad y desarrollo: el valor agregado de la agricultura orgánica*. Universidad Nacional de Colombia.

Mazza, G., Kay, C. D., Cottrell, T., & Holub, B. J. (2002). Absorption of anthocyanins from blueberries and serum antioxidant status in human subjects. *Journal of Agricultural and Food Chemistry*, *50*(26), 7731–7737. https://doi.org/10.1021/jf020690l

Mora González, M. G., Magner Pulgar, N. S., & Marchant Silva, R. (2010). Segmentación de mercado de acuerdo a estilos de vida de consumidores de vino orgánico de la Región Metropolitana de Chile. *Idesia*, *28*(3), 25–33.

Nandi, R., Bokelmann, W., Vishwanath Gowdru, N., & Dias, G. (2017). Factors influencing consumers' willingness to pay for organic fruits and vegetables: empirical evidence from a consumer survey in India. *Journal of Food Products Marketing, 23*(4), 430–451. https://doi.org/10.1080/10454446.2015.1048018

Naranjo Marin, J. M. (2011). *Propuesta de un perfil de riesgo químico establecido para la mora de Castilla (Rubus glaucus Benth) producida en Colombia.* [Master's thesis, Universidad para la Cooperación Internacional]. http://www.fedeorganicos.org/wp-content/uploads/2016/07/TesisMaestria.pdf

Nie, C., & Zepeda, L. (2011). Lifestyle segmentation of US food shoppers to examine organic and local food consumption. *Appetite*, *57*(1), 28–37. https://doi.org/10.1016/j.appet.2011.03.012

Pearson, D., Henryks, J., & Jones, H. (2011). Organic food: what we know (and do not know) about consumers. *Renewable Agriculture and Food Systems*, *26*(2), 171–177. https://doi.org/10.1017/S1742170510000499

Roddy, G., Cowan, C. A., & Hutchinson, G. (1996). Consumer attitudes and behaviour to organic foods in Ireland. *Journal of International Consumer Marketing*, *9*(2), 41–63. https://doi.org/10.1300/J046v09n02_03

Salgado Beltrán, L. (2019). Segmentación de los consumidores de alimentos orgánicos según sus actitudes, valores y creencias ambientales. *Contaduría y Administración*, *64*(2), 1–22. https://doi.org/10.22201/fca.24488410e.2018.1491

Sánchez Castañeda, J. (2017). Mercado de productos agrícolas ecológicos en Colombia. *Suma de Negocios*, *8*(18), 156–163. https://doi.org/10.1016/j.sumneg.2017.10.001

Schifferstein, H. N. J., & Ophuis, P. A. M. O. (1998). Health-related determinants of organic food consumption in The Netherlands. *Food Quality and Preference*, *9*(3), 119–133. https://doi.org/10.1016/S0950-3293(97)00044-X

The Hartman Group. (2020, June 2). *The culture of organic today: who buys organic and why*. https://www.hartman-group.com/newsletters/1291505642/the-culture-of-organic-today-who-buys-organic-and-why

Törrönen, R., Kolehmainen, M., Sarkkinen, E., Poutanen, K., Mykkänen, H., & Niskanen, L. (2013). Berries reduce postprandial insulin responses to wheat and rye breads in healthy women. *The Journal of Nutrition*, *143*(4), 430–436. https://doi.org/10.3945/jn.112.169771

Törrönen, R., Sarkkinen, E., Niskanen, T., Tapola, N., Kilpi, K., & Niskanen, L. (2012). Postprandial glucose, insulin and glucagon-like peptide 1 responses to sucrose ingested with berries in healthy subjects. *British Journal of Nutrition, 107*(10), 1445–1451. https://doi.org/10.1017/S0007114511004557

Vapa-Tankosić, J., Ignjatijević, S., Kranjac, M., Lekić, S., & Prodanović, R. (2018). Willingness to pay for organic products on the Serbian market. *International Food and Agribusiness Management Review, 21*(6), 791–801. https://doi.org/10.22434/IFAMR2017.0068

Veldstra, M. D., Alexander, C. E., & Marshall, M. I. (2014). To certify or not to certify? Separating the organic production and certification decisions. *Food Policy*, *49*(2), 429–436. https://doi.org/10.1016/j.foodpol.2014.05.010

Willer, H., & Lernoud, J. (Eds.). (2019). *The world of organic agriculture: statistics and emerging trends 2019.* Research Institute of Organic Agriculture FiBL, IFOAM - Organics International.

Zepeda, L., Chang, H. S., & Leviten-Reid, C. (2006). Organic food demand: a focus group study involving Caucasian and African-American shoppers. *Agriculture and Human Values*, *23*, Article 385. https://doi.org/10.1007/s10460-006-9001-9

SUPPLEMENTARY MATERIAL 1. Socioeconomic consumer survey on the willingness to pay for organic Andean blackberry 2019.

1. Date survey was conducted _ _ _ _ _ _ _ _
2. Code of the surveyor _ _ _ _ _ _ _ _
3. Questionnaire number _ _ _ _ _ _ _ _
4. City _ _ _ _ _ _ _ _

5. Place of application of the survey

[ ] Carulla Place: _ _ _ _ _ _ _ _
[ ] Euro Place: _ _ _ _ _ _ _ _
[ ] La Vaquita Place: _ _ _ _ _ _ _ _
[ ] Éxito Place: _ _ _ _ _ _ _ _
[ ] Merkepaisa Place: _ _ _ _ _ _ _ _
[ ] Other Place: _ _ _ _ _ _ _ _

A. CONSUMER ID - SOCIOECONOMIC CHARACTERIZATION

6. Name of respondent _ _ _ _ _ _ _ _
7. Cell phone number _ _ _ _ _ _ _ _
8. E-mail _ _ _ _ _ _ _ _ **9. Age** _ _ _ _ _ _ _ _
10. Gender [ ] Male [ ] Female
11. Civil status [ ] Single [ ] Married [ ] Divorced [ ] Other

12. Employment status
[ ] Student [ ] Employee [ ] Independent
[ ] Unemployed [ ] Housewife [ ] Retired

13. Socioeconomic stratum _ _ _ _ _ _ _ _
14. Neighborhood _ _ _ _ _ _ _ _

15. Education level
[ ] Pre-school [ ] Primary [ ] Incomplete primary
[ ] Secondary [ ] Incomplete secondary [ ] Undergraduate
[ ] Graduate [ ] Technical - technologist [ ] Other

16. Profession/occupation _ _ _ _ _ _ _ _
17. Number of members of consumer's household _ _ _ _ _ _ _ _

B. CONSUMER LIFESTYLE

18. Do you eat food without preservatives?
[ ] Always
[ ] Usually
[ ] Sometimes
[ ] Rarely
[ ] Never

19. Do you eat processed foods?
[ ] Always
[ ] Usually
[ ] Sometimes
[ ] Rarely
[ ] Never

20. Do you follow a low-salt diet?
[ ] Always
[ ] Usually
[ ] Sometimes
[ ] Rarely
[ ] Never

21. Do you have regular medical check-ups?
[ ] Always
[ ] Usually
[ ] Sometimes
[ ] Rarely
[ ] Never

22. Do you practice any sport?
[ ] Always
[ ] Usually
[ ] Sometimes
[ ] Rarely
[ ] Never

23. Do you exercise?
[ ] Always
[ ] Usually
[ ] Sometimes
[ ] Rarely
[ ] Never

24. Do you sleep between 7 and 8 hours per day?
[ ] Always
[ ] Usually
[ ] Sometimes
[ ] Rarely
[ ] Never

25. Do you practice any type of physical/mental/spiritual therapy?
[ ] Always
[ ] Usually
[ ] Sometimes
[ ] Rarely
[ ] Never

26. Do you consume alcoholic beverages?
[ ] Always
[ ] Usually
[ ] Sometimes
[ ] Rarely
[ ] Never

27. Do you consume alcoholic beverages?
[ ] Yes
[ ] No

28. Do you consume fruits and vegetables on a regular basis?
[ ] Strongly disagree
[ ] Disagree
[ ] Undecided
[ ] Agree
[ ] Strongly agree

29. Do you consume red meat in moderation?
[ ] Strongly disagree
[ ] Disagree
[ ] Undecided
[ ] Agree
[ ] Strongly agree

30. Do you consume sugar in moderation?

[ ] Strongly disagree
[ ] Disagree
[ ] Undecided
[ ] Agree
[ ] Strongly agree

31. Do you consider that there is a balance between your work and personal life?

[ ] Strongly disagree
[ ] Disagree
[ ] Undecided
[ ] Agree
[ ] Strongly agree

32. Does anyone in your family suffer from any disease?

Yes [ ] No [ ]

C. CONSUMER LIFESTYLE

33. Do you avoid using plastic bags (at the grocery store, at home, etc.)?

[ ] Strongly disagree
[ ] Disagree
[ ] Undecided
[ ] Agree
[ ] Strongly agree

34. Do you dispose your household garbage in different containers?

[ ] Strongly disagree
[ ] Disagree
[ ] Undecided
[ ] Agree
[ ] Strongly agree

35. Do you take actions in your home that allow you to save energy and water?

[ ] Strongly disagree
[ ] Disagree
[ ] Undecided
[ ] Agree
[ ] Strongly agree

D. IMPORTANT CONSUMER CRITERIA WHEN BUYING BLACKBERRY AND BUYING HABITS

36. Do you know the benefits of blackberry?

[ ] None
[ ] Some
[ ] All

37. Where do you buy blackberry?

Supermarkets [ ]
Farmers' markets [ ]
Neighborhood store [ ]
Market place [ ]
Particular supplier [ ]
Other [ ]

E. CONSUMER ATTITUDE TOWARDS ORGANIC FOOD

When buying organic food (fruits and vegetables), how important are the following criteria for you?

38. Region of origin

[ ] Not important at all
[ ] Not very important
[ ] Indifferent
[ ] Important
[ ] Very important

39. Price

[ ] Not important at all
[ ] Not very important
[ ] Indifferent
[ ] Important
[ ] Very important

40. Packing

[ ] Not important at all
[ ] Not very important
[ ] Indifferent
[ ] Important
[ ] Very important

41. Brand

[ ] Not important at all
[ ] Not very important
[ ] Indifferent
[ ] Important
[ ] Very important

42. Nutritional value

[ ] Not important at all
[ ] Not very important
[ ] Indifferent
[ ] Important
[ ] Very important

43. Appearance

[ ] Not important at all
[ ] Not very important
[ ] Indifferent
[ ] Important
[ ] Very important

44. Color

[ ] Not important at all
[ ] Not very important
[ ] Indifferent
[ ] Important
[ ] Very important

45. Firmness

[ ] Not important at all
[ ] Not very important
[ ] Indifferent
[ ] Important
[ ] Very important

46. Maturity level

[ ] Not important at all
[ ] Not very important
[ ] Indifferent
[ ] Important
[ ] Very important

47. Vitamin C content

[ ] Not important at all
[ ] Not very important
[ ] Indifferent
[ ] Important
[ ] Very important

48. Iron content

[ ] Not important at all
[ ] Not very important
[ ] Indifferent
[ ] Important
[ ] Very important

49. Calcium content

[ ] Not important at all
[ ] Not very important
[ ] Indifferent
[ ] Important
[ ] Very important

50. Phosphorus content

[ ] Not important at all
[ ] Not very important
[ ] Indifferent
[ ] Important
[ ] Very important

51. Antioxidant properties

[ ] Not important at all
[ ] Not very important
[ ] Indifferent
[ ] Important
[ ] Very important

52. The information available on the label (nutritional information, ingredients, etc.) of the blackberry packing

[ ] Not important at all
[ ] Not very important
[ ] Indifferent
[ ] Important
[ ] Very important

53. That it does not generate environmental impacts in its production

[ ] Not important at all
[ ] Not very important
[ ] Indifferent
[ ] Important
[ ] Very important

54. Safety

[ ] Not important at all
[ ] Not very important
[ ] Indifferent
[ ] Important
[ ] Very important

55. That its production takes place under a fair-trade framework

[ ] Not important at all
[ ] Not very important
[ ] Indifferent
[ ] Important
[ ] Very important

56. In what presentation do you buy blackberry?

[ ] Fresh
[ ] Fruit flesh
[ ] Juice
[ ] Nectar
[ ] Pastry shop
[ ] Other

57. What are your ways of using and consuming blackberry?

[ ] Fresh
[ ] Fruit flesh
[ ] Juice
[ ] Nectar
[ ] Pastry shop
[ ] Other

58. How much is the monthly blackberry consumption (kg/month) of your household?

_ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _

58. Price of blackberry consumed (per lb, kg)?

_ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _

59. ¿Do you consume organic blackberry?

[ ] Yes [ ] No

59.1 Price you pay for organic blackberry (per lb, kg) _ _ _ _ _ _ _ _

59.2 Where do you buy organic blackberry?

Supermarkets [ ]
Specialized stores [ ]
Farmers' markets [ ]
Particular supplier [ ]
Other [ ]
Name of the supplier

E. CONSUMER ATTITUDE TOWARDS ORGANIC FOOD

When buying organic food (fruits and vegetables), how important are the following criteria for you?

60. Region of origin

[ ] Not important at all
[ ] Not very important
[ ] Indifferent
[ ] Important
[ ] Very important

61. Price

[ ] Not important at all
[ ] Not very important
[ ] Indifferent
[ ] Important
[ ] Very important

62. Packing

[ ] Not important at all
[ ] Not very important
[ ] Indifferent
[ ] Important
[ ] Very important

63. Brand

[ ] Not important at all
[ ] Not very important
[ ] Indifferent
[ ] Important
[ ] Very important

64. Nutritional value

[ ] Not important at all
[ ] Not very important
[ ] Indifferent
[ ] Important
[ ] Very important

65. Appearance

[ ] Not important at all
[ ] Not very important
[ ] Indifferent
[ ] Important
[ ] Very important

66. The information available on the label (nutritional information, ingredients, etc.) of the blackberry package

[ ] Not important at all
[ ] Not very important
[ ] Indifferent
[ ] Important
[ ] Very important

67. That is at discount

[ ] Not important at all
[ ] Not very important
[ ] Indifferent
[ ] Important
[ ] Very important

68. Taste

[ ] Not important at all
[ ] Not very important
[ ] Indifferent
[ ] Important
[ ] Very important

Do you consider organic food (fruits/vegetables)

69. is of superior quality?

[ ] Strongly disagree
[ ] Disagree
[ ] Undecided
[ ] Agree
[ ] Strongly agree

70. does not affect the environment?

[ ] Strongly disagree
[ ] Disagree
[ ] Undecided
[ ] Agree
[ ] Strongly agree

71. is healthier?

[ ] Strongly disagree
[ ] Disagree
[ ] Undecided
[ ] Agree
[ ] Strongly agree

72. is more nutritious?

[ ] Strongly disagree
[ ] Disagree
[ ] Undecided
[ ] Agree
[ ] Strongly agree

73. is more expensive?

[ ] Strongly disagree
[ ] Disagree
[ ] Undecided
[ ] Agree
[ ] Strongly agree

74. is trendy?

[ ] Strongly disagree
[ ] Disagree
[ ] Undecided
[ ] Agree
[ ] Strongly agree

75. helps prevent and reverse the development of diseases?

[ ] Strongly disagree
[ ] Disagree
[ ] Undecided
[ ] Agree
[ ] Strongly agree

76. The main reason why you consume organic food is:

_ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _

77. What organic foods do you eat?

_ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _

78. How do you identify organic foods?

_ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _

F. CONSUMER'S CONFIDENCE IN SUPPLIERS AND ORGANIC CERTIFICATION

79. Do you trust that the people/entities that market organic food are marketing real organic food?

[ ] Always
[ ] Usually
[ ] Sometimes
[ ] Rarely
[ ] Never

80. Do you know about organic food certification?

[ ] Yes
[ ] No

81. Do you trust the label of organic food?

[ ] Yes
[ ] No

82.1 When consuming organic fruits/vegetables, do you only buy those that are certified organic?

Yes [ ] No [ ]

82.2 Is it important to you that the organic food you are buying is certified/sealed as organic?

[ ] Strongly disagree
[ ] Disagree
[ ] Undecided
[ ] Agree
[ ] Strongly agree

G. CONSUMPTION OF ORGANIC FRUITS/VEGETABLES AND WILLINGNESS TO PAY FOR ORGANIC BLACKBERRIES

83. Where do you buy your organic vegetables/fruits?

Supermarkets [ ]
Specialized shops [ ]
Farmers' markets [ ]
Particular supplier [ ]
Other [ ]

84. For whom do you buy organic fruits and vegetables?

For all the family [ ]
For a member of the family [ ]
For me [ ]

85. How often do you buy organic vegetables/fruits?

[ ] Once a year
[ ] Twice a year
[ ] Every 2/3 months
[ ] Every month
[ ] Several times/month
[ ] Every week

86. Are you willing to pay out of your income to acquire organic arrears?

Yes [ ]
No [ ]

86.1. How much are you willing to pay for organic blackberry? (per lb/kg) _ _ _ _ _ _ _ _

H. BARRIERS TO THE CONSUMPTION OF ORGANIC VEGETABLES AND FRUITS

Do you consider any of the following as reasons that hinder the consumption of organic food?

87. Organic fruits and vegetables are very expensive

[ ] Strongly disagree
[ ] Disagree
[ ] Undecided
[ ] Agree
[ ] Strongly agree

88. The appearance of organic fruits and vegetables is not attractive

[ ] Strongly disagree
[ ] Disagree
[ ] Undecided
[ ] Agree
[ ] Strongly agree

89. Low availability in stores/supermarkets

[ ] Strongly disagree
[ ] Disagree
[ ] Undecided
[ ] Agree
[ ] Strongly agree

90. Organic vegetables/fruits generally come in plastic packaging

[ ] Strongly disagree
[ ] Disagree
[ ] Undecided
[ ] Agree
[ ] Strongly agree

91. There is no constant supply

[ ] Strongly disagree
[ ] Disagree
[ ] Undecided
[ ] Agree
[ ] Strongly agree

92. There is little variety of organic fruits/vegetables

[ ] Strongly disagree
[ ] Disagree
[ ] Undecided
[ ] Agree
[ ] Strongly agree

93. Little information/education on organic ruits/vegetables

[ ] Strongly disagree
[ ] Disagree
[ ] Undecided
[ ] Agree
[ ] Strongly agree

94. Many organic foods do not have a certification

[ ] Strongly disagree
[ ] Disagree
[ ] Undecided
[ ] Agree
[ ] Strongly agree

95. Multiple certifications on fruit packages

[ ] Strongly disagree
[ ] Disagree
[ ] Undecided
[ ] Agree
[ ] Strongly agree

Evaluation of the drying process and toxic metal contents in yerba mate cultivated in southern Brazil

Evaluación del proceso de secado y contenido de metales tóxicos en la yerba mate cultivada en el sur de Brasil

Caroline Gieseler Dornelles[1] and Matheus Poletto[2*]

ABSTRACT

The drying kinetics of yerba mate leaves from southern Brazil was investigated theoretically and experimentally in a drying oven at 70°C. The effect of drying conditions on the moisture ratio, drying rate, and effective diffusivity of yerba mate leaves was evaluated. Five drying models were fitted to experimental data. Additionally, the concentrations of As, Cd, Pb, Ni, Cr, and Hg in the yerba mate leaves were determined using inductively coupled plasma optic emission spectrometry (ICP-OES). The results revealed that all samples only showed a falling rate period without a constant rate of drying. The Midilli model showed the best fit of experimental data. All samples showed levels of Hg higher than those permitted by Brazilian legislation. Monitoring programs and other studies are required to prevent the intake of yerba mate products contaminated with toxic metals.

Key words: diffusion, drying, modelling, *Ilex paraguariensis*.

RESUMEN

La cinética del secado de hojas de yerba mate del sur de Brasil se investigó experimental y teóricamente en un horno de secado a 70°C. Se evaluó el efecto de las condiciones de secado sobre la proporción de humedad, la velocidad de secado y la difusividad efectiva de las hojas de yerba mate. Se ajustaron cinco modelos de secado a los datos experimentales. Además, se determinó la concentración de As, Cd, Pb, Ni, Cr y Hg en las hojas de yerba mate mediante espectrometría de emisión óptica de plasma de acoplamiento inductivo (ICP-OES). Los resultados revelaron que todas las muestras solo presentaron un período de tiempo de velocidad decreciente sin periodo de velocidad constante de secado. El modelo Midilli mostró el mejor ajuste de los datos experimentales. Todas las muestras presentaron niveles de Hg superiores a los permitidos por la legislación brasileña. Se requieren programas de monitoreo y otros estudios para prevenir la ingesta de productos de yerba mate contaminados con metales tóxicos.

Palabras clave: difusión, secado, modelado, *Ilex paraguariensis*.

Introduction

Several studies have been recently conducted on yerba mate (*Ilex paraguariensis*) because of its effects on human health (Kahmann *et al*., 2017; Zielinski *et al*., 2020; Croge *et al*., 2021). The alkaloids, terpenes, polyphenols, and essential oils, among other compounds present in its chemical composition are responsible for pharmacological activities that have anti-inflammatory, anti-obesity, and antioxidant effects (Croge *et al*., 2021). The most common use of yerba mate consists of an infusion of its leaves, such as *chimarrão* (a hot infusion of yerba mate leaves), *tereré* (a cold infusion of leaves) and mate tea (Zielinski *et al*., 2020). However, in the last years, yerba mate has been used as raw material in non-traditional uses that include the production of beers, soft drinks, sweets, and functional cheeses (Croge *et al*., 2021).

Yerba mate production is particularly favored in eastern Paraguay, north-eastern Argentina and southern Brazil, where its cultivation and processing are an important economic activity, not only for South America but also for international trade (Heck & De Mejia, 2007; Frizon *et al*., 2017). The geographical origins associated with environmental conditions, soil composition, harvest time, and processing and cultivation can affect the levels of the bioactive compounds (Kahmann *et al*., 2017; Zielinski *et al*., 2020). Additionally, other anthropogenic activities can influence the chemical composition of yerba mate by introducing compounds harmful to health (Toppel *et al*., 2018; Valduga *et al*., 2019).

The drying is a vital stage in the processing of the yerba mate leaves. This process involves simultaneous mass

Received for publication: 10 June, 2021. Accepted for publication: 18 December, 2021. Doi: 10.15446/agron.colomb.v39n3.96535

[1] Exact Sciences and Technology, Chemical Engineering, University of Caxias do Sul (UCS), Caxias do Sul (Brazil).
[2] Postgraduate Program in Engineering of Processes and Technologies (PGEPROTEC), University of Caxias do Sul (UCS), Caxias do Sul (Brazil).
[*] Corresponding author: mpolett1@ucs.br

and heat transfer through the leaves, which may cause significant changes in yerba mate characteristics. The drying stage influences the product quality as well as its cost (Timm *et al*., 2019; Siqueira *et al*., 2020). These concerns could be addressed by using mathematical simulations of the drying process (Siqueira *et al*., 2020). Hence, knowing the drying characteristics of biological materials is essential to design, optimize, and control the drying process (Pilatti *et al*., 2016).

In this context, this study evaluated the drying characteristics of yerba mate leaves cultivated in southern Brazil and proposed a mathematical model of the drying process. This research also determined the concentrations of diverse toxic metals, such as As, Cd, Pb, Ni, Cr and Hg since studies about the presence of toxic metals in yerba mate leaves are scarce.

Materials and methods

Plant material

Yerba mate leaves were evaluated from three different geographical locations from Brazil. The samples from Cruz Machado (26°1'0" S, 51°21'0" W) located in Paraná state, Brazil were identified as PR. Samples from Santiago (29°11'30" S, 54°52'2" W), located in Rio Grande do Sul state, Brazil were identified as RS. Finally, leaves from Antônio Prado (28°50'13" S, 51°17'45" W) also located in Rio Grande do Sul state, Brazil were identified as NT.

Drying equipment and experimental setup

Leaves of each sample were cut with dimensions of 2 x 2 cm to be used in the drying experiments. The initial moisture content of each sample was determined using a convection oven at 70 ± 2°C. The drying was carried out in a Gibertini Eurotherm thermobalance (Novate Milanese, Italy) with 350W power, using approximately 1 g of sample. The temperature used was 70°C. The sample mass was determined at 5 min intervals, directly by the thermobalance front display, until a constant mass was reached.

Modelling of the drying process

To evaluate the drying characteristics of yerba mate, the moisture ratio (MR) values were determined from the moisture content data (kg water/kg dry matter) at time *t* from Equation 1:

$$MR = \frac{Mt-Me}{Mo-Me} \quad (1)$$

where M_t, M_o and M_e are the moisture content at any time of drying (kg water/kg dry matter), the initial moisture content (kg water/kg dry matter), and the equilibrium moisture content (kg water/kg dry matter), respectively. M_e was neglected since its values were relatively small compared to M_t and M_o (Mewa *et al*., 2019; Yilmaz *et al*., 2019).

The drying rate of yerba mate leaves was obtained according to Equation 2:

$$DR = \frac{M_t-M_{t+dt}}{dt} \quad (2)$$

where DR is the drying rate (kg water/kg dry matter per min), M_{t+dt} is the moisture content at t + dt time (kg water/kg dry matter) and *t* is the drying time (min).

The drying data obtained were fitted to five different drying models detailed in Table 1.

TABLE 1. Mathematical models applied to the drying curves.

Model's name	Model's equation
Newton (Yilmaz *et al*., 2019)	MR = exp(-kt)
Page (Mewa *et al*., 2019)	MR = exp(-kt^n)
Henderson and Pabis (Kouhila *et al*., 2020)	MR = a exp(-kt)
Midilli *et al.* (2002)	MR = a exp(-kt^n) + bt
Parabolic (Darvishi *et al*., 2013)	MR = at^2 + bt + c

MR - Moisture ratio; t - time; a, b, c, k, n: constants.

The nonlinear least-squares regression analysis was used to estimate model parameters using the software Origin® 2018 (OriginLab Corporation, Northampton, MA, USA). The coefficient of determination (R^2) was the primary parameter used for selecting the best model to define the drying curves (Darvishi *et al*., 2013). However, to better determine the quality of the fit, reduced chi-square (χ^2) and root mean square error (RMSE) were also calculated. These two statistical features were calculated according to Equations 3 and 4, respectively:

$$RMSE = \left[\frac{1}{N}\sum_{i=1}^{N}\left(\left(MR_{exp,i} - MR_{pre,i}\right)^2\right]^{\frac{1}{2}} \quad (3)$$

$$\chi^2 = \frac{\sum_{i=1}^{N}\left(\left(MR_{exp,i} - MR_{pre,i}\right)^2\right)}{N-Z} \quad (4)$$

where $MR_{exp,i}$ is the experimental moisture ratio, $MR_{pre,i}$ is the predicted moisture ratio, *N* is the number of observations, and *Z* is the number of constants. The model that best described the drying characteristics was chosen as the one with the highest R^2, followed by the lowest χ^2 and RMSE values (Sarimeseli, 2011; Yilmaz *et al*., 2019).

Effective moisture diffusivity

The effective moisture diffusivity can be determined from the slope of the normalized plot of MR, ln(MR) versus time, when the drying time is abundant (Darvishi *et al*., 2013; Incedayi, 2020; Kouhila *et al*., 2020), using Equation 5:

$$\ln(MR) = \ln\left(\frac{8}{\pi^2}\right) - \left(\frac{\pi^2\, D_{eff}}{4\, L^2}\right) t \qquad (5)$$

where (D_{eff}) is the effective moisture diffusivity (m^2/s) and L is the half thickness of the sample (m).

Metal content determination

The arsenic, cadmium, lead, nickel, chromium, and mercury contents in yerba mate leaves were analyzed using ICP-OES equipment (ICAP 7000, Thermo Scientific, Cambridge, UK). The samples were previously dried in a convection oven at 70°C until constant weight. The samples were prepared according to the EPA method 3050B (EPA, 1996) and the APHA method 3120B (Gottler, 2017), and analyzed in triplicate.

Results and discussion

Drying characteristics

The evolution of moisture content as a function of time can be represented by the drying curve. Figure 1A shows the variations in the moisture ratio (MR) with the drying time for all leaves studied. According to Kouhila *et al.* (2020), the drying curve can be separated in three main phases. In the first phase, a transition between transient to permanent regime of drying takes place. The sample temperature reaches the wet bulb temperature. The main part of energy is used to increase the sample's surface temperature through a sensible heat input which will result in water evaporation. As seen in Figure 1A, before the initial 1000 s, all samples show very similar behavior, which is consistent with the first stage of drying.

The second drying phase corresponds to a more prominent drying since the steady state phase begins. At this stage, the free water present in the sample's surface is evaporated. The saturation vapor pressure inside the oven is equal to the vapor pressure at the sample's surface (Kouhila *et al.*, 2020). The drying rate is constant during this stage and only depends on the external conditions, such as temperature, air humidity and flow characteristics (Darvishi *et al.*, 2013; Kouhila *et al.*, 2020). It was not possible to identify the steady state phase in Figure 1A. After the first period, all samples only showed a falling rate period. A similar behavior was observed by Pilatti *et al.* (2016) for yerba mate leaves.

Phase 3 corresponds to the falling drying rate period. As shown in Figure 1A, this period can be identified approximately from 1000-6000 s. At this period, the energy is used to evaporate the water from the inside of the sample. In organic materials, such as yerba mate, the water evaporates from the inner layers of the sample generally by a diffusion-controlled process. Consequently, the drying rate decreases increasing the drying time, as can be seen in Figure 1B. Thus, the constitution of each yerba mate leaf may influence the mass and heat transfer that may cause the decrease in the drying rate. Pilatti *et al.* (2016) reported that, at this stage, the drying is governed by the water diffusion in the solid that agrees with the results of this study. After 6000 s, the moisture ratio was reduced, indicating that the drying process was near reaching the equilibrium moisture content.

Modelling of the drying curves

The best model for describing the drying characteristics of the leaves studied was selected according to the highest R^2 and the lowest χ^2 and RMSE values. The statistical results for the models tested are summarized in Table 2.

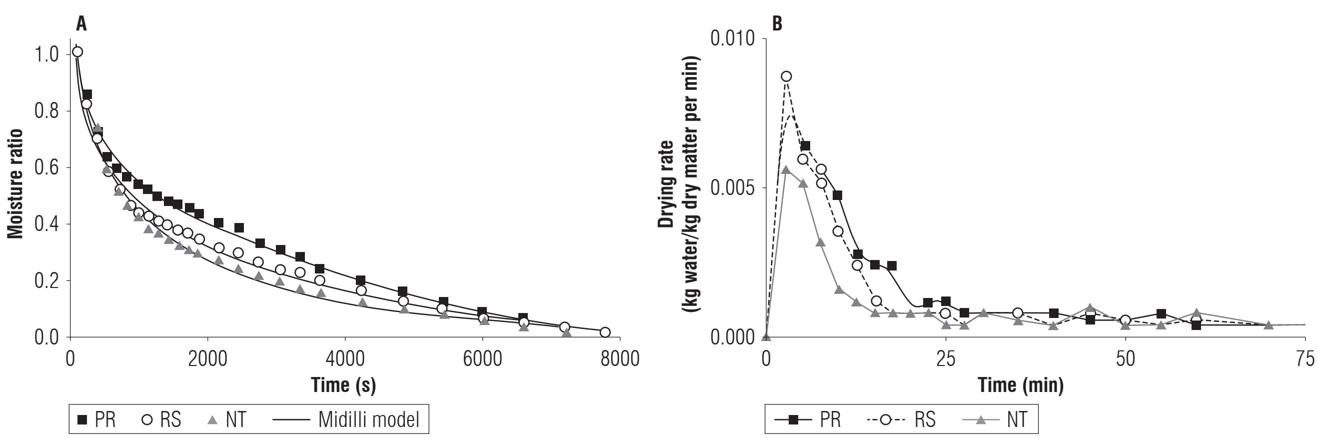


FIGURE 1. Evolution of A) moisture ratio and B) drying rate of the yerba mate leaves versus drying time. PR: Samples from Paraná; RS: samples from Rio Grande do Sul; NT: samples from Antônio Prado.

TABLE 2. Results of the statistical analysis on the modelling of moisture content versus drying time for the samples of yerba mate leaves studied.

Model name	Samples	R^2	χ^2	RMSE	Coefficients				
					a	b	c	n	k
Newton	PR	0.8837	0.0068	0.1641	---	---	---	---	0.00049
	RS	0.8623	0.0081	0.2017	---	---	---	---	0.00067
	NT	0.9217	0.0050	0.1207	---	---	---	---	0.00077
Page	PR	0.9779	0.0013	0.0299	---	---	---	0.646	0.00731
	RS	0.9850	0.0009	0.0211	---	---	---	0.597	0.01289
	NT	0.9892	0.0006	0.0159	---	---	---	0.662	0.00878
Henderson and Pabis	PR	0.9520	0.0028	0.0648	0.8318	---	---	---	0.00037
	RS	0.9286	0.0042	0.1004	0.8053	---	---	---	0.00047
	NT	0.9458	0.0035	0.0800	0.8668	---	---	---	0.00063
Midilli	PR	0.9925	0.0004	0.0093	1.009	-0.000029	---	0.462	0.02409
	RS	0.9894	0.0006	0.0137	1.016	-0.000015	---	0.507	0.02353
	NT	0.9888	0.0007	0.0151	1.024	-0.000002	---	0.635	0.01088
Parabolic	PR	0.9281	0.0042	0.0929	0.78495	-0.00019	0.0002	---	---
	RS	0.8759	0.0073	0.1672	0.72184	-0.00022	0.0002	---	---
	NT	0.8698	0.0084	0.1840	0.74416	-0.00027	0.0003	---	---

PR: samples from Paraná; RS: samples from Rio Grande do Sul; NT: samples from Antônio Prado. R^2: coefficient of determination, χ^2: reduced chi-square; RMSE - root mean square error.

From all modes tested, the Midilli model seems to be the best model describing the drying characteristics of the three samples, as can be seen in the adjustment done in the experimental data shown in Figure 1A, and also verified by the highest R^2 and lowest χ^2 and RMSE values shown in Table 2. However, the Page model also showed similarity with the experimental data and could not be discarded. Pilatti *et al.* (2016) evaluated the drying of yerba mate leaves in a bench dryer and found that the Page model was suitable to predict the experimental data. Holowaty *et al.* (2018) also observed that the Page model better described the drying of yerba mate leaves.

Effective moisture diffusivity results

The values of effective moisture diffusivity are shown in Table 3. The D_{eff} values ranged from 2.96 x 10^{-12} to 3.69 x 10^{-12} $m^2\,s^{-1}$. The values obtained in this research are in the general range of other values found in the literature for yerba mate leaves ranging from 10^{-14} to 10^{-10} $m^2\,s^{-1}$ (Ramallo, 2001). Ramallo *et al.* (2001) found moisture diffusivity between 2.3 x 10^{-11} and 2.5 x 10^{-10} $m^2\,s^{-1}$ for yerba mate leaves cultivated in Argentina, drying the leaves at 100-130°C, while Pilatti *et al.* (2016) obtained moisture diffusivity ranging from 2.3 x 10^{-11} to 2.5 x 10^{-10} $m^2\,s^{-1}$ for yerba mate leaves from Brazil, when drying the samples at 55°C, 65°C and 75°C.

TABLE 3. Values of effective moisture diffusivity (D_{eff}) for the yerba mate leaves.

Sample	D_{eff} ($m^2\,s^{-1}$)	R^2
PR	3.65 x 10^{-12}	0.981
RS	2.96 x 10^{-12}	0.993
NT	3.69 x 10^{-12}	0.991

PR: Samples from Paraná; RS: samples from Rio Grande do Sul; NT: samples from Antônio Prado; R^2: coefficient of determination.

The NT showed the highest D_{eff} value followed by PR and RS. This result corroborated the moisture ratio showed in Figure 1A. When samples were dried, the activity of water molecules increased (Xiao *et al.*, 2010) since the energy was used to heat the sample; consequently, the movement and collisions between water molecules increased, which resulted in higher moisture diffusivity.

Metal content

The concentration of arsenic, cadmium, lead, chromium, mercury, and nickel found in the leaf samples is shown in Table 4. The maximum metal content permitted in yerba mate according to Brazilian legislation (Presidência da República - Brasil, 1965; Ministério da Saúde - Brasil, 2013) is shown in Table 4.

Based on the results shown in Table 4, the contents of As, Cd, Pb and Ni were below those permitted by Brazilian legislation, while the Hg content obtained in the three samples of yerba mate leaves was above that allowed by Brazilian legislation. The highest levels were obtained for the PR and NT samples, with contamination 3 times higher than the maximum content established by Brazilian legislation. The Hg contamination in the RS sample was 50% higher than that allowed by Brazilian legislation.

Mercury is present in the entire biosphere, although it is far from natural or anthropogenic sources. Mercury compounds such as chlorides, nitrates and sulphates are stable and are even more abundant in the environment (Xiao *et*

TABLE 4. Metal concentration (mg kg^{-1} DW) found in the yerba mate leaves.

Metal	Maximum concentration permitted	PR	RS	NT
As	0.60	0.0047 ± 0.0019	0.0186 ± 0.0005	0.0185 ± 0.0004
Cd	0.40	0.1989 ± 0.0015	0.1921 ± 0.0085	0.0458 ± 0.0011
Pb	0.60	0.3313 ± 0.0024	0.3072 ± 0.0158	0.1990 ± 0.0044
Cr	0.10	0.0431 ± 0.0250	0.1523 ± 0.0037	0.1307 ± 0.0018
Hg	0.01	0.0377 ± 0.0015	0.0155 ± 0.0005	0.0305 ± 0.0008
Ni	5.00	3.7791 ± 0.0209	0.5413 ± 0.0302	2.4941 ± 0.0071

Values in red are higher than the maximum permitted by Brazilian legislation (Presidência da República - Brasil, 1965; Ministério da Saúde - Brasil, 2013). PR: Samples from Paraná; RS: samples from Rio Grande do Sul; NT: samples from Antônio Prado.

al., 2010). Thus, contamination of soils and waters with Hg can generate leaf contamination during plant growth.

The Cr content was 50% above that established by Brazilian legislation for the RS leaf samples and 30% above for NT. Saidelles *et al*. (2010) evaluated yerba mate samples and verified higher levels of Cr. The authors obtained Cr content that ranged from 1.3 and 1.6 mg kg^{-1} for samples commercialized in the southern region of Brazil. According to Valduga *et al*. (2019), the elemental composition of the yerba mate leaves can be altered by the chemical properties of the soil, fertilization, and limestone use. Water used for soil irrigation may also contain toxic metals (Kosanić *et al*., 2017; Santos *et al*., 2018) and contribute to the higher levels of metals in the yerba mate leaves analyzed. Therefore, other studies need to be carried out to confirm these hypotheses.

Conclusions

Drying characteristics and concentrations of As, Cd, Pb, Ni, Cr, and Hg in the yerba mate leaves were investigated. The drying curve revealed only the presence of a falling drying rate period. The Midilli model seemed to be the best mathematical model to describe the drying process. The moisture diffusivity ranged from 2.96×10^{-12} to 3.69×10^{-12} $m^2 s^{-1}$. All samples studied showed Hg levels higher than those permitted by Brazilian legislation, while the RS and NT samples showed higher levels of Cr. The higher levels of Hg and Cr may be associated with soil composition, use of fertilizers, limestone addition to soil, or contamination of the water used for soil irrigation. However, new studies are required to confirm these hypotheses.

Acknowledgments

The authors would like to thank the University of Caxias do Sul (UCS) of Brazil for its contribution to this research.

Conflict of interest statement

The authors declare that there is no conflict of interest regarding the publication of this article.

Author's contributions

CGD and MP formulated the overarching research goals and aims. CGD carried out activities to annotate scrub data and maintain research data for initial use and later reuse. MP applied statistical, mathematical, computational techniques to analyze study data. CGD conducted the research and investigation process, specifically performing the experiments. CGD developed the methodology and created the models. MP managed and coordinated the research activity planning and execution. CGD provided the study materials, reagents, laboratory samples, instrumentation, and other analysis tools. CGD and MP verified the overall replication/reproducibility of results and other research outputs. CGD and MP prepared, created, and presented the published work and oversaw its visualization. CGD and MP wrote/translated the initial draft. MP carried out the critical review, commentary, and revision of the manuscript. All authors reviewed the manuscript.

Literature cited

Croge, C. P., Cuquel, F. L., & Pintro, P. T. M. (2021). Yerba mate: cultivation systems, processing and chemical composition. A review. *Scientia Agricola*, *78*(5), Article e20190259. https://doi.org/10.1590/1678-992X-2019-0259

Darvishi, H., Azadbakht, M., Rezaeiasl, A., & Farhang, A. (2013). Drying characteristics of sardine fish dried with microwave heating. *Journal of the Saudi Society of Agricultural Sciences*, *12*(2), 121–127. https://doi.org/10.1016/j.jssas.2012.09.002

EPA. (1996). *Method 3050B: acid digestion of sediments, sludges, and soils*. United States Environmental Protection Agency. https://www.epa.gov/sites/default/files/2015-06/documents/epa-3050b.pdf

Frizon, C. N. T., Dagostin, J. L. A., Nisgoski, S., Ribani, M., & Hoffmann-Ribani, R. (2017). Microwave drying and NIR spectroscopy for the rapid moisture measurement of yerba mate (*Ilex paraguariensis*) leaves during storage. *International Journal of Food Properties*, *20*(sup2), 1403–1412. https://doi.org/10.1080/10942912.2017.1343834

Gottler, R. A. (2017). 3120 - metals by plasma emission spectroscopy. In R. B. Baird, A. D. Eaton, & E. W. Rice (Eds.), *Standard methods for the examination of water and wastewater* (23rd ed., pp. 3–42). American Public Health Association, American Water Works Association, Water Environment Federation.

Heck, C. I., & De Mejia, E. G. (2007). Yerba mate tea (*Ilex paraguariensis*): a comprehensive review on chemistry, health implications, and technological considerations. *Journal of Food Science*, *72*(9), R138–R151. https://doi.org/10.1111/j.1750-3841.2007.00535.x

Holowaty, S. A., Thea, A. E., Alegre, C., & Schmalko, M. E. (2018). Differences in physicochemical properties of yerba maté (*Ilex paraguariensis*) obtained using traditional and alternative manufacturing methods. *Journal of Food Process Engineering*, *41*(8), Article e12911. https://doi.org/10.1111/jfpe.12911

Incedayi, B. (2020). Assessment of pretreatments on drying kinetics and quality characteristics of thin-layer dried red pepper. *Turkish Journal of Agriculture and Forestry*, *44*(6), 543–556.

Kahmann, A., Anzanello, M. J., Marcelo, M. C. A., & Pozebon, D. (2017). Near infrared spectroscopy and element concentration analysis for assessing yerba mate (*Ilex paraguariensis*) samples according to the country of origin. *Computers and Electronics in Agriculture*, *140*, 348–360. https://doi.org/10.1016/j.compag.2017.06.007

Kosanić, M., Ranković, B., Rančić, A., & Stanojković, T. (2017). Evaluation of metal contents and bioactivity of two edible mushrooms *Agaricus campestris* and *Boletus edulis*. *Emirates Journal of Food and Agriculture*, *29*(2), 98–103. https://doi.org/10.9755/ejfa.2016-06-656

Kouhila, M., Moussaoui, H., Lamsyehe, H., Tagnamas, Z., Bahammou, Y., Idlimam, A., & Lamharrar, A. (2020). Drying characteristics and kinetics solar drying of Mediterranean mussel (*Mytilus galloprovincilis*) type under forced convection. *Renewable Energy*, *147*, 833–844. https://doi.org/10.1016/j.renene.2019.09.055

Mewa, E. A., Okoth, M. W., Kunyanga, C. N., & Rugiri, M. N. (2019). Experimental evaluation of beef drying kinetics in a solar tunnel dryer. *Renewable Energy*, *139*, 235–241. https://doi.org/10.1016/j.renene.2019.02.067

Midilli, A., Kucuk, H., & Yapar, Z. (2002). A new model for single-layer drying. *Drying Technology*, *20*(7), 1503–1513. https://doi.org/10.1081/DRT-120005864

Ministério da Saúde - Brasil. (2013). *Resolução - RDC no. 42, de 29 de Agosto de 2013*. https://bvsms.saude.gov.br/bvs/saudelegis/anvisa/2013/rdc0042_29_08_2013.html

Pilatti, D., Johann, G., Palú, F., & Silva, E. A. (2016). Evaluation of a concentrated parameters mathematical model applied to drying of yerba mate leaves with variable mass transfer coefficient. *Applied Thermal Engineering*, *105*, 483–489. https://doi.org/10.1016/j.applthermaleng.2016.02.139

Presidência da República - Brasil. (1965). *Decreto no. 55871 de 26 de março de 1965*. https://www.gov.br/agricultura/pt-br/assuntos/inspecao/produtos-vegetal/legislacao-1/biblioteca-de-normas-vinhos-e-bebidas/decreto-no-55-871-de-26-de-marco-de-1965.pdf/view

Ramallo, L. A., Pokolenko, J. J., Balmaceda, G. Z., & Schmalko, M. E. (2001). Moisture diffusivity, shrinkage, and apparent density variation during drying of leaves at high temperatures. *International Journal of Food Properties*, *4*(1), 163–170. https://doi.org/10.1081/JFP-100002194

Saidelles, A. P. F., Kirchner, R. M., Santos, N. R. Z., Flores, E. M. M., & Bartz, F. R. (2010). Análise de metais em amostras comerciais de erva-mate do sul do Brasil. *Alimentos e Nutrição*, *21*(2), 259–265.

Santos, A. C., Barbosa, S., Pessoa, M. F., Leal, N., Reboredo, F., Lidon, F., & Almeida, J. (2018). Speciation, mobility and adsorption effects of various metals in sediments in an agricultural area surrounding an uranium ore deposit (Nisa, Portugal). *Emirates Journal of Food and Agriculture*, *30*(6), 503–514.

Sarimeseli, A. (2011). Microwave drying characteristics of coriander (*Coriandrum sativum* L.) leaves. *Energy Conversion and Management*, *52*(2), 1449–1453. https://doi.org/10.1016/j.enconman.2010.10.007

Siqueira, V. C., Leite, R. A., Mabasso, G. A., Martins, E. A. S., Quequeto, W. D., & Isquierdo, E. P. (2020). Drying kinetics and effective diffusion of buckwheat grains. *Ciência e Agrotecnologia*, *44*, Article e011320. https://doi.org/10.1590/1413-7054202044011320

Timm, T. G., Pasko, R. Z., Campos, C. S. G., Helm, C. V., & Tavares, L. B. B. (2019). Drying process of *Lentinula edodes*: influence of temperature on β-glucan content and adjustment of mathematical models. *Ciência e Agrotecnologia*, *43*, Article e025719. https://doi.org/10.1590/1413-7054201943025719

Toppel, F. V., Maccari Junior, A., Motta, A. C. V., Frigo, C., Magri, E., & Barbosa, J. Z. (2018). Soil chemical attributes and their influence on elemental composition of yerba mate leaves. *Floresta*, *48*(3), 425–434. https://doi.org/10.5380/rf.v48i3.56677

Valduga, A. T., Gonçalves, I. L., & Magri, E. (2019). Analysis of the presence of toxic metals in yerba mate samples: a case study from south Brazil. *Water, Air, & Soil Pollution*, *230*, Article 153. https://doi.org/10.1007/s11270-019-4204-z

Xiao, H. W., Pang, C. L., Wang, L. H., Bai, J. W., Yang, W. X., & Gao, Z. J. (2010). Drying kinetics and quality of Monukka seedless grapes dried in an air-impingement jet dryer. *Biosystems Engineering*, *105*(2), 233–240. https://doi.org/10.1016/j.biosystemseng.2009.11.001

Yilmaz, M. S., Şakiyan, O., Mazi, I. B., & Mazi, B. G. (2019). Phenolic content and some physical properties of dried broccoli as affected by drying method. *Food Science and Technology International*, *25*(1), 76–88. https://doi.org/10.1177/1082013218797527

Zielinski, A. A. F., Alberti, A., Bona, E., Bortolini, D. G., Benvenutti, L., Bach, F., Demiate, I. M., & Nogueira, A. (2020). A multivariate approach to differentiate yerba mate (*Ilex paraguariensis*) commercialized in the southern Brazil on the basis of phenolics, methylxanthines and *in vitro* antioxidant activity. *Food Science and Technology*, *40*(3), 644–652. https://doi.org/10.1590/fst.15919

Editorial policy

Agronomía 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 triannual publication, this journal is intended to transfer research results in different areas of Agronomy in the tropics and subtropics. Original unpublished papers are, therefore, accepted in the following areas: plant physiology, crop nutrition and fertilization, genetics and plant breeding, entomology, phytopathology, integrated crop protection, agroecology, weed science, environmental management, geomatics, soil science, 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 manuscripts submitted to *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 taken seriously and requests for changes and corrections respected, manuscripts subject to review by the peer reviewers, and articles remained unaltered.

Agronomía Colombiana uses a double-blind peer review process anticipated by the previous quick preliminary review. The manuscripts must be submitted according to the rules established in the instructions to authors. If the articles fulfill the minimum criteria established for the preliminary review in terms of language and scope, these are sent to three or more expert reviewers in the specific area of knowledge to obtain two or three evaluations; two of the experts are external to the Universidad Nacional de Colombia, and the third one belongs to the research and teaching staff of the Universidad Nacional de Colombia. When the manuscript obtains two approval evaluations, the manuscript can be considered for publication by the editors; if two of the reviewers consider that the scientific level of the manuscript is not acceptable for publication, the manuscript will be rejected. Once the peer-review process is completed, the manuscript is sent back to the author(s) who must introduce the suggested corrections and answer all the questions obtained 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 *Agronomía 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 and http://www.revistas.unal.edu.co/index.php/agrocol

Instructions to authors

The manuscripts submitted 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. Contributions to the study and the submitted manuscript and any conflict of interest must be declared by the authors in the sections created for these purposes.

To submit manuscripts, the 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 their ORCID Id when submitting a manuscript. After manuscript submission, papers will be screened for plagiarism using a specialized software. In case of finding a significant level of duplication, the manuscript will be rejected.

At the preliminary review stage, two aspects, English grammar and scope, will be assessed in order to ensure the minimum requirements of any manuscript to be peer-reviewed. If the manuscript is rejected during the preliminary review because of the English grammar aspect, authors are encouraged to edit their manuscript for language using professional services and submit the manuscript again. When the reason for preliminary reject is a mismatch of the scope between the manuscript and the journal interests, resubmission should be avoided.

Agronomía Colombiana accepts the following three 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.
- **Review articles**: Published only at the invitation of the Editor-in-Chief and with the approval of the Editorial Committee of *Agronomía Colombiana*. The review article should present an unbiased summary of the current understanding of a topic considered as a priority by the Editorial or Scientific Committee of *Agronomía Colombiana*.
- **Scientific notes**: Brief document introducing original preliminary or partial results of a scientific or technical research, which usually needs immediate publication.

Format and organization of the text

Research article length should not exceed 5,200 words, whereas scientific notes should have no more than 4,000 words. As review articles contain a large amount of detailed information, their length may be greater than research articles but should not exceed 8,000 words, or 10,000 words including the list of references. For review articles, the list of references (Literature cited section) should include at least 50 references. 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 the MS Word® processor. Manuscripts including tables as embedded images will not be published. All text should be double-spaced including table headers, figure captions and cited literature. All pages must be numbered consecutively. 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 other graphic formats but maintaining a high resolution. Other figures, including photographs and drawings should be submitted in digital JPG (or JPEG) 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 or for those cases in which it is absolutely necessary to present them in color (at the judgment and discretion of the Editor).

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 for 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).

Decimal fractions should be separated by a point (.), not a comma (,).

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 lowercased, except for the first letter of the genus (and of the species author), which must be uppercased.

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, only the name and city location of the employer or supporting institution(s), and the e-mail address of the corresponding author should be included.

Abstract, resumen, and key words

The Abstract should be written in English with Spanish translation for the "Resumen". 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

The introduction must 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 by 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-explanatory, 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 a 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 data.

This section should briefly and concisely summarize the most important findings of the research.

Conclusion (optional)

A short conclusion section is useful for a long or complex discussion. It should provide readers with a brief summary of the main achievements from the results of the study. It can also contain final remarks and a brief description of future complementary studies that should be addressed.

Acknowledgments

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.

Conflict of interest statement

All manuscripts that are submitted to and published in *Agronomía Colombiana* must be accompanied by a conflict of interest disclosure statement by the authors. Please, include such a statement or declaration at the end of your manuscript, following any acknowledgments and prior to the references, under the heading 'Conflict of interest statement'.

Example: The authors declare that there is no conflict of interest regarding the publication of this article.

Author's contributions

This information is mandatory for *Agronomía Colombiana* from 2020 onwards. In order to describe each of the authors' contribution, please follow the CRediT taxonomy and use the following roles as a guide:

Contributor roles

Conceptualization: AAA formulated the overarching research goals and aims.

Data curation: AAA carried out activities to annotate scrub data and maintain research data for initial use and later re-use.

Formal analysis: AAA applied statistical, mathematical, computational, or other formal techniques to analyze or synthesize study data.

Funding acquisition: AAA obtained the financial support for the project leading to this publication.

Investigation: AAA conducted the research and investigation process, specifically performing the experiments or data/evidence collection.

Methodology: AAA developed or designed the methodology; created the models.

Project administration: AAA managed and coordinated the research activity planning and execution.

Resources: AAA provided the study materials, reagents, laboratory samples, instrumentation, computing resources, or other analysis tools.

Software: AAA implemented the computer code and supporting algorithms/software.

Supervision: AAA oversaw and led the research activity planning and execution.

Validation: AAA verified the overall replication/reproducibility of results/experiments and other research outputs.

Visualization: AAA prepared, created, and/or presented the published work and oversaw its visualization/data presentation.

Writing – original draft: AAA wrote/translated the initial draft.

Writing – review & editing: AAA carried out the critical review, commentary, or revision of the manuscript.

Authors have to keep in mind the CRediT taxonomy is not useful to determine who is eligible as author, but to state the contribution of each author in the study or the article. More information about the CRediT taxonomy is available in: https://casrai.org/credit/

Example: MYD and AFT designed the experiments, AFT carried out the field and laboratory experiments, AFT contributed to the data analysis, MYD and AFT wrote the article. All authors reviewed the manuscript.

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 '&': (García & López, 2012) or García and López (2012).

Tables and figures should be cited in parenthesis as follows: (Tab. 1), (Tab. 2), (Tab. 3), etc., or (Fig. 1), (Fig. 2), (Fig. 3), etc. In the text, each table or figure must be referred to using a capital T or F, for example: ...as shown in Table 1, Table 2, Table 3, etc., or in Figure 1, Figure 2, Figure 3, etc.

The complete list of cited references in alphabetical order, according to the authors' surnames, must 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.

Agronomía Colombiana has adopted the American Psychological Association (APA) standards (https://apastyle.apa.org/about-apa-style) to elaborate the final list of references cited in the text ("Literature cited" section). This standard will be required for new manuscripts received from March 1st, 2020 onwards.

Basic information about the use of APA for the list of references is available here: https://apastyle.apa.org/style-grammar-guidelines/references. In order to illustrate these standards, authors can check some examples about how to create each item of the list of references, keeping in mind the type of publication cited as follows (click on each option to open APA web information):

Journal article

Example: García-Arias, F., Sánchez-Betancourt, E., & Núñez, V. (2018). Fertility recovery of anther-derived haploid cape gooseberry (*Physalis peruviana* L.) plants. *Agronomía Colombiana*, *36*(3), 201–209. https://doi.org/10.15446/agron.colomb.v36n3.73108

Published dissertation or thesis references

Example: Franco, C. V. (2012). *Efecto de la colchicina sobre el número cromosómico, número de cloroplastos y características morfológicas del fruto en ecotipos de uchuva (*Physalis peruviana *L.) Colombia, Kenia y Perú* [Undergraduate thesis, Universidad Francisco de Paula Santander]. UFPS Library. http://alejandria.ufps.edu.co/descargas/tesis/1610259.pdf

Whole book

Example: Suescún, L., Sánchez, E., Gómez, M., García-Arias, F. L., & Núñez Zarantes, V. M. (2011). *Producción de plantas genéticamente puras de uchuva*. Editorial Kimpres Ltda.

Edited book chapter

Example: Ligarreto, G., Lobo, M., & Correa, A. (2005). Recursos genéticos del género *Physalis* en Colombia. In G. Fischer, D. Miranda, W. Piedrahita, & J. Romero. (Eds.), *Avances en cultivo, poscosecha y exportación de la uchuva* Physalis peruviana *L. en Colombia* (pp. 329–338). Universidad Nacional de Colombia.

For other types of references such as technical reports, conference presentations or proceedings, magazine articles or preprints see https://apastyle.apa.org/style-grammar-guidelines/references/examples. Archival documents, letters, collections and unpublished documents can be referenced using some standards users can find here: https://apastyle.apa.org/style-grammar-guidelines/references/archival. In this link (http://shorturl.at/xGIPZ) authors can find a summary of the APA style for references created by Mendeley.

Authors can also consider the use of some APA list of reference generators such as Reference Management (Mendeley, https://www.mendeley.com/reference-management/mendeley-desktop), Scribbr (https://www.scribbr.com/apa-citation-generator/#/) or MyBib (https://www.mybib.com/).

Whenever possible, please provide a valid DOI for each reference in the literature cited.

AUTHOR INDEX OF *AGRONOMÍA COLOMBIANA* VOLUME 39, 2021

Continued

Continued

Author name	Article title	Location
Enciso-Maldonado Guillermo	Soybean yield components at different densities and planting seasons in Paraguay	No. 1, pp. 12-21
Falcón-Rodríguez Alejandro Bernardo	Effect of a mix of oligogalacturonides on symbiotic nitrogen fixation in common bean	No. 1, pp. 30-36
Fanti Paolo	The age of tomato plants affects the development of *Macrosiphum euphorbiae* (Thomas, 1878) (Hemiptera) colonies	No. 1, pp. 108-112
Farias David Ingsson Oliveira Andrade de	Glyphosate and atrazine inhibit growth of *Azospirillum brasilense*, *Bacillus subtilis*, *Bacillus thuringiensis*, *Chromobacterium subtsugae* and Saccharopolyspora *spinosa*	No. 1, pp. 113-120
Fernández-Riquelme Francisco	Soybean yield components at different densities and planting seasons in Paraguay	No. 1, pp. 12-21
Fernández-Salinas Paulino	Soybean yield components at different densities and planting seasons in Paraguay	No. 1, pp. 12-21
Ferracini Vera Lúcia	Improvement of growth of common bean in phosphorus-deficient soils by phosphate-solubilizing and phytohormone-producing bacteria	No. 3, pp. 372-380
Figueiredo Josiane Cantuária	Extension of umbu (*Spondias tuberosa* Arruda) postharvest life using a cassava starch-based coating	No. 2, pp. 293-299
Fischer Gerhard	Causes of fruit cracking in the era of climate change. A review	No. 2, pp. 196-207
Flores-Fernández Carol Nathali	Optimization of lactic acid production by *Lactobacillus plantarum* strain Hui1 in a medium containing sugar cane molasses	No. 1, pp. 98-107
Flores-Santos Juan Carlos	Optimization of lactic acid production by *Lactobacillus plantarum* strain Hui1 in a medium containing sugar cane molasses	No. 1, pp. 98-107
Flórez-Méndez Jennyfer	Effect of fertilization on yield and phenology of the halophyte *Sarcocornia neei* as an emerging crop	No. 2, pp. 300-305
Forlano Pierluigi	The age of tomato plants affects the development of *Macrosiphum euphorbiae* (Thomas, 1878) (Hemiptera) colonies	No. 1, pp. 108-112
França Edgar Estevam	Agronomic and physiological characteristics of soybean cultivars and their seeds according to plant population	No. 2, pp. 177-186
Fuentes Alfonso Leticia	Co-inoculation with a product based on native microorganisms improves germination and seedling growth of *Phaseolus vulgaris* L.	No. 3, pp. 405-414
Furtado José Augusto Lima	Weed control and selectivity of different pre-emergence active ingredients in a soybean crop	No. 3, pp. 392-404
Galati Vanessa Cury	Performance of corn hybrids for *in natura* consumption in the Pontal do Triângulo Mineiro region of Brazil	No. 3, pp. 330-336
Galati Vanessa Cury	Physicochemical characterization of cantaloupe fertilized with various potassium sources	No. 3, pp. 364-371
García-Guzmán Sebastián David	Relationships between mineral nutrients in banana (*Musa* AAA Simmonds cv. Williams) bunches fertilized with nitrogen in two production cycles	No. 2, pp. 234-242
García-Vivas Yuly Samanta	Content and distribution of micronutrients in calendula (*Calendula officinalis*) grown in Valle del Cauca, Colombia	No. 1, pp. 59-67
Garzón-Méndez Juan Diego	Consumer profile and factors determining the purchase of agroecological products. A case study: UNIMINUTO Agroecological Fair and Minuto de Dios Solidarity Market, Colombia	No. 2, pp. 265-281
Genuncio Glaucio da Cruz	Growth parameters and organoleptic characteristics of plantain (*Musa* AAB Simmonds cv. Farta Velhaco) at different planting densities	No. 3, pp. 322-329
Gilson Italo Kael	Productive characterization of the blueberry cultivar Bluegem in Brazil	No. 1, pp. 22-29
Gimenes Morgana Baptista	Physicochemical characterization of cantaloupe fertilized with various potassium sources	No. 3, pp. 364-371
Glint Anouschka Leonilda de	Physicochemical characterization of cantaloupe fertilized with various potassium sources	No. 3, pp. 364-371
González Sierra Llliet	Co-inoculation with a product based on native microorganisms improves germination and seedling growth of *Phaseolus vulgaris* L.	No. 3, pp. 405-414
Gradella Aluisio dos Santos Salustiano	Physicochemical characterization of cantaloupe fertilized with various potassium sources	No. 3, pp. 364-371
Grohs Sofia Ghizlan	*In vitro* inhibition attempts and bio-elicitation of *Solanum lycopersicum* L. by chitin and chitosan against *Ralstonia solanacearum*, the causal agent of bacterial wilt	No. 3, pp. 355-363
Gurgel Marcelo Tavares	Naturally colored cotton irrigated with saline water at different growth stages	No. 1, pp. 68-77
Hernández Forte Ionel	Characterization of potassium solubilizing bacteria isolated from corn rhizoplane	No. 3, pp. 415-425
Hernández Georgina	Effect of a mix of oligogalacturonides on symbiotic nitrogen fixation in common bean	No. 1, pp. 30-36
Hernández-Forte Ionel	Rhizospheric rhizobia with potential as biofertilizers from Cuban rice cultivars	No. 1, pp. 78-89
Herter Flávio Gilberto	Productive characterization of the blueberry cultivar Bluegem in Brazil	No. 1, pp. 22-29

Continued

Author name	Article title	Location
Holtz Filipe Garcia	Acaricidal activity and repellency of commercial essential oils on *Tetranychus urticae in vitro* and protected cultivation	No. 2, pp. 226-233
Huaringa-Joaquín Amelia	Interaction of triacontanol with other plant growth regulators on morphology and yield of field pea (*Pisum sativum* L.)	No. 2, pp. 187-195
Hurtado Pérez María Clara	Segmentation of Colombian organic food consumers focused on the consumption of the Andean blackberry	No. 3, pp. 438-452
Huver Andressa	Acaricidal activity and repellency of commercial essential oils on *Tetranychus urticae in vitro* and protected cultivation	No. 2, pp. 226-233
Jaramillo Camilo Ignacio	Morphological variations and abundance of populations of the leafhopper *Dalbulus maidis* (De-Long) (Hemiptera: Cicadellidae) from the corn-producing region of Huila, Colombia	No. 3, pp. 343-354
Jesus Jhenef Gomes de	Population dynamics of the nematodes *Heterodera glycines* and *Pratylenchus brachyurus* in a succession crop of soybean and chickpea	No. 3, pp. 337-342
Justi André Luiz	Effect of a friction-reducing additive on the drip irrigation uniformity with sugarcane vinasse	No. 1, pp. 90-97
Kaneko Flávio Hiroshi	Performance of corn hybrids for *in natura* consumption in the Pontal do Triângulo Mineiro region of Brazil	No. 3, pp. 330-336
Laekemariam Fanuel	Soil fertility management practices for faba bean (*Vicia faba* L.) production in Wolaita zone, Southern Ethiopia	No. 2, pp. 252-264
Lara Danurys	Effect of a mix of oligogalacturonides on symbiotic nitrogen fixation in common bean	No. 1, pp. 30-36
Leija Alfonso	Effect of a mix of oligogalacturonides on symbiotic nitrogen fixation in common bean	No. 1, pp. 30-36
Leite Robson da Costa	Glyphosate and atrazine inhibit growth of *Azospirillum brasilense*, *Bacillus subtilis*, *Bacillus thuringiensis*, *Chromobacterium subtsugae* and Saccharopolyspora *spinosa*	No. 1, pp. 113-120
Liriano González Ramón	Co-inoculation with a product based on native microorganisms improves germination and seedling growth of *Phaseolus vulgaris* L.	No. 3, pp. 405-414
López-Pazos Silvio Alejandro	A basic scheme of soybean transformation for glyphosate tolerance using *Agrobacterium tumefaciens* through an approximation of patents: a review	No. 2, pp. 145-155
Lorenzetti Juliano Bortoluzzi	Pre-sowing application of combinations of burndown and pre-emergent herbicides for *Conyza* spp. control in soybean	No. 1, pp. 121-128
Lugo-Pereira Wilfrido	Soybean yield components at different densities and planting seasons in Paraguay	No. 1, pp. 12-21
Macêdo Amanda Gomes	Population dynamics of the nematodes *Heterodera glycines* and *Pratylenchus brachyurus* in a succession crop of soybean and chickpea	No. 3, pp. 337-342
Magalhães Caroline da Cruz	Population dynamics of the nematodes *Heterodera glycines* and *Pratylenchus brachyurus* in a succession crop of soybean and chickpea	No. 3, pp. 337-342
Maqueira-López Lázaro Alberto	Rhizospheric rhizobia with potential as biofertilizers from Cuban rice cultivars	No. 1, pp. 78-89
Marín Viviana	Agro-morphological characterization of yellow passion fruit (*Passiflora edulis* f. *flavicarpa* Degener) reveals elite genotypes for a breeding program in Colombia	No. 2, pp. 156-176
Márquez-Berber Sergio Roberto	Farm management succession by heritage. A Central Mexico case study	No. 2, pp. 282-292
Martins Albert Lennon Lima	Glyphosate and atrazine inhibit growth of *Azospirillum brasilense*, *Bacillus subtilis*, *Bacillus thuringiensis*, *Chromobacterium subtsugae* and Saccharopolyspora *spinosa*	No. 1, pp. 113-120
Matos Trujillo Madyu	Co-inoculation with a product based on native microorganisms improves germination and seedling growth of *Phaseolus vulgaris* L.	No. 3, pp. 405-414
Matseh Irvan	Effect of selected bacteria from biogas sludge on the growth and nutrition of upland rice	No. 3, pp. 381-391
Mayer Tainah de Oliveira	Growth parameters and organoleptic characteristics of plantain (*Musa* AAB Simmonds cv. Farta Velhaco) at different planting densities	No. 3, pp. 322-329
Melo Mariana Nunes Viera de	Propagation of macadamia (*Macadamia integrifolia* Maiden & Betche) by cuttings	No. 1, pp. 129-135
Meloca Lucas Grogenski	Effect of a friction-reducing additive on the drip irrigation uniformity with sugarcane vinasse	No. 1, pp. 90-97
Mendes Débora Souza	Extension of umbu (*Spondias tuberosa* Arruda) postharvest life using a cassava starch-based coating	No. 2, pp. 293-299
Mendes Jones de Oliveira	Development of soybean plants using a substrate based on green coconut fiber	No. 1, pp. 47-58
Menini Luciano	Acaricidal activity and repellency of commercial essential oils on *Tetranychus urticae in vitro* and protected cultivation	No. 2, pp. 226-233
Menjivar-Flores Juan Carlos	Content and distribution of micronutrients in calendula (*Calendula officinalis*) grown in Valle del Cauca, Colombia	No. 1, pp. 59-67

Continued

Author name	Article title	Location
Mesquita Rosilene Oliveira	Development of soybean plants using a substrate based on green coconut fiber	No. 1, pp. 47-58
Miranda Neyton de Oliveira	Naturally colored cotton irrigated with saline water at different growth stages	No. 1, pp. 68-77
Monje Andrade Buenaventura	Morphological variations and abundance of populations of the leafhopper *Dalbulus maidis* (DeLong) (Hemiptera: Cicadellidae) from the corn-producing region of Huila, Colombia	No. 3, pp. 343-354
Montes-Rodríguez José Mauricio	Sampling methods of symphylids in pineapple (*Ananas comosus* L.) crops in Santander, Colombia	No. 2, pp. 208-215
Morales Romero Sebastián Camilo	Relationships between mineral nutrients in banana (*Musa* AAA Simmonds cv. Williams) bunches fertilized with nitrogen in two production cycles	No. 2, pp. 234-242
Mota Andygley Fernandes	Naturally colored cotton irrigated with saline water at different growth stages	No. 1, pp. 68-77
Muñoz-Araya Mariana	Effect of fertilization on yield and phenology of the halophyte *Sarcocornia neei* as an emerging crop	No. 2, pp. 300-305
Mustamu Novilda Elizabeth	Effect of selected bacteria from biogas sludge on the growth and nutrition of upland rice	No. 3, pp. 381-391
Nápoles María Caridad	Effect of a mix of oligogalacturonides on symbiotic nitrogen fixation in common bean	No. 1, pp. 30-36
Nápoles-García María Caridad	Rhizospheric rhizobia with potential as biofertilizers from Cuban rice cultivars	No. 1, pp. 78-89
Nascimento Elisamara Caldeira do	Effects of swine manure biochar on sorption equilibrium of cadmium and zinc in sandy soils	No. 1, pp. 37-46
Nascimento Elisamara Caldeira do	Growth parameters and organoleptic characteristics of plantain (*Musa* AAB Simmonds cv. Farta Velhaco) at different planting densities	No. 3, pp. 322-329
Nascimento Gabriel Pimenta do	Physicochemical characterization of cantaloupe fertilized with various potassium sources	No. 3, pp. 364-371
Nascimento Warley Marcos	Population dynamics of the nematodes *Heterodera glycines* and *Pratylenchus brachyurus* in a succession crop of soybean and chickpea	No. 3, pp. 337-342
Nasution Zulkifli	Effect of selected bacteria from biogas sludge on the growth and nutrition of upland rice	No. 3, pp. 381-391
Neto Miguel Ferreira	Naturally colored cotton irrigated with saline water at different growth stages	No. 1, pp. 68-77
Ocampo John	Agro-morphological characterization of yellow passion fruit (*Passiflora edulis* f. *flavicarpa* Degener) reveals elite genotypes for a breeding program in Colombia	No. 2, pp. 156-176
Oliveira Analya Roberta Fernandes	Development of soybean plants using a substrate based on green coconut fiber	No. 1, pp. 47-58
Oliveira Gelsimar Faria	Performance of corn hybrids for *in natura* consumption in the Pontal do Triângulo Mineiro region of Brazil	No. 3, pp. 330-336
Oliveira Leonardo Bernardes Taverny de	Weed control and selectivity of different pre-emergence active ingredients in a soybean crop	No. 3, pp. 392-404
Openda Zanabria Yarixa	Characterization of potassium solubilizing bacteria isolated from corn rhizoplane	No. 3, pp. 415-425
Ossa-Yepes Juan Felipe	Sampling methods of symphylids in pineapple (*Ananas comosus* L.) crops in Santander, Colombia	No. 2, pp. 208-215
Ounine Khadija	*In vitro* inhibition attempts and bio-elicitation of *Solanum lycopersicum* L. by chitin and chitosan against *Ralstonia solanacearum*, the causal agent of bacterial wilt	No. 3, pp. 355-363
Paraízo Eliene Almeida	Extension of umbu (*Spondias tuberosa* Arruda) postharvest life using a cassava starch-based coating	No. 2, pp. 293-299
Pazianotto Ricardo Antônio Almeida	Improvement of growth of common bean in phosphorus-deficient soils by phosphate-solubilizing and phytohormone-producing bacteria	No. 3, pp. 372-380
Perdoná Marcos José	Propagation of macadamia (*Macadamia integrifolia* Maiden & Betche) by cuttings	No. 1, pp. 129-135
Pereira Ednângelo Duarte	Extension of umbu (*Spondias tuberosa* Arruda) postharvest life using a cassava starch-based coating	No. 2, pp. 293-299
Pérez Hernández Yunel	Co-inoculation with a product based on native microorganisms improves germination and seedling growth of *Phaseolus vulgaris* L.	No. 3, pp. 405-414
Pérez Martínez Simón	Characterization of potassium solubilizing bacteria isolated from corn rhizoplane	No. 3, pp. 415-425
Pérez Zuñiga José Ives	Plant spacing assessment in cocoa (*Theobroma cacao* L.) agroforestry systems in the Colombian Pacific region	No. 3, pp. 426-437
Pérez-Pérez Reneé	Rhizospheric rhizobia with potential as biofertilizers from Cuban rice cultivars	No. 1, pp. 78-89
Pérez-Pérez Reneé	Characterization of potassium solubilizing bacteria isolated from corn rhizoplane	No. 3, pp. 415-425
Pessoa Herika Paula	Extension of umbu (*Spondias tuberosa* Arruda) postharvest life using a cassava starch-based coating	No. 2, pp. 293-299
Pinheiro Wagner Dutra	Commercial substrates and nutrient concentrations in the production of arugula microgreens	No. 1, pp. 5-11
Pinheiro Charles Lobo	Parental selection and descriptor evaluation in the identification of superior cowpea genotypes	No. 3, pp. 313-321

Continued

Author name	Article title	Location
Pinheiro Jadir Borges	Population dynamics of the nematodes *Heterodera glycines* and *Pratylenchus brachyurus* in a succession crop of soybean and chickpea	No. 3, pp. 337-342
Poletto Matheus	Evaluation of the drying process and toxic metal contents in yerba mate cultivated in southern Brazil	No. 3, pp. 453-458
Radünz André Luiz	Productive characterization of the blueberry cultivar Bluegem in Brazil	No. 1, pp. 22-29
Radünz Lauri Lourenço	Productive characterization of the blueberry cultivar Bluegem in Brazil	No. 1, pp. 22-29
Radünz Marjana	Productive characterization of the blueberry cultivar Bluegem in Brazil	No. 1, pp. 22-29
Ramírez Godoy Augusto	Morphological variations and abundance of populations of the leafhopper *Dalbulus maidis* (DeLong) (Hemiptera: Cicadellidae) from the corn-producing region of Huila, Colombia	No. 3, pp. 343-354
Ramírez Mario	Effect of a mix of oligogalacturonides on symbiotic nitrogen fixation in common bean	No. 1, pp. 30-36
Raulino Ludmila	Population dynamics of the nematodes *Heterodera glycines* and *Pratylenchus brachyurus* in a succession crop of soybean and chickpea	No. 3, pp. 337-342
Ribeiro Evandro Alves	Glyphosate and atrazine inhibit growth of *Azospirillum brasilense*, *Bacillus subtilis*, *Bacillus thuringiensis*, *Chromobacterium subtsugae* and Saccharopolyspora *spinosa*	No. 1, pp. 113-120
Rkhaila Amine	*In vitro* inhibition attempts and bio-elicitation of *Solanum lycopersicum* L. by chitin and chitosan against *Ralstonia solanacearum*, the causal agent of bacterial wilt	No. 3, pp. 355-363
Rojan-Herrera Osmany	Rhizospheric rhizobia with potential as biofertilizers from Cuban rice cultivars	No. 1, pp. 78-89
Rojas Arias Adriana Carolina	A basic scheme of soybean transformation for glyphosate tolerance using *Agrobacterium tumefaciens* through an approximation of patents: a review	No. 2, pp. 145-155
Rojas Molina Jairo	Plant spacing assessment in cocoa (*Theobroma cacao* L.) agroforestry systems in the Colombian Pacific region	No. 3, pp. 426-437
Romero-Padilla Arely	Farm management succession by heritage. A Central Mexico case study	No. 2, pp. 282-292
Saavedra Stephy	Optimization of lactic acid production by *Lactobacillus plantarum* strain Hui1 in a medium containing sugar cane molasses	No. 1, pp. 98-107
Saga Mouhatti	*In vitro* inhibition attempts and bio-elicitation of *Solanum lycopersicum* L. by chitin and chitosan against *Ralstonia solanacearum*, the causal agent of bacterial wilt	No. 3, pp. 355-363
Salcedo Benítez Juan Carlos	Characterization of potassium solubilizing bacteria isolated from corn rhizoplane	No. 3, pp. 415-425
Sanabria-Velázquez Andrés	Soybean yield components at different densities and planting seasons in Paraguay	No. 1, pp. 12-21
Sánchez Jaiver Danilo	Relationships between mineral nutrients in banana (*Musa* AAA Simmonds cv. Williams) bunches fertilized with nitrogen in two production cycles	No. 2, pp. 234-242
Sánchez Reinoso. Isda	Morphological variations and abundance of populations of the leafhopper *Dalbulus maidis* (DeLong) (Hemiptera: Cicadellidae) from the corn-producing region of Huila, Colombia	No. 3, pp. 343-354
Santos Edson Fagne dos	Extension of umbu (*Spondias tuberosa* Arruda) postharvest life using a cassava starch-based coating	No. 2, pp. 293-299
Santos Iasmim Leite dos	Weed control and selectivity of different pre-emergence active ingredients in a soybean crop	No. 3, pp. 392-404
Santos Layane Aparecida Mendes dos	Performance of corn hybrids for *in natura* consumption in the Pontal do Triângulo Mineiro region of Brazil	No. 3, pp. 330-336
Santoyo-Cortés Vinicio Horacio	Farm management succession by heritage. A Central Mexico case study	No. 2, pp. 282-292
Scariot Maurício Albertoni	Productive characterization of the blueberry cultivar Bluegem in Brazil	No. 1, pp. 22-29
Seleguini Alexsander	Physicochemical characterization of cantaloupe fertilized with various potassium sources	No. 3, pp. 364-371
Sembiring Mariani	Effect of selected bacteria from biogas sludge on the growth and nutrition of upland rice	No. 3, pp. 381-391
Silva Alessandro Guerra da	Agronomic and physiological characteristics of soybean cultivars and their seeds according to plant population	No. 2, pp. 177-186
Silva André Felipe Moreira	Pre-sowing application of combinations of burndown and pre-emergent herbicides for *Conyza* spp. control in soybean	No. 1, pp. 121-128
Silva Gisele Carneiro da	Agronomic and physiological characteristics of soybean cultivars and their seeds according to plant population	No. 2, pp. 177-186
Silva Johny de Souza	Development of soybean plants using a substrate based on green coconut fiber	No. 1, pp. 47-58
Silva Vanessa Neumann	Productive characterization of the blueberry cultivar Bluegem in Brazil	No. 1, pp. 22-29
Silva Vitor Hugo Domingos da	Propagation of macadamia (*Macadamia integrifolia* Maiden & Betche) by cuttings	No. 1, pp. 129-135

Continued

AGRONOMIA COLOMBIANA

VOLUME XXXIX, No. 3 SEPTEMBER-DECEMBER 2021 ISSN (print): 0120-9965 / ISSN (online): 2357-3732

PLANT BREEDING, GENETIC RESOURCES AND MOLECULAR BIOLOGY / FITOMEJORAMIENTO, RECURSOS GENÉTICOS Y BIOLOGÍA MOLECULAR

CROP PHYSIOLOGY / FISIOLOGÍA DE CULTIVOS

CROP PROTECTION / PROTECCIÓN DE CULTIVOS

POSTHARVEST PHYSIOLOGY AND TECHNOLOGY / FISIOLOGÍA Y TECNOLOGÍA DE POSCOSECHA

SOILS, FERTILIZATION, AND WATER MANAGEMENT / SUELOS, FERTILIZACIÓN Y MANEJO DE AGUAS

EXPERIMENTAL DESIGN AND BIOMETRY / BIOMETRÍA Y DISEÑO EXPERIMENTAL

AGROECOLOGY / AGROECOLOGÍA

ECONOMY AND RURAL DEVELOPMENT / ECONOMÍA Y DESARROLLO RURAL

FOOD SCIENCE AND TECHNOLOGY / CIENCIA Y TECNOLOGÍA DE ALIMENTOS

APPENDIX / ANEXOS

AGRONOMIA COLOMBIANA

P.O. Box 14490, Bogotá-Colombia

(571) 316 5355 / (571) 316 5000 ext. 10265

E-mail: agrocol_fabog@unal.edu.co

http://www.revistas.unal.edu.co/index.php/agrocol

http://www.scielo.org.co