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E-mail: agrocol_fabog@unal.edu.co

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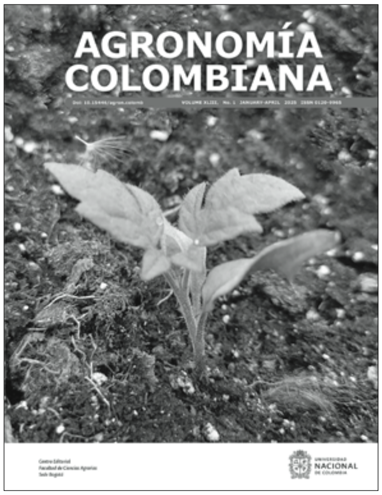

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Hormones mitigate salt stress in tomato (*Solanum lycopersicum* L.) plants during vegetative growth

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Family farming in Colombia: A pillar for food security and sustainable development

Agricultura familiar en Colombia: un pilar para la seguridad alimentaria y el desarrollo sostenible

Family farming is defined as a form of organization of agricultural production characterized by an indissoluble connection between the production unit and the family, either through ownership or leasing, which can use family labor and excludes the permanent existence of employees. Thus, this type of livelihood generation strategy is the one that allows the families to consume their own products, which can be partial or dominant. This organization of farms or agricultural production originates from an organic link between the production unit and a family; it uses family labor, and when it is necessary to pay for labor, this organization is complementary. Unlike conventional schemes, which are more capital-intensive, family farming tends to rely more on labor.

Thus, the provision of nutrients, vitamins, and minerals essential for the proper function of a population that is constantly growing is in the hands of food-producing families. Even though industrial agriculture continues to generate a large amount of agricultural goods and services with high social and environmental costs, the dominance of family farms, which constitute 87% at the national level, continues to supply the household with fresh, healthy, and nutritious food products. In terms of food production, approximately 70% of national production in Colombia comes from family farming.

The relationship between family farming and sustainable development is progressively recognized. The productive units under this scheme are recognized for their multi-functional attributes, which assume a crucial role not only for their contribution to food and nutritional security but also for the effective management of natural resources, the preservation of the environment, and the establishment of sustainable livelihoods. Therefore, the contribution to the sustainability of the rural community in ecological, economic and social terms is evident, and this type of agriculture decisively supports food security while combating the lack of economic and financial resources.

It is generally recognized that small family farms are more environmentally sustainable than their large, corporate counterparts. They are more diverse, employ traditional methods of farming, conserve various landscape features, are planned for the long term, and are usually intended to be passed on to the next generation. Family farming is recognized as a crucial element of climate resilient food systems, providing a method for food production, defending local and sustainable food systems. In rural regions of Colombia, around 12 million people derive their livelihoods from family farming, which offers a food production strategy usually framed by local knowledge and ecologically friendly techniques. Therefore, the family scheme has a vital role to play in both adapting to and reducing the effects of climate change through the implementation of sustainable land management techniques, diversification of production, and the use of local resources and knowledge.

Family farming in Colombia is frequently made up of Afro-descendant and peasant communities, with around 115 indigenous communities and some families that have immigrated to rural areas from the cities. The production models are based on conventional, organic, agroecological and agroforestry schemes. In most cases, conventional schemes predominate, such as potato, carrot, pea, bean and onion production. To a lesser extent, production is focused on organic and agroecological models, which restrict the use of chemical synthesis inputs, trying to take advantage of the internal resources of the production unit to produce manure, fertilizers and inputs for phytosanitary control; under these schemes, vegetables, aromatic and medicinal plants are obtained. On the other hand, agroforestry, by integrating agricultural production with the perennial woody species, i.e. with trees, shrubs and palms, generates crops such as plantain, banana, cassava, corn, citrus crops, cocoa and coffee, and products such as milk and meat.

Family farmers in Colombia have chosen to take differential attitudes that allow them to compete in the market, with innovative products, reduced dependence on raw materials, and collaborative means of production. An important example is the transformation of coffee, cocoa and dairy production, which has allowed Colombian consumers to enjoy high quality products, increased the demand for these foods, and allowed producer families to diversify their economy for the benefit of their life quality.

The importance of family farming is based on its potential to offer a diverse range of nutrient-rich foods, generate employment opportunities, enhance biodiversity and ecosystem services, and improve local food sovereignty. The family scheme relies heavily on agricultural activities; however, it also depends on multiple activities. Thus, family farming serves as a fundamental pillar of the local economy and culture in certain regions of Colombia, while in other locations it constitutes a component of a broad and varied economic scheme.

VANDREÉ JULIÁN PALACIOS BUCHELI, PhD in Agricultural Science
Assistant Professor
Universidad de Ciencias Aplicadas y Ambientales U.D.C.A., Bogotá (Colombia)
E-mail: vpalacios@udca.edu.co

Structural and phylogenetic characterization of a polyphenol oxidase gene in lulo (*Solanum quitoense* Lam.)

Caracterización estructural y filogenética de un gen de polifenol oxidasa en lulo (*Solanum quitoense* Lam.)

Mauricio Antonio Pulido Jiménez[1*], Silvia Gómez Daza[2], and Víctor Núñez[3]

ABSTRACT

The lulo or naranjilla (*Solanum quitoense* Lam.) is one of the most important Colombian native fruits. The sale and industrial processing of fresh fruit is severely limited by enzymatic browning. Until now, there was no knowledge about polyphenol oxidases (PPO) in lulo. The aim of this study was to understand some structural and phylogenetic aspects of the first lulo *ppo* gene that has been characterized. Using two pairs of degenerate primers, two fragments of lulo genomic DNA were isolated by PCR, sequenced and assembled into a partial sequence of 1417 bp (*SquPPO1*) lacking introns. Hybridization of a 920-bp probe generated from a potato *ppo* gene with a 12 kb region of *Bam*HI-*Pst*I, *Bam*HI-*Xba*I and *Xba*I-*Pst*I digested lulo DNA confirmed the presence of at least one *ppo* gene in this species. While two conserved sites (Tyr-1 and Tyr-2) have been identified in the copper-binding domains of other Solanaceae PPOs, no Tyr-2 site was found in lulo PPO because of a conservative substitution DxE in this region. Phylogenetic analysis placed the *SquPPO1* gene in the same cluster as the *SmePPO4*, *SmePPO5,* and *SmePPO6* eggplant (*Solanum melongena* L.) genes. Our results show that *SquPPO1* is phylogenetically closer to eggplant *ppo* genes than to those of potato, tobacco, and tomato and that it exhibits a variation that modifies the distribution of protein-conserved sites. These findings offer new insights into the molecular basis of enzymatic browning in lulo and may inform strategies to reduce postharvest losses.

Key words: Solanaceae, catechol oxidase, browning, phenolic compounds, protein domain, naranjilla.

RESUMEN

El lulo o naranjilla (*Solanum quitoense* Lam.) es una de las frutas nativas colombianas más importantes. La venta y el procesamiento industrial de la fruta fresca son severamente limitados por el pardeamiento enzimático. Hasta ahora, no había conocimiento sobre las polifenol-oxidasas (PPO) en lulo. El objetivo de este estudio fue conocer algunos aspectos estructurales y filogenéticos del primer gen *ppo* de lulo caracterizado. Utilizando dos pares de iniciadores degenerados, dos fragmentos de ADN genómico de lulo fueron aislados por PCR, secuenciados y ensamblados en una secuencia parcial de 1417 pb (*SquPPO1*) que carece de intrones. La hibridación de una sonda de 920 pb generada a partir de un gen *ppo* de papa con una región de 12 kb de ADN de lulo digerido con *Bam*HI-*Pst*I, *Bam*HI-*Xba*I y *Xba*I-*Pst*I confirmó la presencia de al menos un gen *ppo* en esta especie. Mientras que se han identificado dos sitios conservados (Tyr-1 y Tyr-2) en los dominios de unión a cobre de otras PPOs de solanáceas, no se encontró ningún sitio Tyr-2 en la PPO de lulo debido a una sustitución conservativa DxE en esta región. El análisis filogenético situó al gen *SquPPO1* en el mismo grupo que los genes *SmePPO4*, *SmePPO5* y *SmePPO6* de la berenjena (*Solanum melongena* L.). Nuestros resultados muestran que *SquPPO1* es filogenéticamente más cercano a los genes *ppo* de berenjena que a los de papa, tabaco y tomate, y que presenta una variación que modifica la distribución de los sitios conservados de la proteína. Estos hallazgos ofrecen nuevos conocimientos sobre las bases moleculares del pardeamiento enzimático en lulo y pueden servir de base a estrategias para reducir las pérdidas poscosecha.

Palabras clave: Solanaceae, catecol oxidasa, pardeamiento, compuestos fenólicos, dominio proteico, naranjilla.

Introduction

Polyphenol oxidases (PPOs) are a group of metalloenzymes that oxidize phenolic compounds in the presence of molecular oxygen. They are widely distributed in bacteria, fungi, plants, and animals (Hong *et al.*, 2024; Sarsenova *et al.*, 2023; Taranto *et al.*, 2017). The activity of PPOs on phenolic molecules results in the formation of quinones that spontaneously polymerize to form gray, brown or black pigments (Yoruk & Marshall, 2003) responsible for

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[1] Centro de Estudios en Biología Molecular, Gimnasio Campestre, Bogotá (Colombia).

[2] Departamento de Biología, Universidad Pedagógica Nacional, Bogotá (Colombia).

[3] Grupo de Mejoramiento Genético y Biotecnología, Corporación Colombiana de Investigación Agropecuaria (Agrosavia), Centro de investigación Tibaitatá, Bogotá (Colombia).

* Corresponding author: mauricio.pulidojimenez@gmail.com

the browning of plant tissues (Gerdemann *et al.*, 2002; Mayer, 2006). This biochemical phenomenon affects the appearance and the organoleptic properties of fruits and vegetables. These changes significantly reduce the marketing potential of fresh fruit or its use in industrial processing (Hasan *et al.*, 2019; Moon *et al.*, 2020).

PPOs are present in most cell types of higher plants, either free in the cytoplasm or bound to the cell membrane. They perform a variety of functions (Li, 2020; Liu *et al.*, 2023). There is evidence that the PPOs are involved in (1) resistance to biotic (Constabel *et al.*, 2000; Constabel & Barbehenn, 2008; Thipyapong *et al.*, 2007; Zhang & Sun, 2021) and abiotic (Mishra & Singh Sangwan, 2019) stresses, (2) the biosynthesis of pigments (Molitor *et al.*, 2015; Nakayama *et al.*, 2000; Ono *et al.*, 2006), (3) flavonoid oxidation in seed coats (Pourcel *et al.*, 2005) and indirectly in (4) the regulation of cell death (Araji *et al.*, 2014). In addition, PPOs have a key role in plant reproduction control mechanisms (Wei *et al.*, 2023). Their expression is induced by pathogenic and herbivore attacks or by tissue damage (Constabel & Ryan, 1998; Thipyapong *et al.*, 1995; Zhang & Sun, 2021).

PPOs belong to a group of copper-containing proteins that includes hemocyanins and tyrosinases (Lerch, 1983). These enzymes are characterized by the presence of two copper-binding domains (CuA and CuB) at the active site; each has three histidine residues involved in the coordination of a metal ion. Although both domains are very well conserved and define the PPO protein family, the CuA domain is more variable than the CuB domain (Klabunde *et al.*, 1998). Recently, a CRISPR/Cas9 construct with a single guide RNA (sgRNA) targeting the copper-binding domain was used to edit the *ppo1* and *ppo2* genes in wheat 'Fielder' grains. This reduced PPO activity by up to 86.7%. The same construct was transformed into the winter wheat cultivars 'Guardian' and 'Steamboat' and PPO activity was reduced by >90% (Wold-McGimsey *et al.*, 2023).

The *ppo* genes in plants are organized into multi-gene families. The number of *ppo* genes in some species can vary from 5 to 13, while others have only one. Seven *ppo* genes were isolated in tomatoes (Newman *et al.*, 1993), and nine were identified in potatoes (Chi *et al.*, 2014). Despite the progress in genomic analysis techniques, there are many Solanaceae species for which insufficient genetic information is available (Deanna *et al.*, 2022). From an economic point of view, the lulo or naranjilla is one of the most important of these species in Colombia. There are no reports of *ppo* genes in lulo (*Solanum quitoense* Lam.). Lulo is a perennial shrub of the Solanaceae family that grows in the Andean regions of Colombia, Peru, and Ecuador (Heiser, 1985). In these countries, it is considered valuable for agricultural and industrial use. However, in Colombia, where the species has a large market and is widely accepted, industrial processing and the possibilities of selling fresh fruit are very limited due to the damage caused by enzymatic browning during harvest and post-harvest handling.

This study presents the partial sequencing of a lulo *ppo* gene, a characterization of its structural features and an analysis of its evolutionary relationships with homologous genes from other Solanaceae species. Understanding the structural features of PPOs in lulo is crucial for developing strategies to mitigate enzymatic browning, a major limitation in fruit marketing and industrial processing.

Materials and methods

DNA extraction

Lulo (*Solanum quitoense* accession ILS-388) and potato (*Solanum tuberosum* cv. Millenia) seeds obtained from CORPOICA germplasm bank (Colombia) were grown in a greenhouse. Lulo and potato genomic DNAs have been extracted from young leaves (Doyle & Doyle, 1990).

Primers for PCR, PCR amplification, and DNA sequence analysis

The *ppo* gene sequences from species belonging to the Solanaceae, Convolvulaceae, Rosaceae, Fabaceae, Poaceae, Salicaceae and Zygophyllaceae families were retrieved from the GenBank database (https://www.ncbi.nlm.nih.gov/genbank/) (Tab. 1). A multiple sequence alignment was carried out using MUSCLE v5 (Edgar, 2021) to identify regions showing high homology. For the partial amplification of the *SquPPO1* gene by PCR, two pairs of degenerate primers were designed using Oligo v7 (Rychlik *et al.*, 1990, 2007). Primers SupPPO-F (forward) and SupPPO-L (reverse) were designed to amplify a first fragment (f1) corresponding to the half closest to the 5' end of the *SquPPO1* gene. Primers InfPPO-F (forward) and PPO-Rv (reverse) were designed to amplify a second fragment (f2) corresponding to the part closest to the 3' end of the gene (Tab. 2).

TABLE 1. Accession numbers of the genes used in the multiple sequence alignment.

Species	GenBank ID
Eggplant (*Solanum melongena* L.)	GQ246219.1, HQ731444.1, HQ731445.1, HQ731446.1, HQ731447.1, HM015902.1, JQ621950.1, JQ621948.1, JQ621952.1, JQ621949.1
Tomato (*Solanum lycopersicum* L.)	Z12833.1, Z12834.1, Z12835.1, Z12836.1, Z12837.1, Z12838.1
Tomato (*Solanum pennellii* Correll)	XM015229418.1, XM015229419.1, XM027919008.1
Potato (*Solanum tuberosum* L.)	M95196.1, M95197.1, U22921.1, U22922.1, U22923.1
Tobacco (*Nicotiana tabacum* L.)	KC540916.1, Y12501.1
Tobacco (*Nicotiana tomentosiformis* L.)	XM009591772.2
Tobacco (*Nicotiana attenuata* Steud.)	XM019396541.1
Tobacco (*Nicotiana sylvestris* Speg & S. Comes)	XM009769073.1
Tobacco (*Nicotiana benthamiana* Domin.)	HQ245096.1
Chili pepper (*Capsicum annuum* L.)	XM016718336.1
Goldenberry (*Physalis peruviana* L.)	MF682054.1
Sweet potato (*Ipomoea batatas* L. Lam.)	AB038994.1, AY822711.1
Apple (*Malus domestica* Borkh.)	D87670.1, L29450.1
Chinese plum (*Prunus salicina* Lindl.)	AY865623.2
Faba bean (*Vicia faba* L.)	Z11702.1
Wheat (*Triticum aestivum* L.)	HQ228148.1, HQ228150.1, HQ228152.1
Poplar (*Populus euphratica* Olivier)	HQ914443.1
Balsam poplar (*Populus balsamifera* L.)	AY665682.1
Creosote bush (*Larrea tridentata* (DC.) Coville)	AY370019.1

TABLE 2. Sequence of degenerate primers used for PCR amplification of *SquPPO1* gene fragments 1 and 2. Y= C+T; W= A+T; R= A+G; N= A+C+G+T.

Primer name	Sequence (5´- 3')
SupPPO-F	CTC CTA YWC CAY CYC CTG ATC T
SupPPO-L	CAG AAY TCN GAG TTC AAC CAA TC
InfPPO-F	CAA WTG RTN ACT AAK GCT CC
PPO-Rv	TTA ACA ATC CKC AAG CTT GAT

The PCR was carried out in 0.5 ml microcentrifuge tubes containing 60 mM of Tris-SO_4 (pH 8.9), 18 mM $(NH_4)_2SO_4$, 1.5 and 2.0 mM $MgSO_4$ (f1 and f2 respectively), 0.2 mM of each dNTP, 0.4 µM of each primer, 1.0 unit of Platinum Taq DNA Polymerase High Fidelity (Invitrogen, Carlsbad, CA, USA), and 75 ng of lulo and potato genomic DNA. Amplification was carried out in an MJ Research PTC-100 Thermal Cycler using 35-40 cycles of 1.0 min at 94°C, 45 s at 65.5°C (for f1) and 58°C (for f2) and 75 s at 72°C. One additional cycle was carried out at 72°C for 10 min for the complete extension of the PCR products. The negative amplification control was carried out using DNA from *E. coli*. Both PCR products were purified using the Wizard SV Gel and PCR Clean-Up System (Promega, USA) and sequenced without cloning in an ABIPrism 310 sequencer (Applied Biosystems, USA). Both nucleotide and protein BLAST analyses were carried out using tools from the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov).

Southern blot analysis

DNA was extracted from lulo and potato young leaves, previously described. Both genomic DNAs were double digested with combinations of restriction enzymes *BamHI*, *XbaI*, and *PstI*. Digestions were carried out for 8 h at 37°C. Enzymatically digested DNAs were electrophoresed on a 0.8% (w/v) agarose gel and subsequently transferred to a Hybond N$^+$ nylon membrane (# cat. RPN 225B, Amersham Life Science, USA) using saline-sodium citrate (SSC) buffer 20X (pH 7.0) as transfer solution. This procedure was carried out for 16 h. To fix the DNA, the membrane was treated with UV light (254 nm) for 3 min on each side. A 920-bp potato *ppo* gene fragment generated with primers InfPPO-F and PPO-Rv was used as a probe. The probe amplification conditions were the same as those used for the f2 fragment production. The probe was labeled using the Detector Random Primer DNA Biotinylation Kit (KPL, Gaithersburg, MD, USA). The nylon membrane with digested DNA was prehybridized for 1 h at 42°C. The prehybridization solution contained herring sperm DNA (100 µg ml^{-1}). Next, the biotinylated probe was denatured by heating at 95°C for 10 min and added to the hybridization solution. Hybridization was carried out at 42°C for 16 h. The membrane was washed four times (first wash: 2X SSC + 0.5% SDS, room temperature, 15 min; second and third washes: 1X SSC + 0.5% SDS, 55°C, 15 min; last wash: 0.1X SSC, room temperature, 15 min). Labeled probe detection was made with the Detector AP Chemiluminescent Blotting Kit following the manufacturer's instructions (KPL, Gaithersburg, MD, USA).

Phylogenetic analysis

The *ppo* gene sequences mentioned above were aligned using MUSCLE v5 (Edgar, 2021). The MEGA X software (https://www.megasoftware.net) (Kumar *et al.*, 2018) was used to carry out a Maximum Likelihood test with 500 iterations. Wheat *ppo* genes were used as an outgroup since they possess introns (Massa *et al.,* 2007).

Conservation analysis of the Tyr-2 site

The sequences of the PPO proteins of species belonging to the family Solanaceae were obtained from the National Center for Biotechnology Information Protein Database. (https://www.ncbi.nlm.nih.gov/protein/) (Tab. 3). The Tyr-2 site conservation was evaluated using InterPro v 97.0 (https://www.ebi.ac.uk/interpro/release_notes/97.0/) (Paysan-Lafosse *et al.*, 2023).

TABLE 3. Accession numbers of the PPO proteins used in the Tyr-2 site conservation analysis.

Species	Protein database ID
Potato (*Solanum tuberosum* L.)	AAA02877.1, AAA02879.1
Tomato (*Solanum lycopersicum* L.)	CAA78299.1, CAA78300.1
Tomato (*Solanum pennellii* Correll.)	XP015084904.1
Eggplant (*Solanum melongena* L.)	ADY18410.1, ADY18411.1, ADY18412.1
Chili pepper (C*apsicum annuum* L.)	XP016573822.1
Tobacco (*Nicotiana tabacum* L.)	CAA73103.1, AGK83468.1
Tobacco (*Nicotiana benthamiana* Domin.)	AEO00529.1
Goldenberry (*Physalis peruviana* L.)	ASW18458.1
Lulo (*Solanum quitoense* Lam.)	ACN78382.1

Results and discussion

SquPPO1 gene sequence

Two PCR products of 950-bp and 920-bp corresponding to the regions close to the 5' and 3' ends of the *SquPPO1* gene, respectively, were amplified with the primers designed for this study (Tab. 2). Each pair of primers amplified a fragment of the same length in the potato genomic DNA (Fig. 1).

The assembly of nucleotide sequences corresponding to f1 and f2 produced a 1417-bp partial sequence (GenBank ID: FJ573257), which has 425 adenines (30%), 318 cytosines (22.44%), 300 guanines (21.17%), and 374 thymines (26.39%). The guanine-cytosine content (%GC) of the *SquPPO1* gene is 43.61%, a value within the established range for Solanaceae genomes, which varies between 40% and 45% (Carels *et al.*, 1998; Rensink *et al.*, 2005). The %GC found is within the range observed for tomato and potato *ppo* genes, which vary between 41.83% and 43.64%; in eggplant *ppo* genes this value varies between 41.67% and 44.30%, while in tobacco it is slightly higher than in the above species (44.80%).

A comparison of *SquPPO1* with homologous genes from other species revealed similarity values greater than 89% with those of eggplant (*S. melongena* cv. Arka-Shirish); regarding some of the potato, tomato and tobacco *ppo* genes, the similarity ranged between 80% and 82.85%. This range included genes from wild species such as *Solanum pennellii*, a tomato endemic to the Andes, *Nicotiana attenuata*, *N. tomentosiformis*, *N. sylvestris,* and *N. benthamiana* (Tab. 4). Comparison with genes from other Asian eggplant cultivars (such as Azad Kranti, Anupam, Ravaiya and Raveena) showed similarity levels ranging from 74.34% to 76.79%. Although there are similarities with the *ppo* genes from phylogenetically distant families such as Salicaceae (Wang & Constabel, 2004) and Zygophyllaceae, these values are less than 60%. Like the previously characterized Solanaceae *ppo* genes, *SquPPO1* has no introns. Therefore, the presence of two introns in some members of the wheat *ppo* gene family (Chang *et al.*, 2007) explains the low percentage of similarity (less than 40%) between Solanaceae *ppo* genes and those of monocots such as wheat.

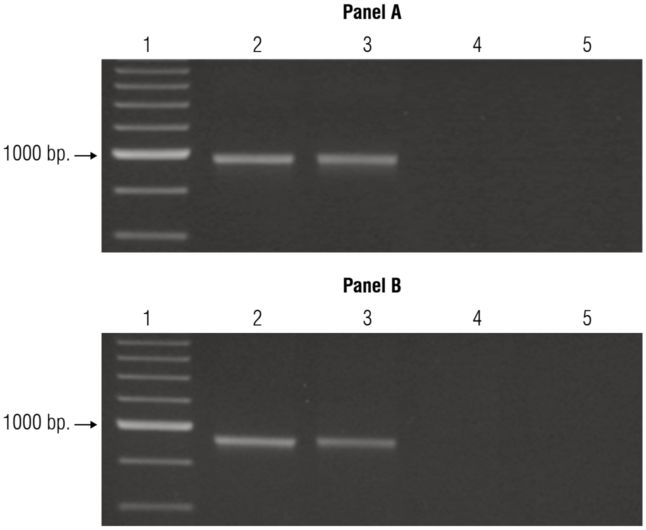

FIGURE 1. Electrophoretic analysis of fragments 1 and 2. Panel A: line 1: molecular size marker; line 2: fragment 1; line 3: positive amplification control (potato genomic DNA); line 4: negative amplification control (*E. coli* DNA); line 5: blank. Panel B: line 1: molecular size marker; line 2: fragment 2; line 3: positive amplification control (potato genomic DNA); line 4: negative amplification control (*E. coli* DNA); line 5: blank.

Southern hybridization analysis

In most plant species, *ppo* genes are part of multigene families, as in the case of tomato (Newman *et al.*, 1993; Thipyapong *et al.*, 1997), potato (Thygesen *et al.*, 1995), faba bean (Cary *et al.*, 1992), wheat (Jukanti *et al.*, 2004) and others. Southern blot revealed hybridization signals of approximately 12 kb corresponding to lulo and potato genomic DNA (Fig. 2). This result is consistent with previous findings in tobacco (Goldman *et al.*, 1998), where a hybridization signal of 11.5 kb was obtained with *BamHI*-digested DNA, and with that of tomato, which showed that the *PPO-E* and *PPO-F* genes are located together in a 12.4

kb region, while the *PPO-A*, *PPO-B* and *PPO-D* genes are located in a second 12.4 kb region (Newman *et al.*, 1993).

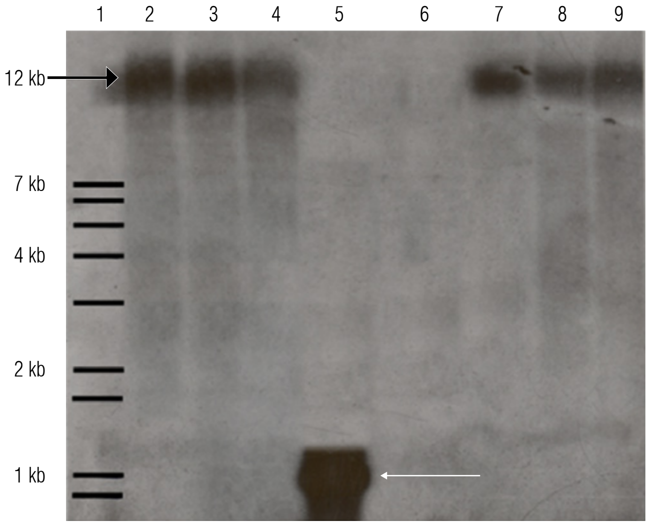

FIGURE 2. Southern analysis: Black arrow shows the Ca.12 kb hybridization signals. Line 1: molecular size marker; lines 2-4: *Bam*HI-*Pst*I, *Bam*HI-*Xba*I and *Xba*I-*Pst*I digested lulo DNA; line 5: 920-bp probe; line 6: blank; lines 7-9: *Bam*HI-*Pst*I, *Bam*HI-*Xba*I and *Xba*I-*Pst*I digested potato DNA.

In monocots like wheat, something similar has been observed: the six *PPO* genes known in this species are organized into two independent clusters, with each one of them having three of these genes (Jukanti *et al.*, 2004). The existence of highly conserved gene clusters in Solanaceae species implies that the location of such clusters on the chromosomes and the positions of some genes relative to others in the same group are also conserved. Using molecular markers, a high degree of collinearity is found between potato and tomato (Bonierbale *et al.*, 1988; Tanksley *et al.*, 1992). In the case of pepper and tomato, 18 common homologous linkage blocks are identified using the same approach (Livingstone *et al.*, 1999). Similarly, tomato markers used in eggplant reveal syntenic regions between these species (Doganlar *et al.*, 2002). The evidence we present, together with the knowledge of the gene family organization in related species allows us to suggest that *SquPPO1* may be accompanied by at least one other *PPO* gene.

Sequence and similarity of lulo PPO to homologous proteins

PPO deduced from the 1417 bp sequence found in lulo has 472 amino acids (GeneBank ID: ACN78382). The BLASTP

TABLE 4. Similarity of the *SquPPO1* gene to related genes from other Solanaceae species.

Species	Gene	Accession number	E-value	Identity (%)
S. melongena cv. Arka Shirish	SmePPO-5	HQ731446.1	0.0	90.28
S. melongena cv. Arka Shirish	SmePPO-4	HQ731445.1	0.0	89.91
S. melongena cv. Arka Shirish	SmePPO-6	HQ731447.1	0.0	89.15
S. tuberosum	ppo-A	M95196.1	0.0	82.85
S. tuberosum	ppo-B	M95197.1	0.0	82.65
S. tuberosum cv. DM 1-3 516 R44	ppo	XM006355177.2	0.0	82.34
C. annuum cv. Zunla-1	ppo-A	XM016718336.1	0.0	82.32
S. lycopersicum cv. Micro-Tom	ppo	AK247410.1	0.0	81.79
S. lycopersicum	ppo-E	Z12837.1	0.0	81.79
S. pennellii	ppo-E	XM015229418.1	0.0	81.72
S. pennellii	ppo	HG975447.1	0.0	81.72
S. pennellii	ppo-F	XM015229419.1	0.0	81.34
S. lycopersicum cv. Tiny Tim LA154	ppo	S40548.1	0.0	81.23
N. tabacum cv. TN90	ppo-E	XM016600648.1	0.0	80.94
N. attenuata	ppo-E	XM019396541.1	0.0	80.87
S. lycopersicum cv. Micro-Tom	ppo-F	NM001331130.1	0.0	80.81
N. tomentosiformis	ppo-E	XM009591772.2	0.0	80.80
N. sylvestris	ppo-E	XM009769073.1	0.0	80.31
N. tabacum cv. Petit Havana SR1	ppo	Y12501.1	0.0	80.31
N. benthamiana	Nbppo-1	HQ245096.1	0.0	80.24
N. tabacum	ppo	KC540916.1	0.0	80.10
S. lycopersicum	ppo-F	Z12838.1	0.0	80.10

analysis showed results consistent with the nucleotide sequence analysis mentioned above (Tab. 5).

Location of copper-binding sites and central domain of tyrosinases in lulo PPO

Lulo PPO has the central domain characteristic of tyrosinases, a protein family that includes all plant PPOs. The region of the lulo PPO where the common central domain would be located extends from P70 to I276. The studies of the three-dimensional structure of sweet potato PPO showed that each one of the two copper-binding sites is coordinated by three histidine residues (Klabunde *et al.*, 1998). These six histidine residues are inside the conserved central domain of the protein. The amino acids involved in the coordination with copper ions called $H_{A1,}$ $H_{A2,}$ H_{A3} (for Copper A) and $H_{B1,}$ $H_{B2,}$ H_{B3} (for Copper B) follow the general rule of $H_{A1} - x(n) - H_{A2} - x(8) - H_{A3}$ and $H_{B1} - x(3) - H_{B2} - x(n) - H_{B3,}$ respectively, where *n* is a variable amino acid number (García-Borrón & Solano, 2002).The histidines that would interact with the two copper ions in lulo PPO fit this distribution. The copper atom in binding site A would be coordinated with H80, H98 and H107; the copper atom in binding site B would be coordinated with H229, H233 and H263. The six histidines mentioned above are well-conserved in all plant PPOs (Fig. 3). The PPO regions involved in the interaction with copper atoms show a high degree of conservation (Halaouli *et al.*, 2006). The alignment of the PPOs (including that of lulo) allowed these regions to be identified by sequence similarity. The Copper A binding site identified in the lulo PPO would be located between H98 and W134, while the Copper B binding site would be located between H229 and D282.

Variation of an amino acid in the hydrophobic shell containing the active site of the lulo PPO

The active site of tyrosinases has been described as a hydrophilic sphere demarcated by four alpha helixes in which the six histidine residues are included (García-Borrón & Solano, 2002). This hydrophilic sphere would be located inside a hydrophobic shell formed by aromatic amino acids

TABLE 5. Similarity of lulo PPO to other Solanaceae PPOs.

Protein	E-value	% identity	Accession
Chloroplast polyphenol oxidase (*S. melongena*)	0.0	86.64%	QLG20182.1
Chloroplast polyphenol oxidase (*S. melongena*)	0.0	85.84%	ADY18411.1
Chloroplast polyphenol oxidase (*S. melongena*)	0.0	85.41%	ADY18412.1
Polyphenol oxidase F, chloroplastic (*C. chinense*)	0.0	84.36%	PHU29006.1
Polyphenol oxidase F, chloroplastic (*C. baccatum*)	0.0	84.14%	PHT58552.1
Catechol oxidase B, chloroplastic (*C. annuum*)	0.0	84.14%	XP016573822.2
Polyphenol oxidase E, chloroplastic (*S. lycopersicum*)	0.0	83.26%	NP001318057.1
Polyphenol oxidase E, chloroplastic (*S. pennellii*)	0.0	83.05%	XP015084904.1
Catechol oxidase B, chloroplastic (*S. commersonii*)	0.0	82.84%	KAG5592182.1
Catechol oxidase B, chloroplastic (*S. stenotomum*)	0.0	82.63%	XP049377390.1
Catechol oxidase B, chloroplastic (*S. verrucosum*)	0.0	82.63%	XP049361834.1
Polyphenol oxidase B precursor (*S. tuberosum*)	0.0	82.42%	Q06355.1
Propolyphenol oxidase (*S. tuberosum*)	0.0	82.20%	AAA02877.1
Polyphenoloxidase (*S. lycopersicum*)	0.0	82.20%	AAB22610.1
Chloroplast polyphenol oxidase (*S. melongena*)	0.0	81.97%	ADY18410.1
Polyphenol oxidase F, chloroplastic (*S. lycopersicum*)	0.0	81.82%	NP001318059.1
Polyphenol oxidase E, chloroplastic (*N. tabacum*)	0.0	81.72%	XP016456134.1
Polyphenol oxidase (*N. tabacum*)	0.0	81.30%	CAA73103.1
Polyphenol oxidase E, chloroplastic (*N. sylvestris*)	0.0	81.30%	XP009767375.1
Polyphenol oxidase E (*N. tomentosiformis*)	0.0	81.30%	XP009590067.1
Polyphenol oxidase F, chloroplastic (*S. pennellii*)	0.0	81.18%	XP015084905.1
Polyphenol oxidase E, chloroplastic (*N. bombycine*)	0.0	81.09%	XP019252086.1
Polyphenol oxidase (*Nicotiana benthamiana*)	0.0	80.88%	AEO00529.1

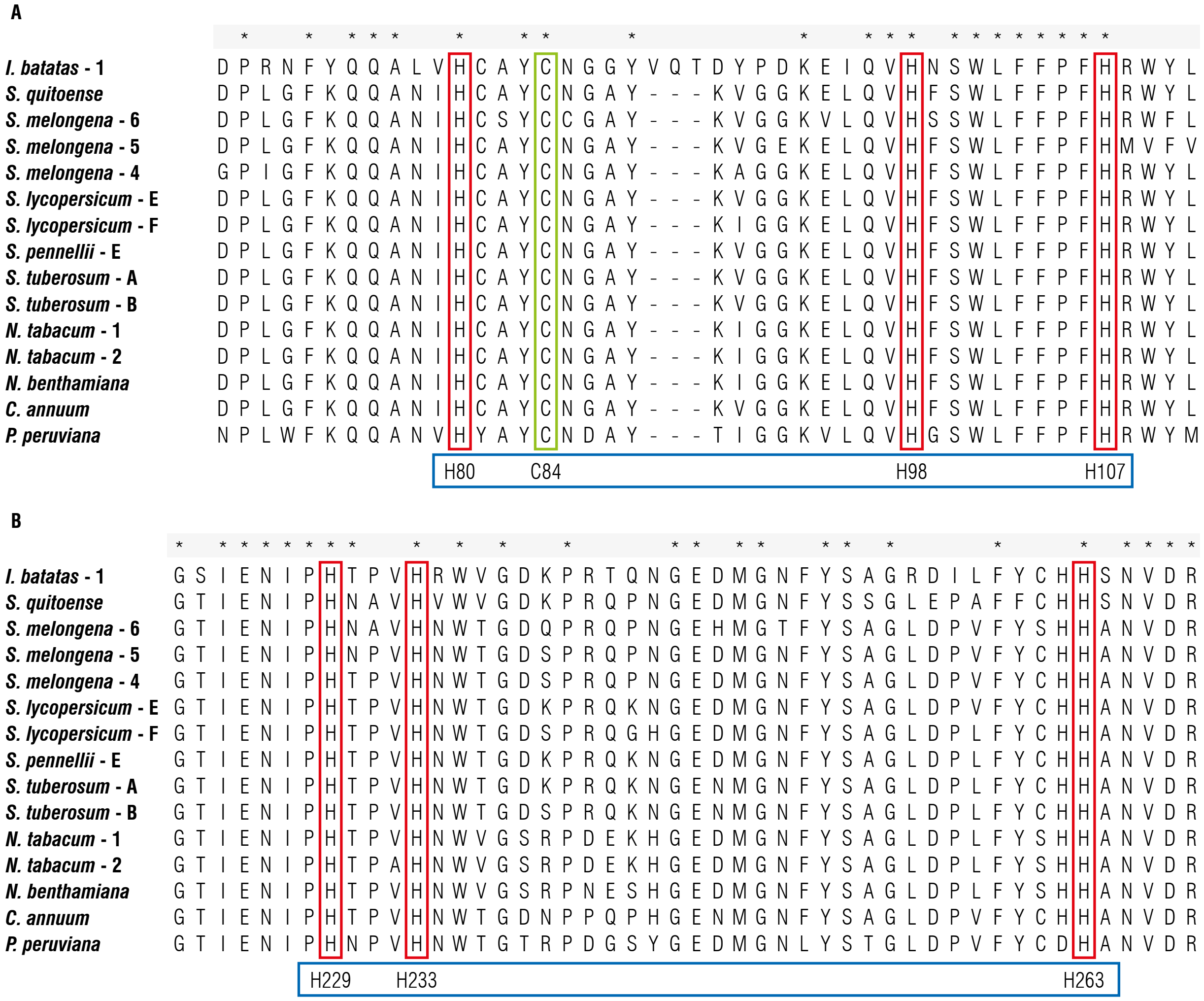

FIGURE 3. Conservation of the histidines involved in the coordination with copper atoms A and B in plant PPOs. Sweet potato PPO was included because its crystal structure has been well studied. A) Histidines coordinated with the Copper A site, B) Histidines coordinated with the Copper B site. Asterisks denote conserved amino acids.

and highly conserved residues. The structure proposed is: $\Phi_{(HA1-7)}$, $R_{(HA3+1)}$, $\Phi_{(HA3+3)}$, $\Phi_{(HA3+7)}$, $E_{(HA3+8)}$, $D_{(HB3-7)}$, $D_{(HB3+4)}$, $\Phi_{(HB3+7)}$, and $W_{(HB3+10)}$ (where "Φ" represents an aromatic residue). The lulo PPO retains the following residues: $\Phi_{(HA1-7)}$ (Φ corresponds to F), $R_{(HA3+1)}$, $\Phi_{(HA3+3)}$ (Φ corresponds to Y), $\Phi_{(HA3+7)}$ (Φ corresponds to Y), $E_{(HA3+8)}$, $D_{(HB3+4)}$, $\Phi_{(HB3+7)}$ (Φ corresponds to W), and $W_{(HB3+10)}$. In the lulo PPO, the $D_{(HB3-7)}$ that is conserved in most of the Solanaceae PPOs, is replaced by E (Fig. 4). This sequence change is conservative because oxidized copper preferentially coordinates with oxygen in aspartic and glutamic acids or with the imidazole nitrogen group in histidines (Festa & Thiele, 2011). Another characteristic trait of fungal tyrosinases and plant PPOs is the presence of a highly conserved cysteine residue, which is involved in a thioether covalent bond with the second conserved histidine at the copper A site. In the lulo PPO, this cysteine is at position 84 (green box in Fig. 3A). As in the previously studied PPOs, the regions of the lulo PPO where the two copper atoms would be coupled are asymmetric concerning the amino acid composition (Fig. 4).

The lulo PPO does not have a Tyr-2 site

One of the characteristic features of PPOs is the presence of three conserved regions: a chloroplast transit peptide (Ctp) at the N-terminal end, the copper-binding domain (dicopper center), and the C-terminal end (Tran *et al.*, 2012; Zhang, 2023). Two conserved sites were identified in the copper-binding domain of all studied Solanaceae PPOs. The conserved site called Tyrosinase 1 (Tyr-1) is 18 residues in size and contains two of the histidines (the second and third) that bind CuA, whereas the conserved site called Tyrosinase 2 (Tyr-2) is 12 residues in size and contains one of the histidines (the third) that binds CuB (Fig. 5).

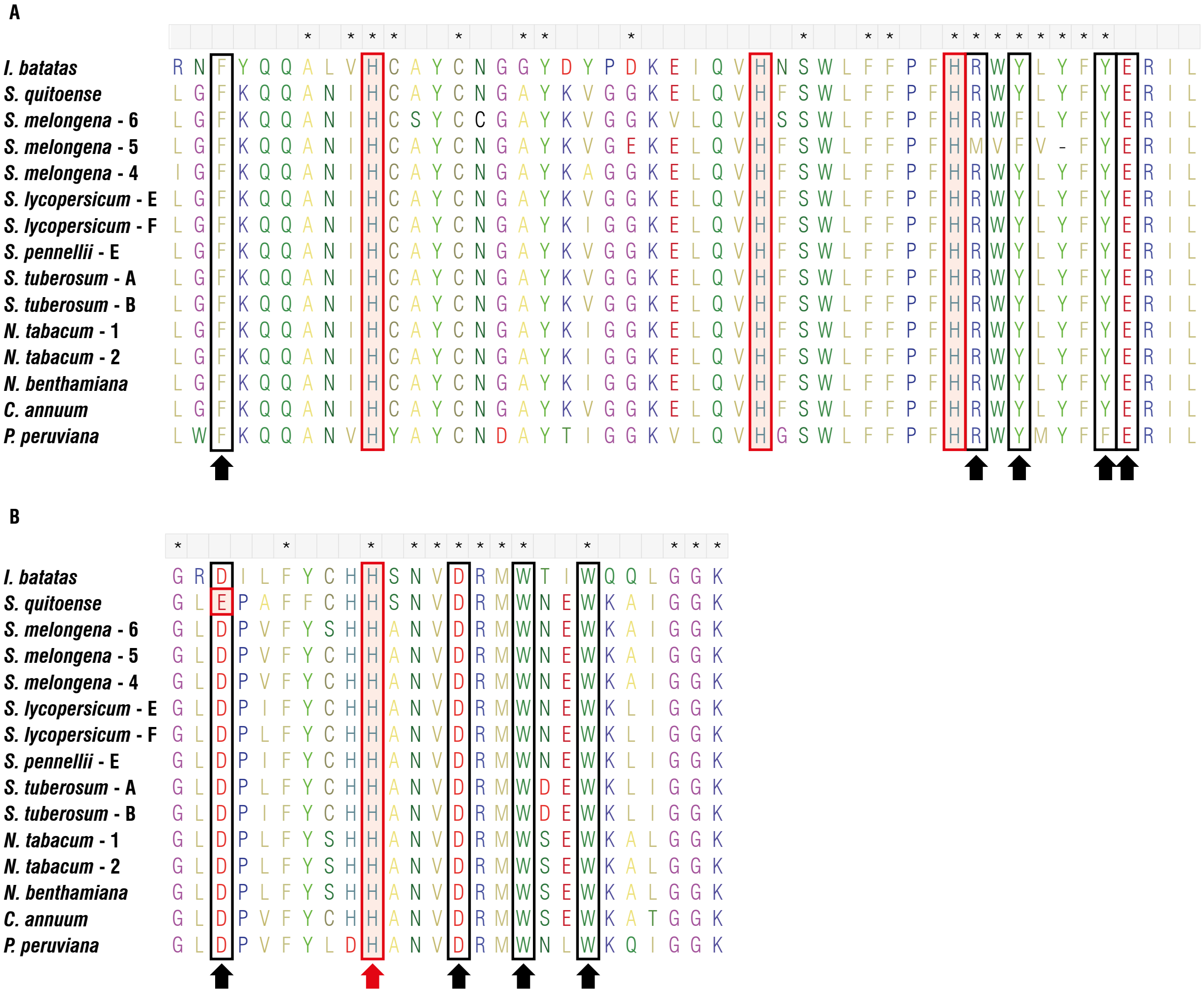

FIGURE 4. Conserved amino acids in the hydrophobic shell containing the active site of plant PPOs (black arrows). A) Copper site A, B) Region near the third histidine (red arrow) of the copper site B. Glutamic acid (E) is located at position HB3-7 of the lulo PPO instead of aspartic acid (D) (red box). Asterisks denote conserved amino acids.

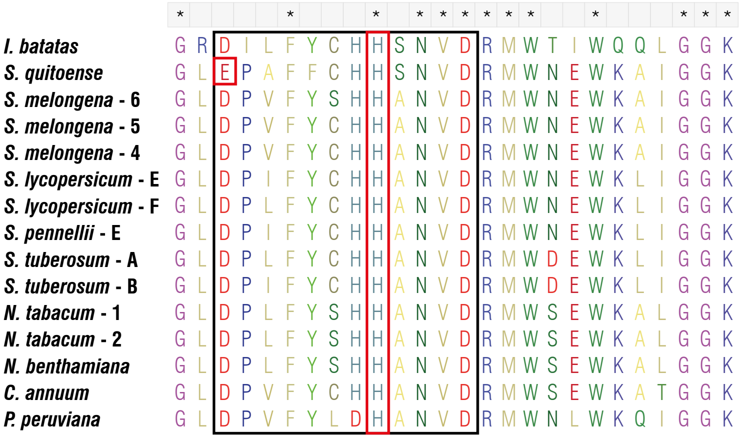

FIGURE 5. The Tyr-2 site (black box) contains the third histidine (pink box) of the CuB-binding site. The red box indicates the DxE substitution that is critical for the conservation of the Tyr-2 site. The sequence of *I. batatas* is included as reference since it is the best characterized plant PPO from a structural point of view. Asterisks denote conserved amino acids.

The absence of the Tyr-2 site in the PPO of lulo (Fig. 6) is due to a conservative DxE substitution, altering the conserved motif found in other Solanaceae PPOs (García-Borrón & Solano, 2002). In most plant PPOs, the conserved Tyr-2 site presents the following sequence pattern: D – P – x – F – [LIVMFYW] – x(2) – H – x(3) – D, as shown in the alignment (Fig. 5). The first residue of the conserved site is D, but in the lulo PPO, D is conservatively substituted by E (red box). In all copper oxidases studied, there are water channels that connect the copper-binding center of the enzyme to the external environment. A conservative substitution D500E in the copper-binding center of a bacterial laccase suggests that acidic groups in the water channel play a key role in the deprotonation events that occur at the copper-binding center (Nasoohi *et al.*, 2013).

On the other hand, the conservative substitution D208E at the copper B-binding site in *S. glaucescens* tyrosinase increases the stability of the oxy-enzyme with respect to the met and deoxy forms, causing a slight perturbation in the kinetic and spectroscopic properties of the enzyme, but without substantially affecting the catalytic activity of the enzyme (Jackman *et al.*, 1992). With the information currently available, it is difficult to precisely establish what effect this amino acid change may have on the function or structure of the lulo PPO. Since crystallography on its own cannot elucidate the enzymatic function, the evaluation of the catalytic activity of this PPO on various *o*-diphenols may offer valuable additional insights.

Phylogenetic relationships between *SquPPO1* and *SmePPO4, SmePPO5* and *SmePPO6*

In our study, a phylogenetic relationship analysis between several Solanaceae *ppo* genes was carried out (Fig. 7). Surprisingly, the phylogenetic tree revealed that *SquPPO1* is more closely related to the eggplant *PPO* genes (*SmePPO4, SmePPO5*, and *SmePPO6*) than to those of potato, tobacco and tomato. This is interesting because lulo, potato, tobacco, and tomato are native to the Andean region of northern South America, while eggplant has as a center of origin the northeastern region of India. Wheat *ppo* genes were used as an outgroup since they possess introns (Massa *et al.,* 2007).

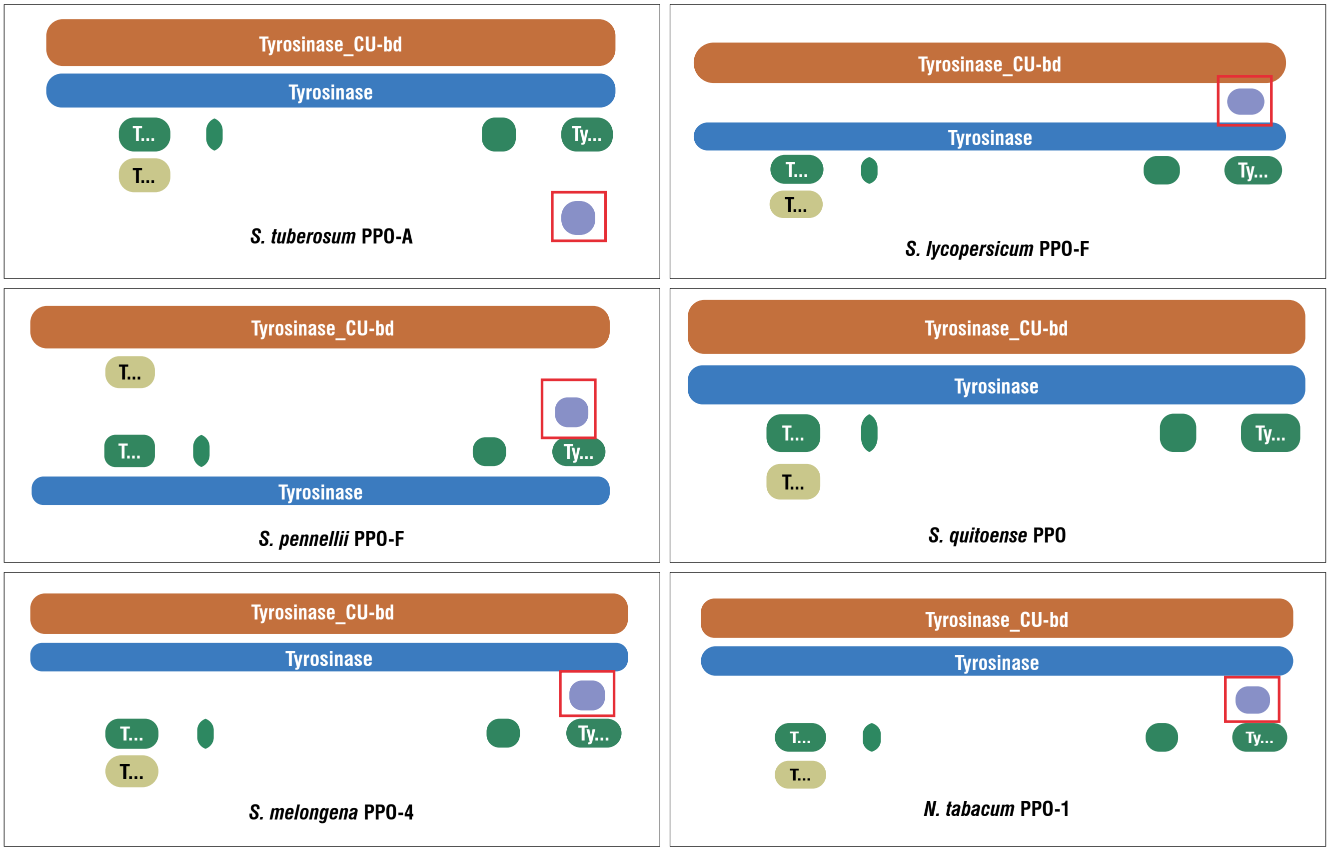

FIGURE 6. The Tyr-2 site (light purple oval inside the red square) is conserved in the Solanaceae PPOs, except in that of lulo (only some of the PPOs are shown). Tyrosinase Cu-bd: copper-binding domain of tyrosinase family; blue oval: common central domain of tyrosinase family. Highly conserved sequence motifs are indicated by the other ovals.

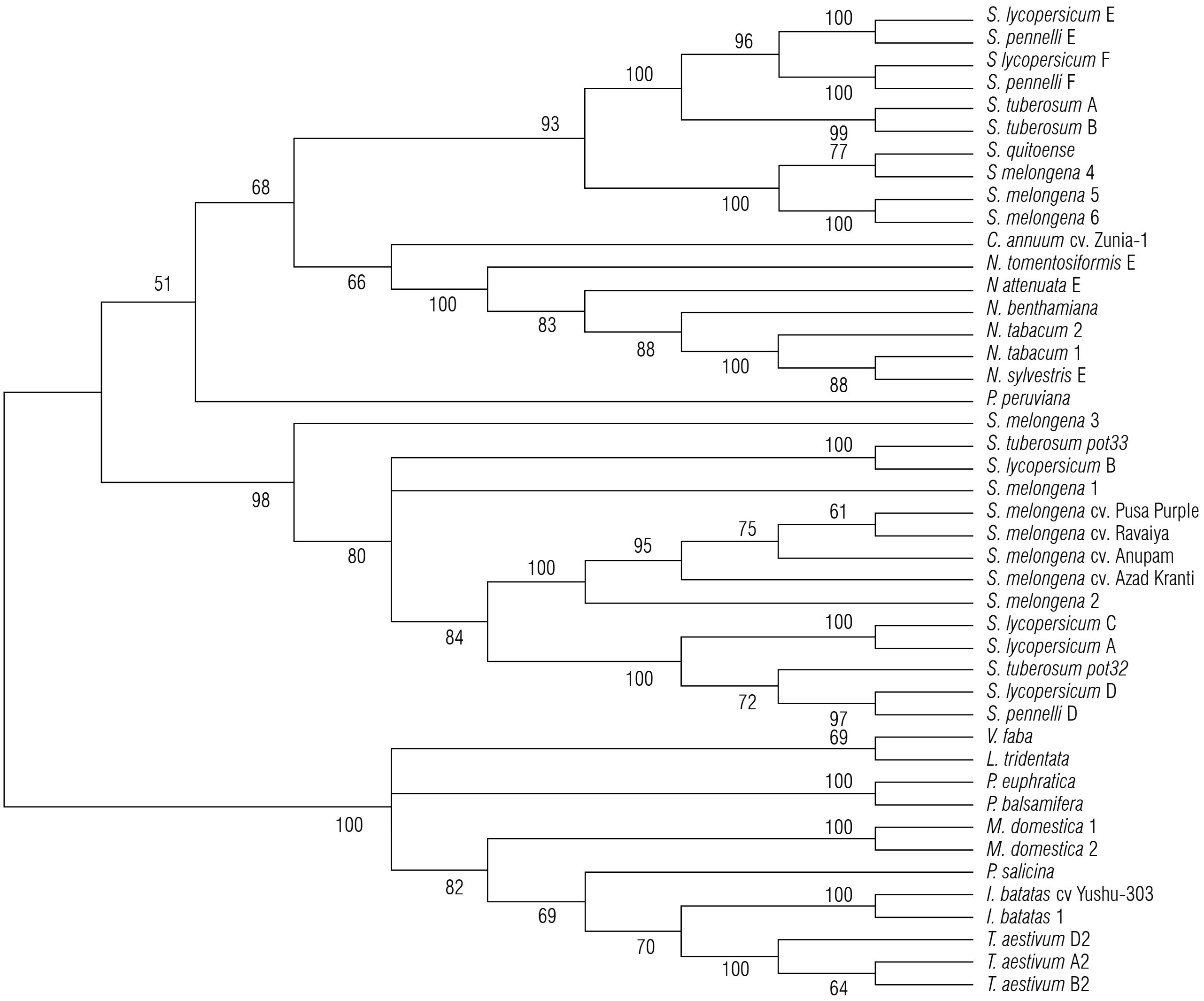

FIGURE 7. Phylogenetic relationships among *ppo* genes are shown as a strict consensus tree. Wheat (*Triticum aestivum*) *ppo* genes were used as an outgroup. Numbers above branches represent bootstrap values.

Depending on the similarity levels between members of the eggplant *ppo* multigene family, the six genes were assigned to two structurally distinct classes: class A (*SmePPO1, SmePPO2,* and *SmePPO3*) and class B (*SmePPO4, SmePPO5,* and *SmePPO6*). This distribution agrees with that observed in our phylogenetic tree. Since the eggplant *SmePPO4* gene is expressed in young and mature leaves, flowers (pre- and post-anthesis), and fruits (Shetty *et al.,* 2011), it is possible to suppose that *SquPPO1* could show a similar pattern of expression and function given the close phylogenetic relationship between the two (Fig. 7).

The close relationship between *SquPPO1* and *SmePPO4* can be interpreted as the consequence of selective pressures of a similar nature acting on them, since they are involved in functions related to the adaptive response of both species to their respective environments. These pressures induce genetic convergence events that maintain the ability of these individuals to oxidize phenolic molecules in certain types of tissues. Similar evolutionary phenomena have been studied in several groups of higher plants. In species of the Iochrominae (Solanaceae) clade, it was observed that flower color transitions are determined by the convergent loss of expression of three genes (*F3'5'h, Dfr,* and *Ans*) of the anthocyanin pathway (Larter *et al.*, 2018). Another well-studied case is that of the furanocoumarin pathway in Moraceae, where the emergence of a new cytochrome P450 is a consequence of convergent evolution (Villard *et al.*, 2021). Although the reactions catalyzed by PPOs are well known, data on the function of these enzymes in cellular or tissue-level processes are still under discussion. The diversity of tissues and conditions in which PPOs are expressed suggests that these enzymes may play a key role in various stress response-related processes.

Conclusions

In this study, the sequence of the lulo *SquPPO1* gene was partially determined. As is the case with other Solanaceae *PPO* genes, the *SquPPO1* gene lacks introns. The results of Southern hybridization suggest that *SquPPO1* may be accompanied by at least one other *PPO* gene, given the size of the genomic region in which it was located. Further studies are required to determine the precise size of the *PPO* gene family in this species. On the other hand, the utilization of molecular markers previously developed in related species (Bonierbale *et al.*, 1988; Doganlar *et al.*, 2002; Tanksley *et al.*, 1992) may be useful in elucidating the degree of collinearity that lulo *PPO* genes share with other Solanaceae species. A comparison of the PPO deduced from the *SquPPO1* gene with PPOs from other species of the Solanaceae family allowed us to determine a DxE substitution (position 256). This substitution involves the disappearance of the Tyr-2 site in the copper-binding domain of the lulo PPO. It remains to be determined whether this amino acid change would confer a structural advantage with evolutionary implications related to the conservation of this gene in the species. Finally, a close phylogenetic relationship was found between *SquPPO1* and the eggplant genes *SmePPO4*, *SmePPO5* and *SmePPO6*. This evolutionary proximity can be interpreted as the result of a genetic convergence event. It is tempting to assume that *SquPPO1* could exhibit a pattern of expression and function similar to that of the eggplant *SmePPO4* gene. The results of our study provide a starting point for subsequent investigations into the effect of DxE substitution on the catalytic activity of lulo PPO. In addition, the use of tools to generate point mutations in the active site of PPO may offer the possibility of reducing its enzymatic activity, thus minimizing the occurrence of enzymatic browning in fruits.

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Conflict of interest statement

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

Author´s contributions

MAPJ and SGD: methodology design, research, formal analysis and data curation. MAPJ: writing the initial draft. VNZ: research design, funding acquisition and project management. All authors reviewed the final version of the manuscript.

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Evaluation of yield and fruit quality in five genotypes of Castilla blackberry (*Rubus glaucus* Benth.)

Evaluación del rendimiento y calidad del fruto de cinco genotipos de mora de Castilla (*Rubus glaucus* Benth.)

Erika Sánchez-Betancourt[1*], Franklin Giovanny Mayorga Cubillos[2], and Francy Liliana García-Arias[1]

ABSTRACT

In Colombia, blackberry (*Rubus* spp.) is one of the crops with the most significant geographic coverage, and the ecotype "Castilla" (*Rubus glaucus*) is the most extensively commercialized. Despite the importance of the crop, there are no registered varieties or hybrids specifically adapted to the diverse growing conditions of the country's various production areas. The purpose of this study was to evaluate and select advanced genotypes of Castilla blackberry based on yield attributes and physicochemical quality of the fruits. Data from five genotypes and one regional check were recorded in the municipality of Silvania, Cundinamarca, during 2022 and 2023. The yield traits, including the number of fruits per kg and the weight of the fruits harvested, were evaluated, as well as the physicochemical variables: fruit diameter, fruit weight, firmness, acidity, total soluble solids, pH, juice and pulp weights, and maturity index. The data were statistically processed using a generalized linear model, principal component and cluster analysis, and Ward's minimum variance clustering method. A selection index based on the traits of production, total soluble solids, fruit weight, and firmness, relevant to the crop, was used. Significant differences were observed between genotypes for the yield traits, total soluble solids, pH, acidity, and maturity index. The results suggested that genotypes G1, G4, and G3 were outstanding in terms of yield and fruit quality. However, genotype G1 led the index selection, outperforming the other genotypes under evaluation.

Key words: physicochemical properties, Brix, selection index.

RESUMEN

En Colombia, la mora (*Rubus* spp.) es uno de los cultivos con mayor cobertura geográfica, siendo el ecotipo "Castilla" (*Rubus glaucus*) el más comercializado. Pese a la importancia del cultivo, no hay registro de variedades o híbridos específicamente adaptados a las diversas condiciones de crecimiento de las diferentes zonas productoras del país. El propósito de este trabajo fue evaluar y seleccionar genotipos avanzados de mora de Castilla por atributos de rendimiento y calidad fisicoquímica de fruto. Se registraron datos de cinco genotipos y un testigo regional en Silvania, Cundinamarca, durante los años 2022 y 2023. Se evaluaron los atributos de rendimiento: número de frutos por kg y peso de los frutos cosechados, así como las variables fisicoquímicas: diámetro del fruto, peso de fruto, firmeza, acidez, contenido de sólidos solubles totales, pH, pesos de jugo y pulpa, e índice de madurez. Los datos fueron estadísticamente procesados mediante un modelo lineal generalizado, análisis de componentes principales y conglomerados, y el método de agrupamiento de varianza mínima de Ward. Se usó un índice de selección basado en las características: producción, sólidos solubles totales, peso de fruto y firmeza, relevantes para el cultivo. Se observaron diferencias significativas entre los genotipos para las características de rendimiento, sólidos solubles totales, pH, acidez e índice de madurez. Los resultados sugirieron que los genotipos G1, G4 y G3 fueron los sobresalientes en rendimiento y calidad de fruto. Sin embargo, el genotipo G1 lideró la selección por el índice, superando a los otros genotipos en evaluación.

Palabras clave: propiedades fisicoquímicas, Brix, índice de selección.

Introduction

The Andean blackberry, *Rubus glaucus* Benth, belongs to the Rosaceae family, is native to the Andes, and grows in the high American tropics (Vaillant, 2020). The fruits are edible, and there is an excellent variety of hybrids within the subgenus *Rubus* as well as hybrids between the subgenera *Rubus* and *Idaeobatus* (Ballington, 2016). The leading exporting countries of blackberries are Spain, Portugal, Morocco in Europe, and Mexico in America. In Colombia, *R. glaucus* Benth, commonly known as Castilla blackberry, is the most cultivated due to its optimal development and lower maintenance requirements (Ramírez, 2023).

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[1] Corporación Colombiana de Investigación Agropecuaria (Agrosavia), Centro de investigación Tibaitatá, Mosquera, Cundinamarca (Colombia).
[2] Federación Nacional de Cultivadores de Cereales, Leguminosas y Soya (FENALCE), Siberia, Cundinamarca (Colombia).
* Corresponding author: esanchez@agrosavia.co

However, *R. alpinus* is gaining ground in the national market (Moreno-Medina *et al.*, 2020).

The global blackberry market was valued at US$1.54 billion in 2023 and is estimated to reach US$2.27 billion by 2032, with an annual growth rate of 4.42% (Business Research Insights, 2025). In Colombia, it is one of the crops with the most extensive geographic coverage, the largest number of producers, and the most significant number of farms. Its continuous production makes the crop a constant source of income for small producers, which directly impacts family farming (MADR, 2021). Between 2019 and 2023, the cultivated area increased by 6.2%, reaching 17,518 ha. The harvested area registered an increase of 17.6% with a production of 170,125 t (EVA & UPRA 2025). The central producing departments in the country are Cundinamarca, Santander, Boyacá, Caldas, and Nariño, which account for 74.3% of the national production, with an average national yield of 10.02 t ha^{-1} (Agronet, 2025).

Blackberry is considered a superfood due to its antioxidant capacity attributed to its high content of secondary metabolites, such as anthocyanins and other phenolic compounds (Gil-Martinez *et al.*, 2023). In addition, it is rich in dietary fiber, vitamins C and K, and manganese (Martins *et al.*, 2023). The characteristics of blackberries in terms of nutritional and nutraceutical properties give them a high potential for development (Blejan *et al.*, 2023). However, technological development is insufficient to respond to the challenges of the crop. Therefore, plant breeding contributes to improved planting material productivity and is complemented by good practices in the technical management of the crop (González-Castro *et al.*, 2019). Consequently, various genetic breeding programs for *Rubus* worldwide are working to address these challenges, meeting market demands for fresh and processed food (for example, frozen, dehydrated, canned), as well as for household consumption (Foster *et al.*, 2019). In Colombia, notable advancements have been made in understanding the key morphological and reproductive traits of *R. glaucus*, essential for the formulation of a successful crossbreeding program (López Gutiérrez *et al.*, 2019). Moreover, the integration of molecular techniques has streamlined the process of identifying and selecting superior genotypes through SNP and SSR markers (López Gutiérrez *et al.*, 2019).

The evaluation and selection of desirable genotypes is perhaps the most crucial activity in plant breeding programs. Selection depends on the genetic diversity present in the population as well as the heritability of the characteristics studied (Rahimi & Debnath, 2023). Some of these characteristics are controlled by multiple genes and show low heritability. Therefore, indirect selection using a selection index is employed, which allows for estimating the genetic gain while simultaneously selecting materials that possess multiple desired attributes (Rahimi & Debnath, 2023). Individuals or materials with the highest scores are reserved for breeding purposes (Moreira *et al.*, 2019). The next step after selection is the evaluation of the characteristics of interest of these materials in production areas and the determination of their adaptation; this last characteristic is a fundamental process in breeding programs, being the first criterion in plant domestication (Turner-Hissong *et al.*, 2020).

The Colombian Agricultural Research Corporation – Agrosavia, aware of the importance of blackberry cultivation and the need to generate planting material, began developing blackberry cultivars (*Rubus* spp.) with desirable attributes, focusing on yield, fruit quality, and favorable disease resistance. Research conducted between 2014 and 2016 enabled the selection of five genotypes, three of which had prickles and two that did not (Sánchez-Betancourt *et al.*, 2020). The five genotypes belong to the *R. glaucus* species. In addition, Espinosa Bayer *et al.* (2009) selected six materials in a participatory manner with producers. From this group of selected materials, the five most outstanding were chosen to continue their evaluation and thus advance in the development of blackberry cultivars. Therefore, the objective of this research was to evaluate the yield and physicochemical quality of the fruits of five advanced genotypes of Castilla blackberry in a representative location of the country's main production areas, to select the genotype with the best characteristics according to a defined selection index.

Materials and methods

Plant genetic material and location of the evaluation lot

Five advanced blackberry genotypes together with a regional check (Tab. 1) were established in 2018 in an experimental plot in the municipality of Silvania, Cundinamarca, with latitude 04°24'10.6" N and longitude 74°19'16.3" W, at an altitude of 2,215 m a.s.l., with an average annual temperature of 18°C and bimodal rainfall distribution. The regional check corresponds to the most cultivated material in Cundinamarca; therefore, it can be used in a comparative analysis of yield under local agroecological conditions. The experimental genotypes are part of a working collection in *Rubus*, maintained at the Agrosavia Tibaitatá Research Center in Mosquera, Cundinamarca, which was derived from the collection of the Germplasm

Banks for Food and Agriculture (BGAA) and from visits to producing areas in the country. These genotypes were previously evaluated in 2009 and 2016 in Cundinamarca and Santander, where they were found susceptible to the primary diseases affecting the crop.

TABLE 1. Genotypes established in the experimental plot in the field.

Experimental code	Work collection code	Genotype	Place of origin
G1	17M020	Spineless experimental 1	UCO* – Rionegro, Antioquia
G2	17M021	Guapante – Seed	Agrosavia – Rionegro, Antioquia
G3	17M022	San Antonio – Seed	Agrosavia – Rionegro, Antioquia
G4	17M046	Monteloro ILS 2277	Farmer – Tuluá, Valle
G5	17M047	Spineless ILS 3400	Farmer – San Bernardo, Cundinamarca
G6	17M023	Regional control Cundinamarca	Silvania, Cundinamarca

*UCO: Universidad Católica de Oriente.

Experimental design

The experiment was conducted using a randomized complete block design with four replicates (blocks) and five plants per genotype (plot), resulting in 20 plants per treatment and a total of 120 plants. These plants were clonally propagated. The planting distance was 3 m between plants and 3 m between rows, resulting in an effective area of 9 m^2 per plant. The treatments consisted of six genotypes, and the experimental unit was the plot, which had an area of 45 m^2.

Data collection

Blackberry genotypes were evaluated from the fourth year of establishment, spanning the period from 2022 to 2023. Evaluations were conducted starting in year four due to financial and operational constraints that delayed the start of field data collection. Agronomic assessments of yield and physicochemical quality of fruits were conducted on maturity stages 4 and 5 according to NTC 4106 (Colombian Institute of Technical Standards – ICONTEC, 1997). Yield was recorded monthly for 10 months, considering two harvests per week. The evaluation variables were the following:

Yield traits

The fruits harvested per plot (five plants per genotype) were counted and weighed. The number of fruits per kilogram (FRKG), the yield (kg ha^{-1}) (YLD), and the annual production expressed in tons per year (PROD) adjusted to the percentage of productive plants at a planting density of 1,111 plants ha^{-1} were calculated.

Physical traits

Of the total number of fruits harvested, those in stage 5 of ripening were selected according to the NTC 4106 standard of the Colombian Institute of Technical Standards (ICONTEC, 1997), and a random sample of five fruits was taken. The polar diameter (PD), equatorial diameter (ED), fruit weight (FWT), and firmness (FIRM) were measured. The last variable was measured on the flattest side of the fruit, near the equatorial zone. For this purpose, the Chatillon® TCD 200 digital force tester was used with a plate probe of 10.92 mm in diameter and 3.45 mm in thickness, at a speed of 60 mm min^{-1}. Ten fruits were squeezed to extract the juice, which was then passed through a cloth filter, and both the filtered juice (JWT) and the pulp (PUWT) remaining in the filter were weighed.

Chemical traits

In the filtered juice of the 10 fruits in stages 4 and 5 of ripening, the contents of total soluble solids (TSS) were measured with an ATAGO PAL-1 refractometer and expressed in degrees Brix. Likewise, the pH was measured at a reference temperature of 20°C. The total titratable acidity (TTA) was determined using 1 ml of juice diluted with 23 ml of distilled water by titration with 0.1 N sodium hydroxide (NaOH) to an endpoint of pH 8.2; with results expressed as g ml^{-1} of malic acid (AOAC, 1995) (Eq. 1):

$$\mathrm{TTA} = \frac{A * B * C}{D} * 100 \qquad (1)$$

where

A is the amount of NaOH consumed in the titration (ml), B is the normality of NaOH (0.1 meq ml^{-1}), C is the equivalent weight expressed in grams of malic acid (0.067 g meq^{-1}), and D corresponds to the volume (ml) of juice used. Finally, the maturity index (MI) was calculated according to the TSS/TTA ratio.

Statistical analysis

The data were analyzed using generalized linear models (GLMs) in the Statistical Analysis System (2016). The main effects were evaluated using the Tukey test ($P<0.05$) and the Dunnett test ($P<0.05$) to compare them with the control. The assumptions of normality and homogeneity of variances were evaluated with the Shapiro-Wilk tests for all variables.

A principal component analysis (PCA) was also performed, using the criterion of eigenvalue≥ 1 (Díaz, 2007). Each value in the matrix was reduced to the variable's mean and then divided by its standard deviation. With the scores obtained in the PCA, a cluster analysis was performed using the squared Euclidean distance and Ward's minimum variance clustering method. The number of groups was determined by Hotelling's pseudo-t^2 statistic.

Heritability

Broad-sense heritability (H^2) was estimated as indicated in Equation 2.

$$\mathrm{H}^2 = \frac{\sigma_g^2}{\sigma_g^2 + \frac{\sigma_e^2}{r}} \qquad (2)$$

where

σ_g^2 is the genotypic variance, σ_e^2 is the error variance or mean square of the error, and r is the number of repetitions or blocks. The genotypic variance was calculated as the mean square of the genotype minus the mean square of the error, divided by the number of repetitions.

Selection index

Traits that are more important in blackberry cultivation and have medium or high heritability were used to develop a selection index. The selection of the best genotypes was based on a standardized selection index (I) (Eq. 3) estimated from the average phenotypic values obtained for the variables of production, total soluble solids, fruit weight, and firmness, which are considered the most significant agronomic interest and fruit quality. The total index was the sum of the standardized genetic merit multiplied by its weighting value, which was 60%, 20%, and 10%, respectively, for each variable. These weights were determined at the discretion of the research team based on previous experience and interactions with producers and industry experts. The standardized index was chosen because it allows comparison among traits with different scales, does not require a genetic covariance matrix, and is suitable when heritability is moderate or high. The genotypes with the best performance were those with the highest selection values.

$$I = \sum_{n=1} \left(\frac{ai - bi}{\sigma_{bi}} \right) * \; g_i \qquad (3)$$

where

I is the selection index value, *t* is the total number of traits, a$_i$ is the genetic merit of trait *i*, *b$_i$* is the average of all selection genotypes for trait *i*, σ_{bi} is the genetic standard deviation of trait *i*, and *g$_i$* is the economic weight of trait *i*.

Results and discussion

Agronomic characterization of yield and fruit physicochemical quality

Statistical significance was observed under the assumptions of normality and homogeneity of variance for all variables. The analysis of variance showed significant differences ($P \leq 0.05$) between the genotypes for the traits such as yield, production, contents of total soluble solids, pH, total titratable acidity, and fruit maturity index (Tab. 2). In the genetic improvement of blackberries, plants with high productivity and long fruits are usually selected since they are the attributes preferred by the market and producers (Hernández-Bautista *et al.*, 2022). Additionally, the chemical composition related to soluble solids, pH, and acidity are other determining factors in the flavor of the fruits (Vergara *et al.*, 2016).

Estimated heritability ranged from low (H^2 = -0.23) in equatorial diameter to high (H^2 = 0.94) in total soluble solids (Resende, 2002; Stanfield, 1971). Variables with heritability values above 60% were production and yield (H^2 = 0.66), pH

TABLE 2. Mean square of the analysis of variance of 13 characteristics studied in the fruits of six blackberry genotypes.

Variable type		Yield			Physical						Chemical			
Source of variation	**Degree of freedom**	**FRKG**	**YLD**	**PROD**	**PD**	**ED**	**FWT**	**FIRM**	**JWT**	**PUWT**	**TSS**	**pH**	**TTA**	**MI**
Genotype	5	448.83^{ns}	1713.27*	18.53*	3.66^{ns}	0.51^{ns}	0.6^{ns}	0.05^{ns}	0.39^{ns}	0.05^{ns}	1.08***	0.004*	0.278*	0.08*
Block	3	1431.17*	4087.14*	44.21*	8.23**	3.84**	2.54**	0.005^{ns}	2.355**	0.16^{ns}	0.61**	0.012***	0.057***	0.05^{ns}
Error	15	316.34	576.58	6.24	1.82	0.63	0.29	0.02	0.29	0.06	0.06	0.001	0.001	0.02
CV (%)	--	8.67	40.34	40.34	5.09	3.89	8.09	14.4	15.25	10.71	3.08	1.22	1.22	5.66
H^2	--	0.30	0.66	0.66	0.50	-0.23	0.51	0.66	0.25	-0.13	0.94	0.68	0.92	0.70

FRKG: number of fruits per kilogram, YLD: yield (kg ha^{-1}), PROD: annual production (t yr^{-1}), PD: fruit polar diameter, ED: fruit equatorial diameter, FWT: fruit weight, FIRM: fruit firmness, JWT: filtered juice weight, PUWT: pulp weight, TSS: contents of total soluble solids, TTA: total titratable acidity, MI: fruit maturity index, CV: coefficient of variation. Significant differences at * 5%; ** 0,5%; *** <0,001. ns: not significant. H^2: heritability.

(H^2 = 0.68), maturity index (H^2 = 0.70), acidity (H^2 = 0.92) and total soluble solids (H^2 = 0.94) (Tab. 2). Most of the traits evaluated showed from medium to high heritability values suggesting the possibility of obtaining reasonable genetic progress to improve these traits through the selection of individual plants. The results of this study agree with those reported by Stephens *et al.* (2012) in raspberry fruits in which firmness (H^2 = 0.54) and acidity (H^2 = 0.54) had medium heritability and total soluble solids had high heritability (H^2 = 0.73) and that reported by Chizk *et al.* (2023) for firmness in blackberry (H^2 = 0.68). The firmness variable did not present significant differences between genotypes; however, this trait is a good indicator of shelf life since it is related to the softness of the fruit at the time of ripening (Zhang *et al*., 2019). Additionally, the average heritability value suggests that the environment plays a role in determining firmness. Some factors that should be considered include water, mineral nutrition (with a focus on nitrogen and calcium), presence of fungal pathogens, and respiration rate (Chizk *et al*., 2023). Negative heritability was obtained for equatorial diameter (H^2 = -0.23) and pulp weight (H^2 = -0.13). These values may be related to the phenotypic homogeneity observed for these traits between genotypes. In the heritability estimation, the error variance for equatorial diameter and pulp weight was greater than the genotypic variance, suggesting a possible low number of segregating loci due to the low genotypic variance (Abbott & Pistorale, 2010).

When analyzing the means of the variables using Tukey's test ($P\leq0.05$) (Tab. 3), it was observed that the genotype with the highest average yield and production over 10 harvests was G1, followed by G4. Regarding the fruit quality parameters, genotype G2 had the highest total soluble solids TSS value, while G5 had the lowest value. The pH ranged from 2.80 to 2.88, with genotype G5 having the highest value and G2 and G3 having the lowest value. Genotype G2 had the highest TTA value, while G1 had the lowest value. Genotype G1 achieved the highest maturity index with no significant differences from genotypes G2, G4, and G5. The results showed that genotype G2 has the highest firmness and G3 has the lowest value. Fruit weight ranged between 6.18 (±1.08) and 7.16 (±0.43) g, with genotype G3 having the highest weight. Polar diameter ranged between 27.53 (±1.22) and 24.90 (±2.70) mm.

When comparing the mean performance of the genotypes against the check (G6), using the Dunnett mean comparison test, statistically significant differences ($P\leq0.05$) were observed with the G1 genotype, which showed a yield of 56.65 kg ha^{-1} and 5.89 kg ha^{-1} in production above the control. Regarding soluble solids, there were differences from the control for G1 (-0.62), G2 (0.57), and G5 (-0.92) and in the maturity index for G1 (0.37). No significant differences were observed compared to the check for the other variables. These results show the superiority of G1 over the check genotype.

In this research, the G1 genotype stood out in yield, production, and maturity index. These characteristics can be considered the main ones to determine the value of a genotype. The TSS and acidity relationship, known as the maturity index, helps determine the perceived sweetness and acidity of fruits and thus establishes consumer

TABLE 3. Mean of seven significant traits evaluated in six blackberry genotypes according to Tukey's test and difference between means based on Dunnett's test.

Genotype	YIELD (kg ha^{-1})	PROD (t yr^{-1})	TSS	pH	TTA	MI	Firmness
G1	95.24 (53.11) a* *56.65**	9.91 (5.52) a *5.89*	7.87 (0.36) cd *-0.62**	2.84 (0.09) ab *0.00*	2.76 (0.06) ab *0.00*	2.94 (0.15) a *0.37**	0.83 (0.09) a *0.02*
G2	41.80 (29.16) ab *3.21*	4.35 (3.03) ab *0.33*	9.07 (0.40) a *0.57**	2.80 (0.05) b *-0.04*	3.44 (0.09) ab *-0.03*	2.71 (0.08) ab *0.14*	1.05 (0.13) a *0.24*
G3	54.37 (13.11) ab *15.78*	5.66 (1.36) ab *1.64*	8.32 (0.25) bc *-0.18*	2.80 (0.04) ab *-0.03*	3.30 (0.14) ab *-0.02*	2.56 (0.08) b *-0.01*	0.76 (0.21) a *-0.04*
G4	68.79 (47.64) ab *30.20*	7.15 (4.95) ab *3.14*	8.13 (0.46) bcd *-0.37*	2.85 (0.05) ab *0.01*	3.08 (0.12) a *0.01*	2.76 (0.23) ab *0.19*	0.95 (0.17) a *0.14*
G5	58.36 (22.30) ab *19.77*	6.07 (2.32) ab *2.06*	7.58 (0.36) d *-0.92**	2.88 (0.04) a *0.04*	2.87 (0.05) ab *0.03*	2.67 (0.21) ab *0.11*	0.96 (0.08) a *0.15*
G6	38.59 (18.97) b	4.01 (1.97) b	8.50 (0.48) ab	2.84 (0.04) ab	3.35 (0.08) ab	2.57 (0.19) b	0.80 (0.12) a
HSD	55.16	5.74	0.58	0.08	0.05	0.35	0.29

TSS: contents of total soluble solids, TTA: total titratable acidity, MI: fruit maturity index. The value in parentheses is the standard deviation of the data (n = 4). The values in the column sharing the same letter are not significantly different according to Tukey's test (α = 0.05). The value in italics corresponds to the difference between means, using the regional genotype G6 as a control. The highest value for each variable is highlighted. HSD: Tukey's Honestly Significant Difference based on the minimum significant difference.

preferences (Threlfall *et al.*, 2016). The market prefers a balance between sweet and acidic, achieving an intense flavor through the combination of high total soluble solids TSS. However, a flat flavor is obtained if the acidity is low and the TSS is high, as in the case of the G2 genotype. The genotypes in this study exhibited maturity indices above 2.56, which can be considered appropriate for consumers, as the acid flavor is less perceived in these genotypes. This is particularly notable considering that the range for Colombia is between 2.2 and 3.1 (ICONTEC, 1997). The advantage of sour fruits is their longer shelf life as acidity prevents the action of microorganisms (Lund *et al.*, 2020). However, this is not optimal for the blackberry consumers.

The correlation between variables was analyzed, highlighting a negative correlation between equatorial diameter and number of fruits per kilogram (-0.77). The larger the diameter, the fewer fruits are needed to complete a kilogram. A positive correlation was observed between fruit weight and polar diameter (0.76), and a negative correlation was observed between weight and firmness (-0.75). Yield was positively correlated with the polar diameter of the fruit (r = 0.64). In a segregating population, individuals exhibiting desirable traits can be pinpointed through indirect selection using easily measurable secondary characteristics that are closely correlated with the primary trait (Fellahi *et al.*, 2020).

The principal component analysis for the total of the variables showed that the first three components have eigenvalues greater than 1 and are the most relevant, as they account for 91.90% of the total variation. The first principal component accounted for 51% of the total variance explained, the second component for 26%, and the third component for 14%. The variables that contributed to the first component were fruits per kilogram, yield, production, total soluble solids, and equatorial diameter. The variables fruit weight, juice weight, polar diameter, and firmness contributed to component two. The variables--maturity index, pH, and acidity--contributed to component three (Tab. 4). Consistent trends in variable clustering have been observed in *Rubus* studies, with Principal Component Analysis (PCA) effectively pinpointing pivotal traits that impact fruit quality and yield (González-Castro *et al.*, 2019; López Gutiérrez *et al.*, 2019).

TABLE 4. Matrix of eigenvectors, eigenvalues, and percentage of variance associated with each principal component for the variables measured in *Rubus*. Silvania (Cundinamarca), Colombia, 2022-2023.

Variables	PC1	PC2	PC3
Number of fruits per 1 kg	0.320412	-0.162466	0.250332
Fruit yield	0.332384	0.113778	0.323398
Production	0.332384	0.113778	0.323398
Fruit polar diameter	0.237981	0.382138	0.010638
Fruit equatorial diameter	-0.318104	0.266553	0.014365
Fruit weight	0.010042	0.528337	-0.049594
Fruit firmness	-0.001128	-0.460838	0.118683
Filter juice weight	0.235991	0.390202	-0.017339
Pulp weight	-0.303973	0.222434	0.208558
Total soluble solids	-0.335249	-0.082394	0.281856
pH	0.299811	-0.090725	-0.432990
Acidity	0.299811	-0.090725	-0.432990
Maturity index	0.279634	-0.118897	0.461623
Eigen value	6.6487	3.405508	1.893214
Proportion	51.14	26.20	14.56
Cumulative variance	51.14	77.34	91.90

* The variables that contribute the most to each principal component are highlighted.

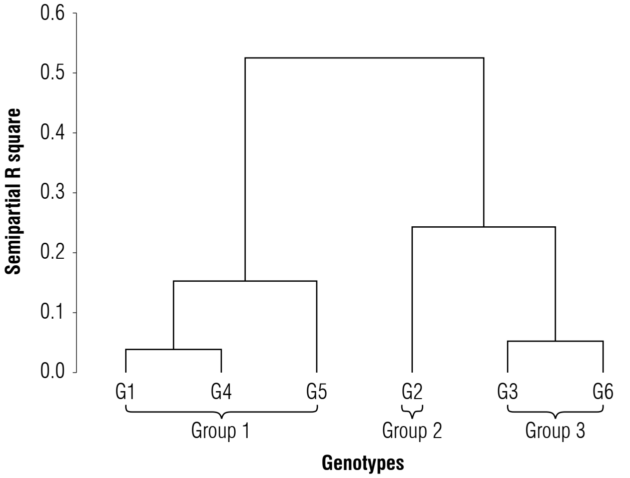

FIGURE 1. Dendrogram of six blackberry genotypes obtained by the Ward method. G1 to G5 were the experimental blackberry genotypes, and G6 was the regional control. Silvania (Cundinamarca), Colombia, 2022-2023.

Three homogeneous groups were generated by cluster analysis (Fig. 1). The first group contains genotypes G1, G4 and G5 (Fig. 1), of which two genotypes are spineless (G1 and G5), with low total soluble solids content, and the highest yield values (74.1 kg ha^{-1}), being higher than the general average (59.5 kg ha^{-1}); this group also presents the highest production (7.7 t yr^{-1}), and maturity index (2.79). The second group contains only genotype G2, which stands out for having the highest total soluble solids value (9.07 °Brix) and greater firmness (1.05 N). In the third group are the genotypes G3 and G6 (Fig. 1), which are characterized as having thorns (Tab. 1). This last group is characterized by having a lower number of fruits per kilogram (193), greater fruit weight (7.01 g) and with the largest equatorial diameter (20.8 mm).

In the dendrogram obtained from the principal component analysis, three groups were observed. These groups exhibit variability for the discriminating characteristics; however, it was observed that the thornless trait is a factor that marked the formation of the groups. The genotypes with and without thorns have the same productivity and fruit size (Marulanda & López, 2009). In this study, the thornless genotypes (G1 and G5) presented the highest production with low fruit weight. The thornless characteristic has been significant at a commercial level for the development of varieties, mainly in temperate zones (Clark *et al*., 2007; Coyner *et al*., 2005). The absence of thorns facilitates agronomic tasks and prevents potential mechanical damage at harvest, thus extending the fruit's shelf life (Clark *et al*., 2007). However, it has been observed that these materials are more susceptible to diseases; therefore, they could be used as parents in improvement programs to gather production and plant health characteristics.

Selection index

Selection of desirable genotypes is perhaps the most critical activity in plant breeding programs. The results of the selection index showed that the G1 genotype was outstanding in the combination of the characteristics of production, contents of soluble solids, fruit weight and firmness (Tab. 5). In second place was the G4 genotype, which presented higher values in relation to the G1 genotype for TSS, fruit weight and firmness. The regional control presented the lowest selection index mainly due to its lower yield during the evaluation. Despite the results obtained, variability was observed in the characteristics for each genotype without achieving the grouping of all the desirable attributes for a blackberry ideotype.

TABLE 5. Selection index values and average of the variables considered for each blackberry genotype.

Genotype	Selection index	Production (t yr^{-1})	Content of total soluble solids (°Brix)	Fruit weight (g)	Firmness (N)
G1	0.91	9.91	7.87	6.69	0.83
G4	0.39	7.15	8.13	7.04	0.95
G3	-0.14	5.66	8.32	7.16	0.76
G2	-0.21	4.35	9.07	6.18	1.05
G5	-0.36	6.07	7.58	6.35	0.96
G6	-0.60	4.01	8.50	6.87	0.80

In blackberry cultivation, the selection criteria are centered around the performance of the plant material concerning both yield and market-relevant variables, which can be chosen at the breeder's discretion. In this case, in addition to yield, soluble solids, fruit weight, and firmness were considered, as they are indicators of the organoleptic quality of the fruits. Each characteristic was weighted according to a relative economic value. The use of selection indices increases the chances of success of breeding programs because it simultaneously uses different traits to identify the superior genotype (Rahimi & Debnath, 2023). These indices have been used in several species, for example, rice (Sabouri *et al*., 2008), soybean (Bizari *et al*., 2017), corn (Gazal *et al*., 2017; Vieira *et al*., 2016), and strawberry (Vieira *et al*., 2017). Their usefulness is reflected in the achievement of genotypes with desirable characteristics that contribute to the development of the material ultimately delivered to the producer. Based on the results obtained, genotype G1 was selected as a potential material for variety registration. This genotype performed well in the field during the evaluation in the Cundinamarca department. Therefore, the next step will be to validate its performance in various production areas to evaluate its adaptation and thus determine the recommendation domain.

Conclusions

Significant differences were observed between the five genotypes for the variables of yield and physicochemical quality of the fruits. These differences allowed the selection of the best genotypes to continue their breeding process. The yield of the G1 genotype was superior to the regional control, followed by the G4 and G3 genotypes. In the combination of the four parameters of interest for the crop, such as production, soluble solids, fruit weight and firmness, the best genotype was G1, therefore its stability and adaptability in the different productive areas of the country should be evaluated in future studies to include it in the National Registry of Cultivars of Colombia.

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Conflict of interest statement

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

Author's contributions

ESB conceived, executed, collected data, interpreted results, and wrote the original draft. FGMC and FLGA collected and analyzed the data, interpreted the results, wrote, reviewed, and edited. All authors have read and approved the final version of the manuscript.

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Morphological characterization of tropical maize (*Zea mays* L.) inbred lines

Caracterización morfológica de líneas endocriadas de maíz (*Zea mays* L.) de origen tropical

José Miguel Sarmiento Buitrago[1*], Daniel Gerardo Cayón Salinas[1],
Laura Marcela Torres González[1], and Eddie Yacir Alvarez[2]

ABSTRACT

Morphological traits expressed by plants result from genotype, the environment where they grow, and the interaction between genotype and environment. These traits are expressed according to the plant's physiological responses to various environmental stimuli. A comparative evaluation was conducted on the main morphological traits of 20 inbred lines from Semillas Valle S.A. hybrid maize breeding program in Colombia. Correlations were found between some traits using a principal component analysis (PCA), where the two main components were plotted in a two-dimensional scatter diagram, and the results were corroborated using Pearson's correlation coefficient. Significant relationships were observed between male flowering time (DTT) and female flowering time (DTS), between plant height (PH) and ear height (EH), and between plant height (PH) and the internode number (IN). No relationships were found between ear height (EH) and number of rows (NR), or between ear weight (EW) and leaf length (LL) or seed index (SI).

Key words: plant physiology, plant growth, plant morphology.

RESUMEN

Los rasgos morfológicos expresados por las plantas son la respuesta a su genotipo, el ambiente donde se desarrollan y la interacción del genotipo y el ambiente. Dichos rasgos se expresan de acuerdo con las respuestas fisiológicas de las plantas a los distintos estímulos ambientales. Se realizó una evaluación comparativa de los principales rasgos morfológicos de 20 líneas endocriadas pertenecientes al programa de mejoramiento de maíz híbrido de Semillas Valle S.A. en Colombia. Se encontraron correlaciones entre algunos de los rasgos evaluados a partir de un análisis de componentes principales (ACP), cuyos dos principales componentes fueron graficados en un diagrama de dispersión de dos dimensiones, y sus resultados corroborados a partir de un coeficiente de correlación de Pearson. Se observaron relaciones significativas entre tiempos de floración masculina (DFM) y femenina (DFF), entre altura de la planta (AP) y altura de la mazorca (AM), y entre la altura de la planta (AP) y el número de entrenudos (NE). No se encontraron relaciones entre la altura de la mazorca (AM) y el número de hileras (NHM) ni entre el peso de mazorca (PM) y la longitud de hojas (LH) o el índice de semilla (IS).

Palabras clave: fisiología vegetal, crecimiento vegetal, morfología vegetal.

Introduction

Plant phenology studies the growth and development of plants by classifying their life cycle into different stages determined by the morphological characteristics of each species. Maize phenology starts with seed germination and finishes when the plant reaches physiological maturity; this process includes two developmental states. The first is called the vegetative stage (Vn, from emergence to tassel formation); each Vn is defined by the number of fully expanded leaves *i.e.*, V3 indicates a plant with three expanded leaves. The second is called the reproductive stage (Rn, from flowering to physiological maturity (R6)) (Ritchie *et al*., 1986).

The morphological characteristics of maize, including yield components, are widely studied in breeding programs to determine their variability, heritability, and correlation with yield, both in parental lines and hybrid materials. Characteristics such as leaf angle and orientation (Lambert & Johnson, 1978), grain yield, thousand-grain weight, ear length, and grain morphology are important to assess the suitability of an inbred line for use as a parent in seed production (Pinnisch *et al*., 2012). Ottaviano and Camussi (1981) suggest that maize grain yield can be considered as the result of two main components: the number and development of potential grains and the amount of photoassimilates translocated to the ear.

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[1] Universidad Nacional de Colombia, Palmira (Colombia).
[2] Universidad Nacional de Colombia, Grupo de Investigación Fitotecnia Tropical, Medellín (Colombia).
[*] Corresponding author: jomsarmientobu@unal.edu.co

Some morphological traits, such as root length, shoot length, dry weights, and fresh weights, show high heritability (Ali *et al*., 2014; Masood *et al*., 2020), indicating their usefulness in evaluating parent lines to produce hybrids with desirable characteristics. Similarly, other studies have assessed the relationship between grain yield and yield components (Jiufeng *et al*., 2008; Peng *et al*., 2011), finding correlations between different morphological traits.

In Colombia, maize breeding has primarily focused on the development of open-pollinated varieties, with some progress in hybrid development driven by private initiatives. As a result, there is limited scientific literature on the morphology of inbred lines. Hybrids represent the genetic improvement standard promoted by the "Maize for Colombia Vision 2030" project led by CIAT and CIMMYT (Goavertz *et al.,* 2019). The objective of this study was to characterize the morphological traits of 20 tropical-origin maize inbred lines. This research represents an important starting point for the morphological study of inbred lines aimed at developing high-yielding hybrids with broad adaptability to Colombian conditions.

Materials and methods

Location and plant material

The trial included 20 elite lines from the Semillas Valle S.A. plant breeding program; each having undergone at least six inbreeding cycles. These lines were selected based on their participation in experimental hybrid crosses conducted between 2013 and 2020, prioritizing those that were most frequently used as parental lines and ensuring representation from South America, Central America, Asia, and Africa. The trial was conducted at the experimental center of Semillas Valle S.A., located in the municipality of El Cerrito, in the department of Valle del Cauca (3°41'22.841" N, 76°18'33.476" W) with an altitude of 990 m a.s.l.

Sowing took place on April 21st, 2022, and harvest on August 9th, 2022. The experimental field was maintained under optimal irrigation and health conditions until physiological maturity. Four irrigations were applied during the crop cycle, each one ranging between 25 and 30 mm. Additionally, the fertilization consisted of 160 kg ha^{-1} of nitrogen, 68 kg ha^{-1} of P_2O_5, 125 kg ha^{-1} of K_2O, 45 kg ha^{-1} of sulfur, divided into two applications: the first at sowing and the second at the V3-V4 stage.

Traits

Several morphological and agronomic traits of maize were evaluated at different phenological stages. At the VT stage, leaf insertion angle (LIA), leaf shape (LS), leaf margin waviness (LMW), leaf width (LW), leaf length (LL), and internode number (IN) were measured. Days to tassel (DTT) and days to silk (DTS) were recorded daily until their appearance. At the R2 stage, internode length (IL), stem diameter (SD), plant height (PH), and ear height (EH) were evaluated. After harvest, the ear length (EL), ear diameter (ED), ear shape (ES), number of rows (NR), number of grains per row (GPR), grain type (GT), grain color (VG), ear weight (EW), grain weight (GW), and seed index (SI) were measured. All traits were assessed according to the CIMMYT maize descriptors (IBPGR, 1991).

Experimental design and statistical analysis

A completely randomized design was established. The experimental unit consisted of two 5-m rows per line, with 27 plants per row (totaling 54 plants), spaced 18 cm between plants and 70 cm between rows (equivalent to 80,000 plants ha^{-1}). The sampling unit was one plant, with three replicates. Three plants were selected for analysis, as the lines—having undergone six cycles of inbreeding—were considered genetically homogeneous, and thus low morphological variability was expected within each genotype.

Data were recorded by evaluating three randomly selected plants per row for each line at each phenological stage. Data obtained were processed using R software (RStudio Team, 2023) and the *ggplot2* library (v3.3.3; Wickham, 2016). For morphological characterization, descriptive statistics were initially presented, and the lines were grouped using the Pearson correlation coefficient. Subsequently, a principal component analysis was performed using the *prcomp* library (R Core Team, 2023), and a scatter plot was generated to show the relationship between the two main components.

Results and discussion

Qualitative traits

Leaf insertion angle (LIA), leaf shape (LS), and leaf margin waviness (LMW)

Table 1 presents the morphological traits of the leaf for each of the 20 inbred lines, where high variability in the combination of traits LIA, LS, and LMW can be observed. Among the evaluated lines, 5% exhibited erect LIA, 50% exhibited semi-erect LIA, 45% exhibited semi-horizontal LIA, and no line exhibited fallen LIA. Additionally, 25% presented rectilinear LS, 35% presented slightly curved LS, 10% presented curved LS, and 30% presented very curved LS. 25% of the lines showed no leaf margin waviness, 50% exhibited wavy LMW, and 25% exhibited very wavy LMW.

TABLE 1. Leaf morphology of maize inbred lines.

Line	LIA	LS	LMW
1	Semi-erect	Curved	No waviness
2	Erect	Slightly curved	Wavy
3	Semi-horizontal	Curved	Wavy
4	Semi-erect	Slightly curved	Wavy
5	Semi-erect	Very curved	Very wavy
6	Semi-horizontal	Very curved	Wavy
7	Semi-erect	Slightly curved	Wavy
8	Semi-erect	Very curved	Very wavy
9	Semi-horizontal	Rectilinear	No waviness
10	Semi-erect	Rectilinear	No waviness
11	Semi-horizontal	Slightly curved	Very wavy
12	Semi-horizontal	Slightly curved	Very wavy
13	Semi-horizontal	Rectilinear	Wavy
14	Semi-horizontal	Rectilinear	No waviness
15	Semi-erect	Slightly curved	No waviness
16	Semi-erect	Very curved	Very wavy
17	Semi-erect	Slightly curved	Wavy
18	Semi-erect	Very curved	Wavy
19	Semi-horizontal	Rectilinear	Wavy
20	Semi-horizontal	Very curved	Wavy

LIA=leaf insertion angle, LS=leaf shape, LMW=leaf margin waviness.

The architecture of the maize plant has been an important objective in hybrid improvement programs. The leaf insertion angle, along with the size and shape of the leaves, are important components of maize plant architecture, and their distribution determines how photosynthetically active radiation is intercepted, consequently influencing total canopy photosynthesis and yield (Ku *et al*., 2012). Li *et al.* (2015) and Seka *et al.* (2019) indicated that erect or vertically oriented leaves may contribute to grain yield due to improved light capture for photosynthesis and their role as nitrogen reservoirs for grain filling, allowing for the establishment of the crop at a higher density with a greater leaf area index. Identifying cultivars with a leaf distribution around the plant that reduces overlap between leaves and allows for greater interception of solar radiation and CO_2 uptake to increase photosynthesis is an objective in hybrid development. Different combinations of leaf area and leaf angle define the structure of the canopy; however, the leaf insertion angle has proven to be the most important trait in reorganizing canopy structure for better adaptation to higher planting densities while maintaining high photosynthetic activity and yield (Seka *et al*., 2019).

The predominance of lines with erect and semi-erect LIA, along with the prevalence of slightly curved and rectilinear leaves, constitutes an important finding for the breeding program. Based on this information, along with the determination of the heritability of these characteristics, it is possible to establish a starting point for selecting parental lines with more erect leaves and subsequently developing hybrids with the same characteristic that can be planted at higher densities while maintaining high photosynthetic rates, thereby contributing to the production of high-yield hybrids. In this study, the lines that exhibit morphologically desirable leaf characteristics are line 2, which presents erect LIA and slightly curved LS; line 10, which presents semi-erect LIA and rectilinear LS; and lines 4, 7, 15, and 17, which exhibit semi-erect LIA and slightly curved LS.

Ear shape (ES), grain type (GT), and grain color (GC)

Table 2 presents the morphological traits of the ear for each of the 20 evaluated lines. Thirty percent of the evaluated genotypes exhibited a cylindrical ear shape, 30% exhibited a conical shape, and 40% exhibited an intermediate shape. Some authors indicate that the size, shape, and texture of maize grains play an important role in grain production (Srinivas *et al*., 1991). Variation in ear shape is related to the size and shape of the grains, as this varies according to their position on the ear. Grains formed at the tip of the ear tend to be smaller and rounder, especially when the ear is

TABLE 2. Morphological traits of the ear of maize inbred lines.

Line	ES	GT	GC
1	Cylindrical	Crystalline	Light orange
2	Intermediate	Crystalline	Yellow
3	Cylindrical	Crystalline	Yellow
4	Conical	Semi-crystalline	Yellow
5	Cylindrical	Semi-crystalline	Yellow
6	Conical	Crystalline	Orange
7	Intermediate	Crystalline	Yellow
8	Intermediate	Crystalline	Yellow
9	Conical	Crystalline	Orange
10	Conical	Semi-crystalline	Yellow
11	Intermediate	Floury	White
12	Intermediate	Crystalline	White
13	Conical	Semi-crystalline	White
14	Conical	Crystalline	White
15	Cylindrical	Semi-crystalline	White
16	Intermediate	Crystalline	White
17	Cylindrical	Semi-floury	White
18	Intermediate	Crystalline	White
19	Cylindrical	Semi-crystalline	White
20	Intermediate	Crystalline	White

ES= ear shape, GT= grain type, GC= grain color.

conical. Those formed at the basal part of the ear tend to be larger and rounder, while those formed in the middle tend to be flat. In industry, seeds are usually classified by shape and size, since the use of uniformly sized seeds allows for their application in mechanized and precision agriculture. Additionally, seeds of medium or large size and flat shape are considered to be of higher quality (El-Abady, 2015). Therefore, characterizing the lines allows for the use of ear shape as a selection criterion when choosing lines as parents for commercial hybrids.

Regarding the grain characteristics (Tab. 2), 60% of the lines exhibited a crystalline grain type, 30% semi-crystalline, 5% semi-floury, and 5% floury. Ten percent of the lines had orange grain, 5% had light orange grain, 35% had yellow grain, and 50% had white grain. Maize is typically classified based on the properties of its endosperm, which determine the grain type and color. These characteristics are related to the quality and quantity of the endosperm and generally determine the intended use of the grain (Zilic *et al.*, 2011). Depending on the target market, hybrid development should focus on obtaining genotypes that meet the required characteristics. Consequently, appropriate and comprehensive characterization, along with knowledge of the inheritance patterns of grain color and type, is of great importance for genetic improvement programs. This information forms the basis for directing crosses, increasing efficiency in the use of genetic resources, and accelerating the production of high-quality hybrids. In this regard, the lines that exhibit morphologically desirable ear characteristics are primarily lines 1 and 3, which have a cylindrical ear shape; secondly, lines 3, 7, 8, 11, 12, 16, 18, and 20, which present an intermediate ear shape. Furthermore, evaluating the heritability of ear shape and its relationship with yield in hybrids is highly relevant for future assessments.

Quantitative traits

Table 3 presents the main morphological traits of the leaves and stem for the 20 inbred lines. The lines with the highest leaf width (LW) were lines 6 and 12, followed by line 4, while the lines with the greatest leaf length (LL) were line

TABLE 3. Main morphological traits of leaves and stem of maize inbred lines.

Line	LW (cm)	LL (cm)	SD (mm)	PH (cm)	EH (cm)	IN
1	7.7 ^{a}	85.7 ^{defg}	12.0 ^{a}	219.0 ^{bcd}	83.0 ^{bc}	9.7 ^{ab}
2	8.3 ^{abc}	82.7 ^{bcdef}	14.7 ^{bcdef}	218.3 ^{bc}	101.3 ^{efghi}	12.7 ^{ghi}
3	8.3 ^{abc}	73.0 ^{a}	12.7 ^{ab}	220.3 ^{bcd}	89.3 ^{cd}	9.7 ^{ab}
4	10.0 ^{e}	90.7 ^{gh}	14.7 ^{bcdef}	222.0 ^{bcd}	63.7 ^{a}	9.3 ^{a}
5	9.3 ^{cde}	102.0 ^{i}	14.7 ^{bcdef}	238.7 ^{def}	96.0 ^{def}	12.0 ^{efg}
6	12.0 ^{f}	84.0 ^{cdefg}	15.0 ^{cdef}	219.0 ^{bcd}	93.7 ^{cde}	12.3 ^{fgh}
7	9.0 ^{bcde}	81.3 ^{bcde}	13.0 ^{abc}	236.0 ^{cdef}	112.0 ^{i}	10.3 ^{bc}
8	9.7 ^{de}	77.7 ^{abc}	16.0 ^{efg}	212.7 ^{b}	94.7 ^{de}	12.3 ^{fgh}
9	9.7 ^{de}	77.0 ^{ab}	16.3 ^{fg}	190.3 ^{a}	73.0 ^{ab}	9.0 ^{a}
10	8.3 ^{abc}	81.3 ^{bcde}	13.0 ^{abc}	234.3 ^{cde}	104.0 ^{efghi}	11.0 ^{cd}
11	12.0 ^{f}	80.7 ^{bcd}	17.7 ^{g}	300.3 ^{h}	147.7 ^{k}	13.7 ^{j}
12	9.7 ^{de}	78.0 ^{abc}	13.3 ^{abc}	256.0 ^{fg}	107.0 ^{fghi}	12.0 ^{efg}
13	9.0 ^{bcde}	79.0 ^{abcd}	12.3 ^{a}	222.0 ^{bcd}	99.3 ^{defgh}	12.3 ^{fgh}
14	9.3 ^{cde}	72.3 ^{a}	16.0 ^{efg}	268.3 ^{g}	123.3 ^{j}	13.3 ^{ij}
15	9.0 ^{bcde}	88.0 ^{efgh}	15.7 ^{defg}	268.0 ^{g}	107.7 ^{ghi}	12.7 ^{ghi}
16	8.0 ^{ab}	85.0 ^{defg}	13.0 ^{abc}	218.3 ^{bc}	110.0 ^{hi}	11.3 ^{de}
17	9.7 ^{de}	80.3 ^{bcd}	12.0 ^{a}	251.7 ^{efg}	98.0 ^{defg}	13.0 ^{hij}
18	8.7 ^{abcd}	94.3 ^{h}	14.0 ^{abcde}	221.0 ^{bcd}	100.7 ^{efgh}	11.7 ^{def}
19	8.3 ^{abc}	88.7 ^{fgh}	16.7 ^{fg}	271.0 ^{g}	99.0 ^{defgh}	12.7 ^{ghi}
20	8.3 ^{abc}	83.3 ^{bcdef}	13.7 ^{abcd}	178.3 ^{a}	74.0 ^{ab}	11.3 ^{de}
Mean	9.1	83.1	14.2	233.3	98.9	11.6
CV %	14	9	13	13	19	12.0
Significance	$P<0.01$	$P<0.01$	$P<0.01$	$P<0.01$	$P<0.01$	$P<0.01$

LW= leaf width, LL= leaf length, SD= stem diameter, PH= plant height, EH= ear height, IN= internode number.

5, followed by line 18. Both LW and LL are traits that determine leaf size; these traits are directly related to leaf area and the plant's ability to produce photoassimilates (Bos *et al.*, 2000). As leaf dimensions increase, the plant's capacity to produce dry matter also increases, which in turn can lead to higher yields. Therefore, developing hybrids with greater LL and LW could be beneficial for grain production and forage production. These traits are highly relevant since they have been shown to correlate with transpiration and photosynthesis (Al-Kaisi *et al.*, 1989).

The lines with the greatest plant height (PH) were lines 14, 15, and 19, while the average ear height (EH) was 98.9 cm. PH and EH are traits of great importance for the selection of new genotypes, as they are directly related to plant lodging. A correlation of up to 88% has been reported between lodging and PH and EH traits (Xue *et al.*, 2020). Therefore, the combination of tall plants with high ear insertion is considered an undesirable trait combination in maize hybrid development. Although PH and EH are not significantly related to grain or seed production, PH is a desirable trait in the selection of genotypes for forage production.

TABLE 4. Flowering of inbred maize lines.

Line	DTS	DTT	IL (cm)
1	70 ^{e}	71 ^{d}	25.7 ^{a}
2	69 ^{d}	73 ^{f}	31.3 ^{bcdefg}
3	69 ^{d}	71 ^{d}	28.2 ^{abcd}
4	62 ^{a}	70 ^{c}	39.2 ^{i}
5	70 ^{e}	73 ^{f}	34.0 ^{fgh}
6	69 ^{c}	73 ^{f}	29.3 ^{abcdef}
7	78 ^{j}	76 ^{i}	32.7 ^{cdefg}
8	69 ^{d}	75 ^{h}	28.8 ^{abcde}
9	69 ^{d}	73 ^{f}	27.0 ^{ab}
10	76 ^{i}	80 ^{k}	33.7 ^{efgh}
11	68 ^{c}	68 ^{b}	38.3 ^{ghi}
12	73 ^{g}	76 ^{i}	34.0 ^{fgh}
13	69 ^{d}	71 ^{d}	33.0 ^{defgh}
14	74 ^{h}	74 ^{g}	27.3 ^{ab}
15	68 ^{c}	70 ^{c}	26.2 ^{a}
16	73 ^{g}	75 ^{h}	26.3 ^{a}
17	70 ^{e}	78 ^{j}	27.8 ^{abc}
18	74 ^{h}	76 ^{i}	33.7 ^{efgh}
19	67 ^{b}	67 ^{a}	35.0 ^{ghi}
20	71 ^{f}	72 ^{e}	25.7 ^{a}
Mean	70.4	73.1	30.7
CV %	4	4	14
Significance	$P<0.01$	$P<0.01$	$P<0.01$

DTS= days to silk, DTT= days to tassel, IL=internode length.

The line that exhibited the highest stem diameter (SD) was line 11, followed by lines 9 and 19. SD is an important characteristic in hybrid selection processes, as it is inversely related to lodging (Novacek *et al.*, 2013). The average internode number (IN) was 11.6. IN is directly related to plant height and is significantly affected by water deficit conditions (Bennouna *et al.*, 2004; Robertson, 1994).

Table 4 shows the traits associated with flowering. The traits DTS and DTT indicate the number of days required for the plant to reach female and male flowering, respectively. These traits are particularly relevant in the hybrid production process, as they must be considered when sowing the lines to achieve synchronization between pollen emission and maturity of stigmas (Guzmán *et al.*, 2017). Therefore, it is not possible to determine one line as superior to others. On the other hand, IL is a trait highly affected by water stress (Bennouna *et al.*, 2004) and is directly related to the amount of pollen emitted (Vidal Martínez *et al.*, 2004), making a higher IL highly desirable. The line with the highest IL was line 4.

Table 5 presents the yield components of the 20 maize inbred lines. The lines with the highest NR were lines 15 and 18. Comparatively high values for yield components are desirable, as these directly affect crop yield. NR is determined at V7 (Stevens *et al.*, 1986) and shows low variability in each genotype, as it is genetically determined (Zhang *et al.*, 2021). The line with the highest GPR was line 11. Although this trait is genetically defined, it can be affected by environmental stresses (Smith, 2004), pollination issues or grain abortion. The line with the best SI was line 11, followed by lines 7 and 20. SI is a determinant trait in yield formation, which is influenced by planting density, environmental conditions (Milander, 2015), ear filling capacity, and the translocation of assimilates to the sink. The highest EW were lines 15 and 17. EW directly depends on the traits SI, NG, and NR, and also includes the weight of the husk and cob.

The line with the highest EL was line 4, followed by line 18. EL has a positive correlation with GPR and a negative correlation with NR (Milander, 2015), and it also depends on grain size. The line with the highest GW was line 18. followed by line 20. Grain weight reflects yield potential, as the grain is the commercially important organ for hybrids, while in inbred lines, it corresponds to the seed.

TABLE 5. Yield components of maize inbred lines.

Line	SI (g)	GW (g)	EW (g)	GPR	NR	ED (mm)	EL (mm)
1	27^{g}	100.7^{def}	129.3^{cdef}	25.7^{cdef}	13.3^{abc}	40.5^{ab}	117.1^{bcde}
2	23^{d}	91.6^{cde}	135.3^{cdef}	29.0^{fgh}	16.0^{de}	47.2^{gh}	125.8^{defgh}
3	26^{f}	101.7^{def}	117.3^{bcdef}	20.7^{ab}	12.0^{ab}	42.4^{bcde}	95.0^{a}
4	28^{h}	89.9^{cde}	134.3^{cdef}	27.7^{efg}	13.3^{abc}	40.1^{ab}	152.7^{j}
5	30^{j}	82.2^{cde}	149.0^{ef}	23.0^{abcd}	15.3^{cd}	43.3^{bcdef}	116.4^{bcde}
6	28^{h}	70.4^{abc}	102.4^{abcde}	20.7^{ab}	16.0^{de}	46.1^{fg}	142.3^{hij}
7	31^{k}	77.3^{bcd}	120.1^{bcdef}	26.7^{cdefg}	14.0^{bcd}	43.2^{bcdef}	141.5^{ghij}
8	30^{j}	107.0^{efg}	99.3^{abcd}	26.7^{cdefg}	13.3^{abc}	42.7^{bcdef}	101.6^{ab}
9	23^{d}	65.7^{abc}	65.8^{a}	27.3^{efg}	13.3^{abc}	44.2^{cdefg}	123.2^{cdef}
10	18^{a}	47.4^{a}	78.6^{ab}	24.3^{bcde}	16.0^{de}	41.2^{bcd}	113.4^{bcd}
11	33^{l}	76.9^{bcd}	113.0^{abcde}	32.0^{h}	13.3^{abc}	44.6^{efg}	132.2^{efghi}
12	27^{g}	63.7^{abc}	116.7^{bcdef}	27.0^{defg}	13.3^{abc}	41.8^{bcde}	144.1^{ij}
13	20^{b}	89.4^{cde}	161.6^{f}	30.0^{gh}	16.0^{de}	40.9^{abc}	136.2^{fghij}
14	22^{c}	77.0^{bcd}	125.2^{bcdef}	22.7^{abc}	11.3^{a}	37.6^{a}	113.7^{bcd}
15	24^{e}	53.9^{ab}	101.5^{abcd}	20.0^{a}	18.0^{e}	49.9^{h}	109.8^{abcd}
16	29^{i}	69.8^{abc}	94.6^{abc}	25.7^{cdef}	13.3^{abc}	42.7^{bcde}	124.3^{cdefg}
17	22^{c}	74.1^{abcd}	114.9^{bcdef}	25.7^{cdef}	22.0^{f}	50.0^{h}	110.9^{abcd}
18	28^{h}	134.8^{g}	139.2^{cdef}	26.3^{cdefg}	12.0^{ab}	42.1^{bcde}	135.6^{fghij}
19	29^{i}	101.5^{def}	145.2^{def}	22.7^{abc}	18.0^{e}	53.5^{i}	107.0^{abc}
20	31^{k}	127.7^{fg}	145.2^{def}	22.7^{abc}	13.3^{abc}	44.4^{defg}	132.0^{efghi}
Mean	26.4	85.1	96.1	25.3	14.6	43.9	123.7
CV %	15	31	28.0	15	18	9	14
Significance	$P<0.01$	$P<0.01$	$P<0.01$	$P<0.01$	$P<0.01$	$P<0.01$	$P<0.01$

SI=seed index, GW=grain weight, EW=ear weight, GPR=number of grains per row, NR=number of rows, ED=ear diameter, EL=ear length.

Among the general lines, line 11 stands out for having the highest SI and GPR, line 15 for presenting the highest ear weight and the greatest number of rows, and line 18 for having the highest NR, the highest GW, and the second largest grain size. However, due to the low heritability reported for these traits, it is recommended that they be evaluated in hybrids.

Clustering of inbred lines based on their correlation

A dendrogram (Fig. 1) was constructed using the Pearson correlation coefficient for the evaluated population, revealing three groupings. The first group consists of lines 1, 2, 3, 4, 5, 13, 18, and 20, which are characterized by the largest average internode length, with an average of 31.3 cm. In contrast, groups 2 and 3 have averages of 30.9 cm and 29.8 cm, respectively. The second group includes lines 7, 10, 12, 16, and 17, characterized by greater ear length, with an average of 126.8 mm compared to groups 1 and 3, which have averages of 126.3 mm and 117.6 mm, respectively. The third group consists of lines 6, 8, 9, 11, 14, 15, and 19, characterized thicker stems, with an average of 16.1 cm compared to groups 1 and 2, which have averages of 13.5 cm and 12.7 cm, respectively. Additionally, this group has greater leaf width, with an average of 9.7 cm compared to groups 1 and 2, which have averages of 8.5 cm and 9 cm. respectively.

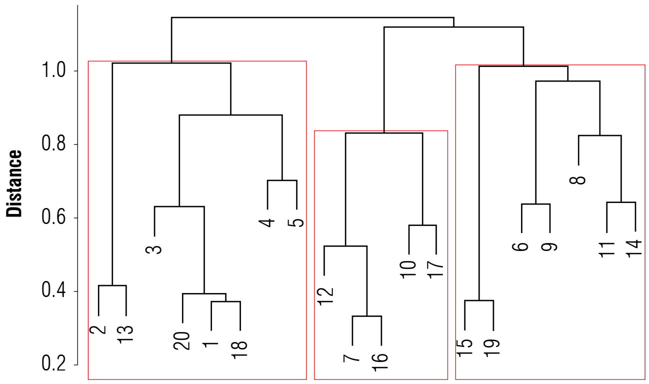

FIGURE 1. Grouping lines according to the Pearson correlation coefficient.

Among the main traits of the groupings, characteristics of ears, cobs, and vegetative traits were highlighted. This suggests that the lines in group 1 may be useful as males; the lines in group 2 may be useful as females due to their seed production capacity or as parental lines for hybrids aimed at grain production; and the lines in group 3 may be useful for developing hybrids focused on forage production.

The groupings shown in Figure 5 could be confirmed through progeny analysis of these lines, in collaboration with the breeding program, which would establish the relationship between this characterization and the performance of the hybrids formed from the evaluated lines.

Principal component analysis

To minimize the dimensionality of the data and reveal potential correlations that may exist among the traits measured in this study, a principal component analysis (PCA) was conducted (Fig. 2) based on the morphological information of the 20 evaluated lines, which included 18 traits represented in a scatter plot (Fig. 5).

The scatter plot was constructed using component 1 (PC1, 7.9%) and component 2 (PC2, 16%), as the principal component analysis (Fig. 2) determined that the first two components accounted for the greatest proportion of variance (35.7%).

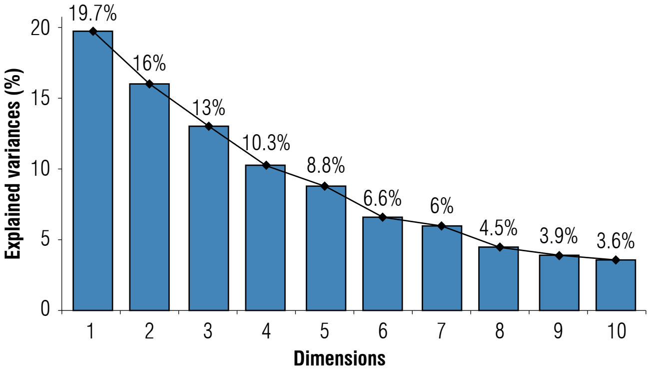

FIGURE 2. Principal component analysis.

Regarding the contribution of the variables to the first two principal components (Fig. 2), it was found that for the first component (Fig. 3), plant height (PH) had the highest contribution (19.09%), followed by the internode number (IN) (15.14%), stem diameter (SD) (10.81%), ear height (EH) (9.48%), ear diameter (ED) (8.18%), and days to silk (DTS) (7.53%).

In the second principal component (Fig. 4), the variable with the greatest contribution to the model was days to silk (DTS) (14.17%), followed by days to tassel (DTT) (13.44%), ear height (EH) (12.96%), grain weight (GW) (12.1%), and seed index (SI) (11.3).

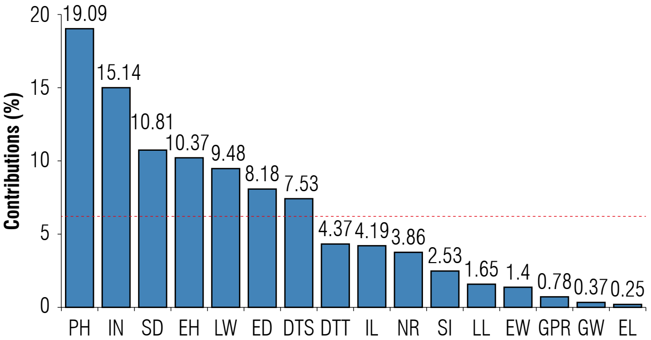

FIGURE 3. Contribution of traits to principal component 1. DTT = days to tassel, DTS = days to silk, IL = internode length, SD = stem diameter, PH = plant height, EH = ear height, EL = ear length, ED = ear diameter, NR = number of rows, GPR = number of grains per row, EW = ear weight, GW = grain weight, LL = leaf length, SI = seed index, LW = leaf width, IN = internode number.

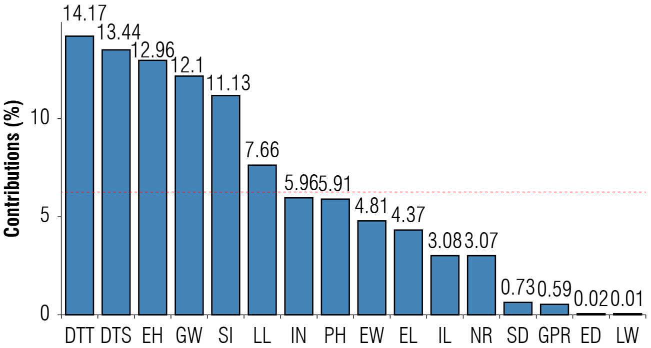

FIGURE 4. Contribution of traits to principal component 2. DTT = days to tassel, DTS = days to silk, IL = internode length, SD = stem diameter, PH = plant height, EH = ear height, EL = ear length, ED = ear diameter, NR = number of rows, GPR = number of grains per row, EW = ear weight, GW = grain weight, LL = leaf length, SI = seed index, LW = leaf width, IN = internode number.

The scatter plot (Fig. 5) demonstrates the independence or correlation between traits; angles close to 0° with similar magnitudes indicate a high correlation between traits, while angles close to 90° indicate independence. According to this, DTT and DTS show a high correlation, representing a strong synchrony between male and female flowering times. Additionally, these two traits showed a correlation coefficient of 0.71, which aligns with Guzmán *et al.* (2017), who mention that the anthesis-silking interval is a highly important trait for selecting crosses. The high synchrony among the evaluated materials highlights the importance for selecting genotypes for crossing, thus maintaining high levels of efficiency in pollination and fertilization.

Traits EH and NR had an angle close to 0° between them; however, their magnitudes were different, indicating a low correlation between the traits. This result is confirmed by a correlation coefficient of 0.0. Although both traits depend directly on the genotype, one might assume that if the ear is located higher on the plant, the ear leaf would have greater light capture and, therefore, a higher capacity for kernel filling. However, this is disproven since all of the plant's leaves contribute to the ear's kernel filling (Nielsen, 2004). Additionally, it has been demonstrated that the number of rows is genetically determined, as genes involved in the genetic control of this characteristic have been identified (Zhang *et al*., 2021), confirming the results obtained.

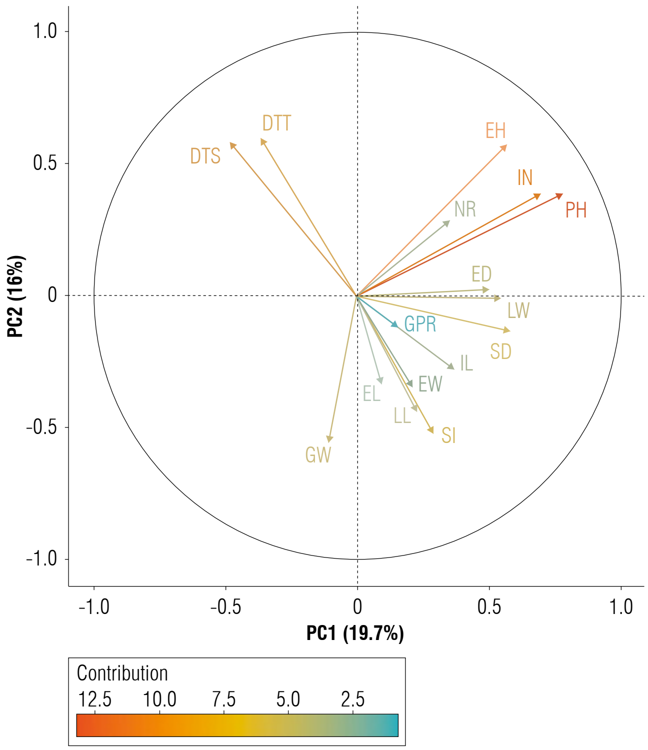

FIGURE 5. Scatter plot for principal components 1 and 2. DTT = days to tassel, DTS = days to silk, IL = internode length, SD = stem diameter, PH = plant height, EH = ear height, EL = ear length, ED = ear diameter, NR = number of rows, GPR = number of grains per row, EW = ear weight, GW = grain weight, LL = leaf length, SI = seed index, LW = leaf width, IN = internode number.

The traits IN and PH had an angle close to 0° and a similar magnitude, matching the correlation coefficient of 0.61. This agrees with Izzam *et al.* (2017), who reported a high correlation between the number of leaves and plant height, a valid relationship as maize plants have one leaf at each internode, and as there is only one stem per plant, plant height is directly defined by the number and length of the internodes. On the other hand, EH and PH had an angle close to 15° with similar magnitudes. Additionally, they had a correlation coefficient of 0.71, which aligns with Carpici and Celik (2010) and Kandel *et al.* (2018), who found high correlations between these traits, as the ear is generally located between the seventh and eighth leaf. Since it is a trait with little variability, a taller plant tends to have a higher ear height.

The traits EW, LL, and SI had angles close to 0° between them; however, their magnitudes were not equal. Additionally, their correlation coefficients did not exceed 0.4, indicating no evident correlation between them. The scatter plot also suggests an inverse relationship with the male and female flowering times, although their correlation coefficients were not significant. Furthermore, the plot indicates that the traits in quadrant 2 (EH, NR, IN, and PH) do not correlate with those in quadrants 1 and 4 (DTT, DTS, SI, LL, and EW), suggesting they are independent of each other (Fig. 5).

The scatter plot (Fig. 5) helps identify redundant traits, allowing for dimension reduction, which means a decrease in the number of traits evaluated in the field, thereby optimizing characterization processes. This analysis suggests that the trait days to silk (DTS) explains more variability in the model compared to days to tassel (DTT). Due to their high correlation, only days to silk (DTS) could be evaluated, consistent with Izzam *et al.* (2017), who found a 91% correlation between these traits. Similarly, plant height (PH) explains more variability in the model than the internode number (IN), and the seed index (SI) explains more variability than ear weight (EW) and leaf length (LL). Therefore, the traits internode number (IN), ear weight (EW), and leaf length (LL) could also be excluded.

Izzam *et al.* (2017) reported that the traits DTS and DTT do not show a statistically significant correlation with EH, EL, and PH, which aligns with the results obtained in the scatter plot (Fig. 4), where DTS and DTT form angles close to 90° with PH and EH, demonstrating independence between these traits. Additionally, EL has a much lower magnitude and an opposite direction to DTS and DTT, suggesting a low negative correlation between the traits.

Conclusions

This study enabled the identification of the distribution, correlation, and redundancy of key traits, suggesting the possibility of optimizing field evaluations by reducing the number of traits assessed.

The morphological diversity observed among the lines highlights their potential for developing hybrids aligned with market demands and underscores the need for complementary genetic analyses.

This research marks an important starting point in the study of inbred lines, aimed at developing high-yielding hybrids with broad adaptability to the conditions of Colombia.

Conflict of interest statement

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

Author's contributions

JMSB, LMTG, and DGCS carried out the planning and formulation of the experiments; JMSB and LMTG performed the data collection; JMSB and EYA conducted the statistical analysis and data management. All authors analyzed and discussed the information. JMSB wrote the draft of the manuscript. All authors reviewed and approved the final version of the manuscript.

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Accumulation and distribution of calcium, magnesium, and sulfur in potato (*Solanum tuberosum* Group Andigenum) cultivars Diacol Capiro and Pastusa Suprema

Acumulación y distribución de calcio, magnesio y azufre en cultivares de papa (*Solanum tuberosum* grupo Andigenum) Diacol Capiro y Pastusa Suprema

Kristal Castellanos Ruiz[1*], Manuel Iván Gómez Sánchez[1], and Luis Ernesto Rodríguez Molano[1]

ABSTRACT

The potato crop has a high response to nutrient application; however, knowledge about requirements and management of calcium (Ca), magnesium (Mg), and sulfur (S) is scarce. It is important to study the accumulation, as well as translocation and use efficiencies, of these nutrients, considering the influence of genotype and environment variables. The objective of this research was to establish the nutrient curves for accumulation, as well as for translocation, use, and recovery efficiencies, of Ca, Mg and S for two potato cultivars of the Andigenum Group in two production cycles (2013-2016) and two localities (Chocontá and Facatativá) with contrasting edaphoclimatic conditions. Diacol Capiro and Pastusa Suprema cultivars were evaluated under two fertilization regimes (0 and 100% of the recommended dose for all essential nutrients). Data on fresh and dry weight, and Ca, Mg, and S content were recorded at five sampling moments, from main stem formation to tuber maturation. The highest accumulation of Ca, Mg, and S was obtained in Facatativá, however the highest translocation, use and recovery efficiencies of nutrients were obtained in Chocontá. Capiro showed higher sink strength, with a higher translocation efficiency compared to Suprema. Nutrient accumulation in tubers (kg ha^{-1}) followed the order of Ca (147) > Mg (66) > S (52), while translocation efficiencies at harvest were S (44%) > Mg (32%) > Ca (6%). Cultivars had different patterns of accumulation, translocation, and Ca, Mg, and S use, which also varied with locality. Capiro was better adapted to high fertility soil conditions and Suprema had better performance in low fertility soils.

Key words: absorption curves, translocation efficiency, nutrition use efficiency, recovery efficiency.

RESUMEN

El cultivo de papa presenta una alta respuesta al manejo de la nutrición; sin embargo, el conocimiento sobre los requerimientos y manejo del calcio (Ca), magnesio (Mg) y azufre (S) es escaso. Es necesario profundizar en los procesos de acumulación, traslocación y uso eficiente de estos nutrientes teniendo en cuenta la influencia de variables como genotipo y ambiente. El objetivo de la presente investigación fue establecer las curvas de absorción, eficiencia de traslocación, uso eficiente y eficiencia de recuperación de Ca, Mg y S en dos cultivares de papa del Grupo Andigenum en dos ciclos (2013-2016) y dos localidades (Chocontá y Facatativá) con condiciones edafoclimáticas contrastantes. Se evaluaron los cultivares Diacol Capiro y Pastusa Suprema bajo dos regímenes de fertilización (0 y 100% de la dosis recomendada para todos los nutrientes esenciales). Se registró información de peso fresco, seco y contenido de Ca, Mg y S en cinco puntos de muestreo, desde la formación de tallos hasta la maduración de los tubérculos. La mayor acumulación de Ca-Mg-S se dio en Facatativá; sin embargo, la mayor eficiencia de traslocación, uso eficiente y recuperación de nutrientes se obtuvo en Chocontá. Capiro mostró una mayor fuerza vertedero al presentar una mejor eficiencia de traslocación respecto a Suprema. La acumulación de nutrientes (kg ha^{-1}) siguió el orden de Ca (147) > Mg (66) > S (52), mientras la eficiencia de traslocación en cosecha fue de S (44%)> Mg (32%) > Ca (6%). Los cultivares evaluados presentaron procesos diferentes de acumulación, translocación y uso eficiente de Ca-Mg-S, los cuales también variaron con la localidad. Capiro tuvo mejor adaptación a las condiciones de suelo de alta fertilidad, mientras Suprema mostró mejor desempeño en condiciones de baja fertilidad.

Palabras clave: curvas de absorción, eficiencia de traslocación, uso eficiente de nutrientes, eficiencia de recuperación.

Introduction

Negative impacts on crop yield and quality are expected in the coming years due to temperature and precipitation changes associated with climate change (Hameed *et al.*, 2018; Handayani *et al.*, 2019). For the years 2040 to 2069, potato crop yield is estimated to decrease by 18-32% (Dahal *et al.*, 2019). Potato has great relevance for global food

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[1] Universidad Nacional de Colombia, Facultad de Ciencias Agrarias, Bogotá (Colombia).

[*] Corresponding author: kcastellanosr@unal.edu.co

security due to its nutritional and yield qualities (Gaj *et al*., 2020; Handayani *et al*., 2019). In addition, with a projected increase of 30% in the world population by the year 2050, there is a need to increase food production. This scenario presents a challenge requiring more sustainable and efficient crop systems (Dahal *et al*., 2019).

The potential yield of potato crop is high (> 40 t ha^{-1}); nevertheless it depends on crucial factors like nutrient management (Campos & Ortiz, 2020). Differences in edaphoclimatic conditions between localities, adaptation of cultivars to these localities, and specific nutritional requirements of each cultivar, are causes of heterogeneity in yield and tuber quality (Gaj *et al*., 2020; Villamil *et al*., 2005). It is important to define the specific nutrient requirements for each cultivar in a locality to improve yield, increase fertilizer efficiency, and reduce the environmental impact of this practice (Dahal *et al*., 2019).

Adequate management of calcium (Ca), magnesium (Mg), and sulfur (S) can increase crop production, improve quality, and reduce the incidence of phytosanitary problems and physiological disorders such as hollow or cracked heart of the tubers (Wendimu Seifu & Deneke, 2017). Cultivars of Andigenum Group are among some of the most widely grown in Colombia, nevertheless there is limited information about uptake, translocation, and use of the mineral elements Ca, Mg, and S for these cultivars. Due to their low mobility in phloem, symptoms of nutritional deficiencies of Ca and S appear in the youngest leaves (Hamdi *et al*., 2015), while deficiency symptoms of Mg are expressed in the mature leaves of the plants (Senbayram *et al*., 2015). The accumulation and translocation of calcium, magnesium and sulfur are affected by soil and weather factors such as pH, CEC, soil texture, organic matter, microbial activity, rainfall regime, temperature, among others (Castro & Gómez, 2013). Additionally, it is important to consider the source-sink relationship in each cultivar, which determines the accumulation of dry matter and nutrients in the tubers (Bender *et al*., 2015).

There are different approaches to establish the nutritional requirements for a crop. Potato growers often rely on general fertilizations, traditionally used or reported in literature. However, this information does not consider factors like edaphoclimatic conditions per location, cultivar features, or productive cycle (Silva *et al*., 2020). Accumulation and translocation curves of nutrients are models that describe the amount of a given nutrient that is translocated to the tuber through the productive cycle. These curves allow for specific nutritional corrections at any moment of the production cycle (Duarte *et al*., 2019; Silva *et al*., 2020). Additionally, nutrient management should consider the measurement of the nutrient use efficiency, i.e., the amount of tubers produced by nutrient unit, as well as nutrient recovery efficiency, *i.e*., the amount of nutrient applied that the plants actually uptake (Lemaire *et al*., 2019).

The objective of the present research was to establish curves for accumulation, as well as for translocation, use and recovery efficiencies of Ca, Mg, and S in two cultivars of potato (*S. tuberosum* Group Andigenum) in two localities of the Cundinamarca-Boyacá highlands in Colombia.

Materials and methods

Study site

This research was carried out in two localities representative of the production of potatoes in Colombia and with records of high yields (> 50 t ha^{-1}): Facatativá with high fertility (Andic Eutrudepts) and Chocontá with low fertility (Humic Dystrudepts) (Castellanos Ruiz *et al*., 2022). In each locality, two production cycles were evaluated between the years 2013-2016, each with a total duration of 150-160 d after planting (DAP). Climatic variables and soil characteristics for the arable layer (0-30 cm) were monitored and recorded in each locality and cycle, prior to the establishment of the trial. Details of climatic variables and soil characteristics by locality and crop cycle can be found in Gómez *et al.* (2019).

Experimental design and crop management

Four experiments were conducted across different seasons using a split-plot design with four replicates distributed in randomized complete blocks. The main plot was the cultivar (Diacol Capiro and Pastusa Suprema) and the subplot was the level of fertilization (0 and 100% of the recommended doses of macro and micronutrients). The 100% fertilization treatment corresponded to the full nutrient recommendation for each locality-cycle, established using the soil-plant balance method (Castro & Gómez, 2013) and the recommendation given by Gomez *et al.* (2019) (see detailed information in Castellanos Ruiz *et al.* (2022)), while the 0% treatment was unfertilized soil. The dose fractionation was carried out according to the historical management of the study sites (historical yields > 50 t ha ^{1}) as follows: N 55% at sowing and 45% at 45 DAP; P 80% at sowing, and 20% at 45 DAP; K 12% at sowing and 88% at 45 DAP; for Ca, Mg, S, 63% was applied at sowing and 37% at 45 DAP. Experimental units of 5 m x 10 m were established, with a distance between rows of 1 m and 0.37 m between plants, for a density of 27,000 plants ha^{-1}. Tubers averaging

70 g were used as seed and were sown manually. Each plot had a total of 135 plants. Phytosanitary management of the crop was carried out according to local practices.

Sampling and measurements

Five destructive samplings were carried out at five phenological stages (Valbuena *et al*., 2009) (Tab. 1). Four plants were harvested per experimental unit in each sampling moment; leaves, stems and tubers were removed, and their fresh weights were recorded separately. To quantify mineral nutrient content in each organ, plant material was washed with deionized water, the same organs from the four plants were mixed, and a subsample of 200 g was separated and dried in an oven at 70°C to constant weight to determine the dry weight (Gómez *et al*., 2019). For chemical analysis, the total concentration of Ca, Mg, and S per organ was determined according to IGAC (2006). The total content of each nutrient was estimated by multiplying the concentrations of nutrients in the organs (g 100 g^{-1} dry weight) by the amount of dry biomass accumulated per organ at each phenological stage (Abdallah *et al*., 2016).

TABLE 1. Sampling stages in relation to days after planting (DAP) and corresponding phenological stages.

Stage	DAP	Phenological stage
I	50-55	Formation of primary stems
II	70-75	Formation of secondary stems and initial tuberization
III	90-100	Flowering, maximum tuberization and beginning of tuber filling
IV	120-125	End of flowering, tuber filling
V	150-160	Leaf senescence, maximum filling, and tuber maturation

Statistical analysis

For each variable, an analysis of variance (ANOVA) was performed using the PROC GLM procedure (SAS Institute, 2017). When significant differences were identified, a comparison test of means between treatments was performed using the Tukey-Kramer test ($P<0.05$). All statistical analyses were performed using SAS 9.4 software (SAS Institute, 2017) and the graphs were constructed using ggplot2 (Wickham, 2016).

Nutrient accumulation curves

Total (ACtot) and tuber (ACtub) nutrient accumulation curves were developed from the concentration values of the nutrients per unit dry weight at each sampling stage. The ACtot and ACtub models were fitted using the NLIN procedure in SAS (SAS Institute, 2017). For ACtub, the model proposed by Yin *et al.* (2003) was fitted (Eq. 1), where *Ctub* represents the concentration of the nutrient in the tuber. *Cmax* is the maximum concentration of the nutrient obtained at time *te* (end of period growth), *tb* is the start time of the growth period, and *tm* is the time corresponding to the highest accumulation rate of the nutrient. In contrast, for ACtot, the Yin *et al.* (2003) model showed poor fit and low convergence. Therefore, the model proposed by Ziadi *et al.* (2008) (Eq. 2) was used instead. In this model, *C* represents the total accumulation of the nutrient, *W* is the total dry biomass, and *a* and *b* are fitting coefficients. Due to the difference between the models, the independent variables were days after planting for the ACtub model and total dry biomass for the ACtot model. Confidence intervals at 95% and the standard error of the coefficients were established to evaluate nutrient accumulation between cultivars and localities.

$$Ctub = Cmax\left(1 + \frac{te-tb}{te-tm}\right)\left(\frac{tb}{te}\right)^{\frac{te}{te-tm}} \quad \text{with} \quad 0 < t_m < t_e \tag{1}$$

$$C = aW^b \tag{2}$$

Growth curve and nutrient accumulation by phenological stage

A three-parameter logistic growth model was fitted (Ritz *et al*., 2015) to calculate the growth curve as a function of days after planting (Eq. 3), where *b, d* and *e* are the model coefficients and *x* is days after planting. Using the growth curve and the total accumulation model (Eq. 2), values for nutrient accumulation by phenological stage were established for the plants. Nutrient accumulation was also calculated for the tuber based on the tuber accumulation model (Eq. 1).

$$W = c + \frac{d-c}{1 + \exp\left(b(\log(x)-\log(e)\right)} \tag{3}$$

Translocation efficiency

Translocation efficiency (CaTE, MgTE, STE) was calculated by dividing the accumulation of nutrients in the tubers (Ntub) (kg ha^{-1}) by the total accumulation of nutrients in the plant (Nupt) (kg ha^{-1}) at each sampled phenological stage (Eq. 4). The translocation efficiency curve was fitted using the NLIN procedure in SAS (SAS Institute, 2017), adjusting it to a logarithmic function as proposed by Gómez *et al.* (2019).

$$\text{Nutrient TE} = (N_{tub} / N_{upt}) \times 100\% \tag{4}$$

Acquisition or recovery efficiency

The fertilizer nutrient recovery efficiency, or acquisition efficiency, for Ca (RFCa), Mg (RFMg), and S (RFS) was calculated using Equation 5, as proposed by Gómez *et al.* (2018), where Et1 is the extraction of nutrients by tubers in fertilized soils (kg ha^{-1}) and Et0 is the extraction of nutrients by tubers in unfertilized soils (kg ha^{-1}).

$$RF = \frac{E_{t1}\text{-}E_{t0}}{\text{amount of nutrients supplied with the fertilizer}} \times 100 \qquad (5)$$

Nutrient use efficiency

Nutrient use efficiency (UE) of Ca (CaUE), Mg (MgUE) and S (SUE) in the tubers was calculated as the dry matter accumulated in the tuber/total kg of the extracted nutrient, as proposed by Poljak *et al.* (2011).

Ethical considerations

The activities in the laboratory and the management of chemical and biodegradable waste were carried out according to the protocol of the Universidad Nacional de Colombia B-FT-15-003.033 and the Colombian Technical Guide 53-7 for the use of non-hazardous organic solid waste.

Results

Growth curves

Growth models were established based on the DAP for each cultivar-locality (Fig. 1). The models had a high level of fit, and no significant differences were found between localities or cultivars (Tab. 2). The accumulation of dry biomass was faster in Facatativá than in Chocontá, and Suprema showed greater and faster accumulation of biomass compared to Capiro. The highest accumulation of dry biomass occurred between 75-100 DAP, which coincided with the formation of secondary stems and the beginning of tuberization (stages I and II).

Nutrient accumulation curves

For the total nutrient accumulation curves, significant differences were found between localities and in the locality x cultivar interaction for the three nutrients ($P<0.001$). A cultivar effect was observed on the accumulation of Mg and S, and Ca. The Mg accumulation models showed significant differences between the localities (coefficient *a* and *b* with $P<0.05$) for both cultivars (Tab. 3, Fig. 2). For S, only the Capiro accumulation model showed differences between localities. Between cultivars, significant differences were observed in the models for Chocontá (coefficients *a* and *b* with $P<0.05$); the accumulation of Ca and Mg was greater in Suprema (Fig. 2). At a dry biomass of 25 t ha^{-1}, Capiro and Suprema presented the highest accumulation of Ca (223 and 185 kg ha^{-1}) and Mg (86 and 78 kg ha^{-1}) in Facatativá, without significant differences between cultivars. In Chocontá, Suprema had significantly higher accumulation of Ca and Mg, with 50% and 59% more, respectively, compared to Capiro. Sulfur accumulation in Capiro was higher in Facatativá (59 kg ha^{-1}), while in Suprema, no significant differences were found between localities.

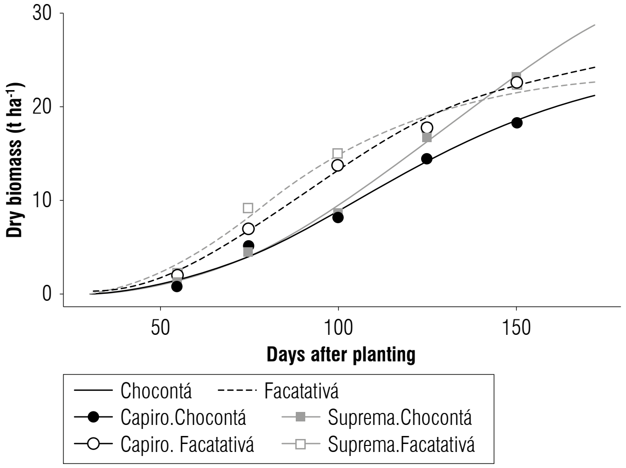

FIGURE 1. Growth model for Diacol Capiro and Pastusa Suprema potato cultivars in two localities with contrasting edaphoclimatic conditions.

The data collected for tuber nutrient accumulation demonstrated the effect of the cultivar and locality x cultivar interaction ($P<0.001$). The accumulation models of Ca, Mg and S in the tubers showed no significant differences between cultivars or localities; however, a lower accumulation of Mg and S was observed for Suprema in Facatativá (Tab. 4). The models indicated that the accumulation rate of Mg and S in the Capiro tuber in Chocontá decreased

TABLE 2. Growth models coefficients for potato cultivars Diacol Capiro and Pastusa Suprema in two localities.

Cultivar	Locality	b	d	e	SE b	SE d	SE e
Capiro	Facatativá	-3.59	28.07	102.69	0.66 ns	4.21 ns	22.40 ns
	Chocontá	-3.38	28.70	125.67	0.70 ns	7.96 ns	10.82 ns
Suprema	Facatativá	-3.76	24.62	89.32	0.77 ns	2.98 ns	8.07 ns
	Chocontá	-3.36	47.22	150.81	0.71 ns	21.10 ns	38.54 ns

ns: no significant differences between cultivars or localities. SE: Standard error. b, d, and e: Fitted equation coefficients.

towards the end of the cycle (Fig. 3). Based on the models, the accumulation of Ca, Mg, and S in the tuber and their proportion by phenological stage was established (Tab. 5). The highest accumulation of nutrients coincided with the phenological stages of maximum tuberization and tuber filling (stages III and IV).

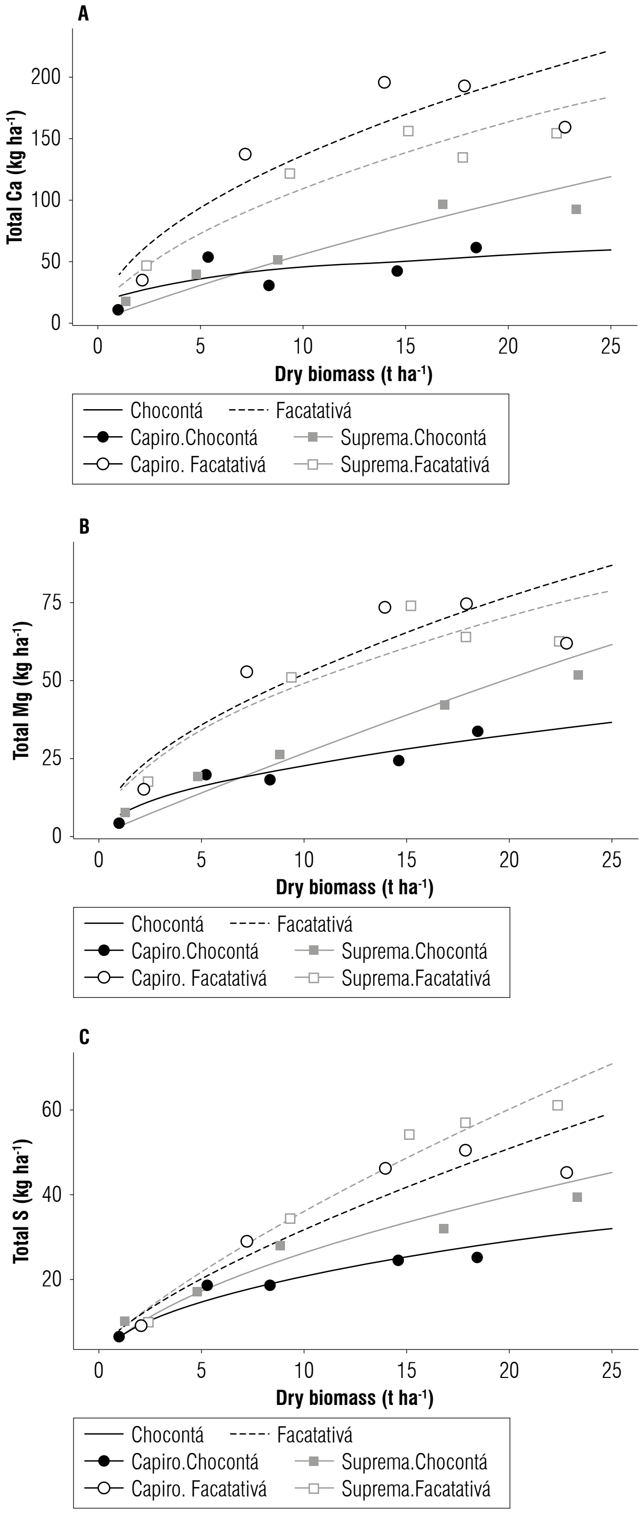

FIGURE 2. Calcium (A), magnesium (B), and sulfur (C) total accumulation curves in potato cultivars Diacol Capiro and Pastusa Suprema in two localities with contrasting edaphoclimatic conditions.

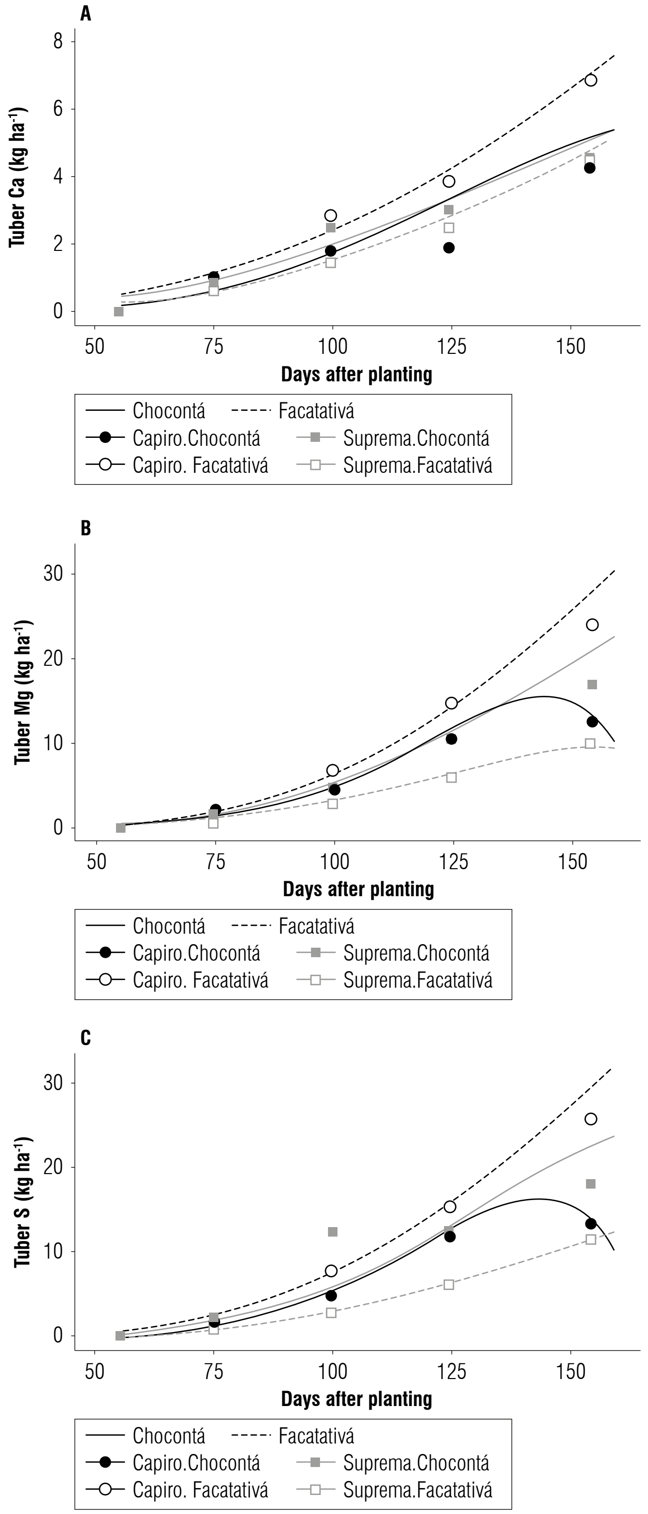

FIGURE 3. Calcium (A), magnesium (B), and sulfur (C) tuber accumulation curves in potato cultivars Diacol Capiro and Pastusa Suprema in two localities with contrasting edaphoclimatic conditions.

TABLE 3. Ca, Mg, and S total accumulation curves coefficients for Diacol Capiro and Pastusa Suprema cultivars in two localities with contrasting edaphoclimatic conditions.

Nutrient	Locality	Cultivar	Curve ($N_{ac}= aW^{b}$)	RMSE	CI (*a*) 95%	CI (*b*) 95%	SE *a*	SE *b*
Calcium	Chocontá	Capiro	$21.84W^{0.31}$	26.24	11.35-32.32	0.12 -0.50	5.26 *, **	0.10 *
		Suprema	$8.27W^{0.83}$	28.22	3.30 -13.24	0.63 -1.04	2.49 *, **	0.10 *
	Facatativá	Capiro	$40.44W^{0.53}$	44.78	24.58-56.30	0.39 -0.67	7.92 **	0.07 ns
		Suprema	$29.53W^{0.57}$	37.14	16.09-42.96	0.41 -0.73	6.71 **	0.08 ns
Magnesium	Chocontá	Capiro	$7.05W^{0.51}$	7.66	4.50 - 9.61	0.37 -0.64	1.28 *, **	0.07 *
		Suprema	$3.50W^{0.89}$	11.91	1.61 -5.38	0.71- 1.07	0.94 *, **	0.09 *, **
	Facatativá	Capiro	$14.72W^{0.55}$	18.78	8.29- 21.16	0.39 -0.70	3.21 **	0.08 ns
		Suprema	$14.71W^{0.52}$	20.80	6.50 -22.91	0.32 -0.72	4.10 **	0.10 **
Sulfur	Chocontá	Capiro	$7.27W^{0.46}$	4.93	5.55 -8.99	0.37 -0.55	0.86 ns	0.05 **
		Suprema	$6.60W^{0.60}$	11.25	3.62 -9.58	0.43 -0.75	1.49 ns	0.08 ns
	Facatativá	Capiro	$7.30W^{0.65}$	9.20	4.69 -9.91	0.53 -0.77	1.30 ns	0.06 **
		Suprema	$6.76W^{0.73}$	8.80	4.44 -9.08	0.61 -0.85	1.16 ns	0.06 ns

* Significant differences between cultivars at $P<0.05$, ** significant differences between localities at $P<0.05$, ns: No significant differences between cultivars or localities. RMSE: Root mean square error. CI: Confidence interval. SE: Standard error.

TABLE 4. Ca, Mg, and S tuber accumulation curves coefficients for Diacol Capiro and Pastusa Suprema cultivars in two localities with contrasting edaphoclimatic conditions.

Nutrient	Locality	Cultivar	*cm*	*E*	*M*	RMSE	SE *cm*	SE *e*	SE *m*
Calcium	Chocontá	Capiro	5.58	170.10	128.10	0.69	1.73 ns	34.07 ns	16.65 ns
		Suprema	6.73	202.80	136.40	0.95	7.67 ns	146.20 ns	72.44 ns
	Faca	Capiro	32.85	382.00	245.10	1.74	501.80 ns	2772.20 ns	1636.10 ns
		Suprema	6.43	195.00	142.80	1.24	12.29 ns	195.10 ns	110.20 ns
Magnesium	Chocontá	Capiro	15.50	144.60	120.50	2.10	0.88 ns	3.29 ns	2.38 ns
		Suprema	26.61	182.80	143.70	2.43	20.37 ns	62.20 ns	38.00 ns
	Facatativá	Capiro	40.25	193.20	151.30	6.40	102.60 ns	201.20 ns	129.00 ns
		Suprema	9.64	157.10	125.00	1.05	1.06 ns	13.49 ns	5.95 ns
Sulfur	Chocontá	Capiro	16.56	143.80	119.90	1.72	0.76 ns	2.17 ns	1.86 ns
		Suprema	24.83	170.00	134.90	3.53	12.56 ns	44.77 ns	24.81 ns
	Facatativá	Capiro	43.33	196.60	151.10	6.25	98.67 ns	193.50 ns	121.20 ns
		Suprema	13.44	175.70	138.50	1.12	6.36 ns	40.28 ns	23.37 ns

ns: No significant differences between locality or cultivar. *cm, E* and *M*: Equation coefficients. RMSE: Root mean square error. SE: Standard error.

Nutrient translocation efficiency

An effect of the locality ($P<0.01$) was observed on the translocation efficiency of the nutrients, which was significantly higher in Chocontá than in Facatativá (Fig. 4). For Mg and S, an effect of the cultivar ($P<0.01$) was observed from 100 DAP in favor of Capiro. The translocation efficiency during the production cycle was fitted to a positive logarithmic model. From the beginning of tuberization (75 DAP), values progressively increased, reaching their maximum at harvest (155 DAP). Calcium showed the lowest translocation efficiency, with an average value of 5.5%, followed by Mg with 33.5% and S with 44.5%.

The models in Chocontá were significantly different from those in Facatativá (parameters *a* and *b* with $P<0.01$), except for the S model in Capiro, where there were no significant differences between the localities (Tab. 6). For the MgTE and STE models in Facatativá, significant differences were found between the cultivars (parameters *a* and *b* at $P<0.01$), with Capiro showing 57% and 67% higher translocation efficiency of Mg and S, respectively, at 155 DAP compared to Suprema. In Chocontá, there were no differences between the cultivars, in contrast to what was found in the total accumulation of nutrients. The Ca translocation efficiency did not differ between cultivars within the same locality.

TABLE 5. Forecast of total accumulation and in the tubers of Ca, Mg, and S by phenological stage for Diacol Capiro and Pastusa Suprema cultivars in two localities with contrasting edaphoclimatic conditions.

Cultivar	Locality	PS	DAP	AC total (kg ha^{-1})			ACtub (kg ha^{-1})			ACtub stage (%)		
				Ca	Mg	S	Ca	Mg	S	Ca	Mg	S
Capiro	Chocontá	I	0-55	25.5	9.1	9.2	0.0	0.0	0.0	0.0	0.0	0.0
		II	55-75	34.2	14.8	14.2	0.4	1.0	1.0	8.6	8.3	10.5
		III	75-100	43.3	21.7	20.0	1.1	3.7	4.0	32.0	40.1	32.3
		IV	100-125	49.7	27.3	24.7	1.6	6.9	7.5	65.5	77.9	80.2
		V	125-150	54.0	31.2	27.8	1.6	3.3	3.1	100	100	100
	Facatativá	I	0-55	68.4	25.4	13.9	0.0	0.0	0.0	0.0	0.0	0.0
		II	55-75	112.2	42.5	25.5	0.6	1.4	1.7	10.6	5.7	6.6
		III	75-100	159.8	61.3	39.4	1.3	4.3	4.8	31.4	22.9	24.8
		IV	100-125	191.4	73.9	49.1	1.8	8.0	8.5	61.2	54.9	56.7
		V	125-150	209.8	81.3	55.0	2.3	11.2	11.5	100	100	100
Suprema	Chocontá	I	0-55	11.9	5.2	8.6	0.0	0.0	0.0	0.0	0.0	0.0
		II	55-75	26.8	12.4	15.5	0.5	1.1	1.3	12.3	6.0	6.2
		III	75-100	53.6	26.0	25.5	1.0	3.4	3.9	35.8	23.8	25.2
		IV	100-125	84.3	42.2	35.4	1.4	6.2	7.1	66.5	56.5	59.3
		V	125-150	113.2	57.9	43.8	1.5	8.3	8.5	100	100	100
	Facatativá	I	0-55	59.6	27.9	16.6	0.0	0.0	0.0	0.0	0.0	0.0
		II	55-75	99.4	44.5	32.0	0.4	0.7	0.7	9.1	7.5	6.2
		III	75-100	137.6	59.9	48.5	0.9	2.0	2.0	30.0	29.5	24.8
		IV	100-125	159.1	68.4	58.4	1.4	3.4	3.5	61.8	66.2	58.1
		V	125-150	170.0	72.6	63.6	1.6	3.1	4.4	100	100	100

PS: Phenological stage. DAP: Days after planting. Total AC: Total accumulation. ACtub: Accumulation in the tubers. I: Stem formation, II: Beginning of tuberization, III: Flowering-maximum. Tuberization, IV: Filling of tubers-flowering cessation, V: Maximum filling-ripening of tubers.

TABLE 6. Ca, Mg, and S translocation efficiency models coefficients for Diacol Capiro and Pastusa Suprema potato cultivars in two localities with contrasting edaphoclimatic conditions.

Nutrient	Locality	Cultivar	Curve NC = a (ln (x)+b)	RMSE	CI (*a*) 95%	CI (*b*) 95%	SE *a*	SE *b*
Calcium	Chocontá	Capiro	8.33 (ln (x)-33.20)	2.86	6.43 - 10.24	(-41.89) - (-24.50)	0.96**	4.36**
		Suprema	6.18 (ln (x)-24.21)	2.94	4.22 - 8.14	(-33.14) - (-15.28)	0.98**	4.48 ns
	Facatativá	Capiro	3.81 (ln (x)-15.66)	1.05	3.05 - 4.57	(-19.13) - (-12.2)	0.38**	1.73**
		Suprema	3.55 (ln (x)-14.72)	1.55	2.43 - 4.67	(-19.84) - (-9.59)	0.56**	2.56 ns
Magnesium	Chocontá	Capiro	45.78 (ln (x)-185.1)	9.42	39.51 - 52.06	(-213.7) - (-156.4)	3.15**	14.37 ns
		Suprema	38.88 (ln (x)-157.9)	9.37	32.64 - 45.12	(-186.4) - (-129.4)	3.13**	14.28**
	Facatativá	Capiro	35.34 (ln (x)-146.9)	7.60	29.86 - 40.83	(-171.9) - (-121.8)	2.74*, **	12.52*
		Suprema	16.21 (ln (x)-67.17)	4.13	13.23 - 19.19	(-80.79) - (-53.56)	1.49*, **	6.80*, **
Sulfur	Chocontá	Capiro	58.43 (ln (x)-238.2)	10.38	51.52-65.34	(-269.8) - (-206.7)	3.46 ns	15.82 ns
		Suprema	53.78 (ln (x)-216.0)	16.88	42.54- 65.02	(-267.3) - (-164.6)	5.63**	27.73**
	Facatativá	Capiro	52.69 (ln (x)-217.8)	9.82	45.61-59.78	(-250.2) - (-185.4)	3.54*	16.17 *
		Suprema	18.0 (ln (x)-74.31)	3.73	15.30-20.69	(-86.61) - (-62.01)	1.34*, **	6.14*, **

* Significant differences between cultivars at $P<0.05$. ** Significant differences between localities at $P<0.05$. ns: No significant differences. RMSE: Root mean square error. CI: Confidence interval. SE: Standard error.

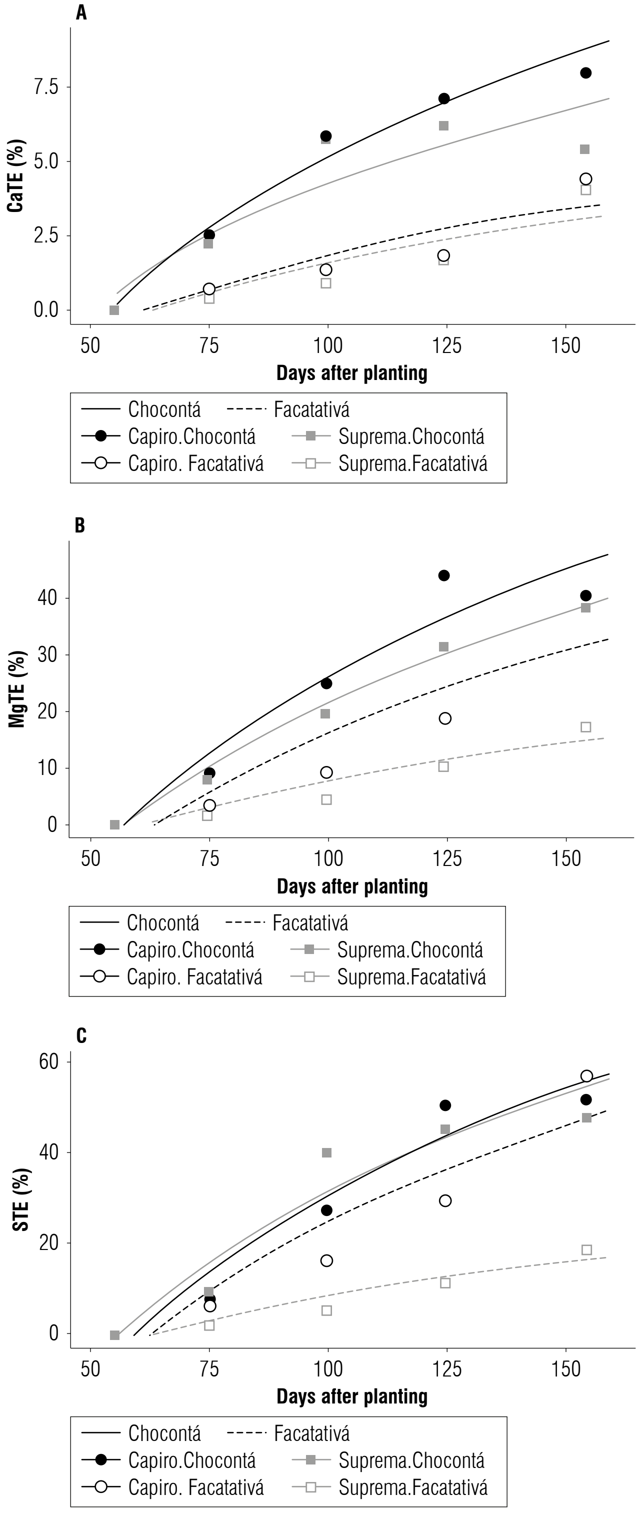

FIGURE 4. Translocation efficiency of Ca (CaTE) (A), Mg (MgTE) (B) and S (STE) (C) in Diacol Capiro and Pastusa Suprema potato cultivars in two localities with contrasting edaphoclimatic conditions.

Nutrient use efficiency

The effect of locality and cultivar were highly significant (*P*<0.01) for CaUE, MgUE and SUE, showing locality x cultivar interaction only in SUE. The use efficiency of the three nutrients was significantly higher (*P*<0.01) in Chocontá compared to Facatativá for the two cultivars (Fig. 5). For CaUE, no differences were found between cultivars in the two localities, however, in Facatativá, Capiro showed significantly (*P*<0.05) higher MgUE and SUE compared to Suprema. In Chocontá, no differences in UE were observed. Sulfur was the nutrient with the highest use efficiency with an average of 426 kg of tuber dry matter per kg of extracted nutrient, followed by Mg with 351 kg and Ca with 176 kg.

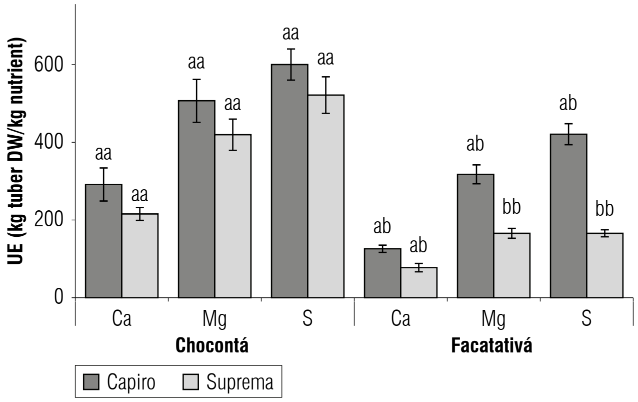

FIGURE 5. Ca, Mg and S use efficiency (UE) in Diacol Capiro and Pastusa Suprema potato cultivars in two localities with contrasting edaphoclimatic conditions. The first letter indicates difference between cultivars within the same locality; the second letter indicates significant differences between localities for the same cultivar. Error bars correspond to standard error.

Nutrient acquisition or recovery efficiency

There was no effect of the locality or the cultivar on RFCa; however, RFMg and RFS did show an effect of the locality (*P*<0.01). Suprema had a significantly higher RFMg and RFS in Chocontá (*P*<0.01) compared to Facatativá (Fig. 6). No significant differences were found between cultivars. The highest recovery efficiency was found for S, followed by Mg and Ca. In Facatativá, negative values were recorded for the three nutrients.

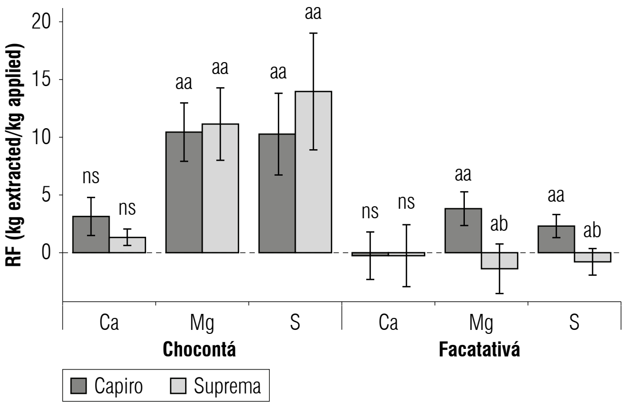

FIGURE 6. Ca, Mg, and S recovery efficiency for Diacol Capiro and Pastusa Suprema potato cultivars in two localities with contrasting edaphoclimatic conditions. The first letter indicates the difference between cultivars within the same locality and the second letter indicates significant differences between localities for the same cultivar. Error bars correspond to standard error.

Discussion

Biomass accumulation was directly related to the tuber formation as these are storage organs and constitute a high proportion of the total biomass (Ferreira *et al.*, 2019). The low translocation between 0-75 DAP is due to the fact that this stage involves the process of cellular differentiation and formation of primary stems. This stage is characterized by a low growth rate due to the limited number of storage cells, resulting in a low nutrient sink strength in the tubers (Gómez *et al.*, 2019). The greater accumulation of dry biomass between 75-100 DAP coincides with stages I and II of secondary stem formation and maximum tuberization. The faster growth observed in Suprema may be related to its indeterminate growth, and the greater accumulation of nutrients in Facatativá may be attributed to a higher cation exchange capacity (CEC) of the soil, due to its higher content of organic matter (Soriano Soto, 2020). Additionally, the higher average temperature in Facatativá may positively affect this process by accelerating metabolic processes, growth and nutrient demand; it may also be related to a higher average soil temperature (variable not monitored) that increases the intake of nutrients by the roots (Bassirirad, 2000). Although different values were found for the Ca and Mg total accumulation between the cultivars and localities, these were similar for the tuber nutrients accumulation. This suggests that the first parameter does not determine the second one. The locality did not affect the nutrient accumulation in tubers, which may be related to the fact that nutrient translocation through the phloem is mainly given by nutrient characteristics (atomic weight, valency, ionic radius, among others) and the sink force. The absence of differences between the cultivars initially indicates that the demand or sink strength by the tuber is similar for both.

The total accumulation of Ca, Mg, and S was higher than values reported by other authors. For the Asterix variety, with yields of 62 t ha^{-1}, Silva *et al.* (2020) reported a total accumulation of Ca of 37 kg ha^{-1} and 2.7 kg ha^{-1} in tubers. For the cv. MNF-80, with a yield of 36 t ha^{-1}, Cabalceta *et al.* (2005) reported a total accumulation of 12, 10, and 11 kg ha^{-1} of Ca, Mg, and S, respectively. For the Agatha variety, with a yield of 35 t ha^{-1}, Soratto *et al.* (2020) reported a total Mg accumulation of 8 kg ha^{-1} and 5 kg ha^{-1} in tubers, which were lower than those found in the present study. The differences in nutrient accumulation compared to other research may be due to the greater yield in the present study (60-70 t ha^{-1} in both cultivars), which increases the nutrient requirements. Likewise, the influence of genotype and experimental conditions in terms of locality, climate, soil, among others, must be considered.

The accumulation and distribution of nutrients among treatments confirm the effect of the genotype and the environment on crop growth and development (Campos & Ortiz, 2020). The higher accumulation of Ca in the aerial part is consistent with the findings of Silva *et al.* (2020), who reported a Ca partition of 93-95% in the aerial part and 5-7% in the tubers. This can be explained by the low mobility of Ca in phloem and its movement by transpiration stream (Silva *et al.*, 2020). Since the tubers have a low transpiration rate compared to the aerial parts, the accumulation of Ca in tubers is limited. These observations are consistent with that reported by different authors (Cabalceta *et al.*, 2005; Fernandes *et al.*, 2011). The greater accumulation of Ca in the tuberization stage and its low mobility highlight the importance of nutrient management that favors the availability and use of Ca by the plants from the early stages of growth. In this case, the importance of the fertilizer applications directed to the tubers is highlighted.

The total accumulation of Mg and tuber Mg was higher than that reported by Silva *et al.* (2020) for four potato varieties. However, the results were in line with the accumulation pattern proposed by the authors, in which the accumulation of Mg in the aerial part increased as the development of the crop advanced, but when tuberization began, Mg accumulation was mainly in the tubers. The decrease in Mg accumulation in the tubers towards the end of the cycle (maximum tuber maturation) may be attributed to a lower Mg requirement due to the reduction in the transport of photoassimilates, where Mg acts as an allosteric activator of ATPases (Koch *et al*., 2020). The lower production of proteins may be linked to the decrease in the tuber content of Mg and S at the end of the cycle, as Mg participates in the synthesis of amino acids, and sulfur is a structural part of amino acids such as methionine and cysteine (Koch *et al*., 2020; Wszelaczyńska *et al*., 2020).

The highest TE of nutrients to the tuber coincided with the tuberization and tuber filling stages, stages in which translocation of photoassimilates, cell wall formation and protein synthesis are required (Naumann *et al*., 2020). Capiro was the genotype with greater sink force and was more efficient in the translocation of nutrients to the tubers, regardless of the locality. In contrast, Suprema showed better performance under the edaphoclimatic conditions of Chocontá (soils of low fertility and higher altitude). The differential behavior of cultivars in the Chocontá locality corroborates that, even though both belong to the Andigenum Group, there are genotypic differences that influence their performance in this locality. These results agree with what was previously reported for nutrients K and P by Gómez *et al.* (2019).

Calcium was the element with the greatest variation in TE between localities. This is related to the mean differential temperature between both. Since Ca moves by the transpiration current, the higher evapotranspiration in Chocontá could have favored its translocation. This variable is influenced by the direct accumulation of Ca in the tubers through the roots that develop on tubers and stolons (Koch *et al*., 2020; Palta, 2010). The low average translocation of Ca was related to the fact that Ca is used mainly as a structural component of cell walls and membranes and has low mobility to the tubers. On the other hand, the higher values of Mg and S are due to their requirement for the translocation of photoassimilates, protein formation, and the regulation of cation-anion transport (Campos & Ortiz, 2020; Koch *et al*., 2020). The translocation of Ca in both cultivars could indicate similar requirements that could be seen in homogeneous characteristics such as postharvest quality and resistance to mechanical damage; however, this must be confirmed with future research. Likewise, it is of interest to establish in future studies whether Capiro's higher Mg and S translocation is reflected in higher protein and starch contents.

The UE values in Chocontá were higher because the production of tubers (dry weight) generally was higher and resulted from a significantly lower total accumulation of nutrients. This may be related to the greater evapotranspiration in Chocontá, which favors the accumulation and partitioning of nutrients and photoassimilates. On the other hand, Capiro proved to be the cultivar with the highest nutrient efficiency in both localities. These results should not be confused with the performance in total production, where Suprema in Chocontá had the highest production in terms of dry weight (Gómez *et al*., 2018), while, in Facatativá, it was Capiro. It is of interest for future research to investigate why, in Facatativá, the difference was significant only for Mg and S. In recent years, research has increased on the intake of nutrients; however, studies on internal nutrient use efficiency are scarce (Tamagno *et al*., 2017).

RF must be interpreted according to three conditions or scenarios that can occur separately or together: amount of nutrient that is taken up based (1) on the plant requirements, (2) on plant capacity (plant-roots), or (3) on plant possibility (external soil-climate conditions). Even though in the Chocontá locality the fertilization dose was higher, the RF of the three nutrients was higher than in Facatativá. This shows the greater need due to the condition of low soil fertility at the locality (more acidic pH, lower CEC and nutrient content). The higher RF in Chocontá is not considered as a response to a better root development since, even though the root weight was not recorded, the amount of the plant-absorbed nutrients was higher in Facatativá. Negative or low RF values in Facatativá should be understood as low or no need for the nutrient supply since the nutrients supplied were not used, possibly due to an excess of application or an imbalance between nutrients. To establish the effect of soil characteristics on RF, it is necessary in the future to establish the amount of nutrient that is lost by leaching or immobilization. It should be noted that the RF of Ca, Mg and S is lower (<15%) compared to that reported in previous publications for the macronutrients N and K (> 40%) (Gómez *et al*., 2018), due to their easy loss in the soil by leaching or by immobilization (Senbayram *et al*., 2015; White & Broadley, 2003). Ramaekers *et al.* (2010) suggest that to increase the RF, it is crucial to promote greater roots development, better hierarchical distribution of roots and a greater production of absorbent hairs, which would favor nutrient uptake. Additionally, a greater availability of nutrients in the soil must be ensured, considering its composition, incompatibility, and imbalance between nutrients, among others. Finally, the effect of continuous cultivation cycles on the properties of the soil and on its fertility should be considered, as over time these can affect RF (Duarte *et al*., 2019; Stewart, 2007).

Conclusions

The cultivars Diacol Capiro and Pastusa Suprema show different accumulation and translocation processes for Ca, Mg, and S, which depend on the genotype x environment interaction. Capiro shows greater adaptation to the climate and soil conditions of Facatativá, while Suprema performs better in Chocontá. Nutrient requirements follow the order of Ca > Mg > S, while translocation follows S > Mg > Ca. A more in-depth analysis of the nutrition physiology of these nutrients reveals that even when accumulation was favored by Facatativá conditions, the use, recovery and translocation efficiencies of nutrients were higher in Chocontá, positively impacting plant performance in this locality. The model curves for accumulation, as well as translocation, use and recovery efficiencies of Ca, Mg and S were established and can be used as a tool to adjust Ca, Mg, and S fertilizer doses based on crop nutrient requirements.

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Conflict of interest statement

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

Author contributions

KC: Methodology, visualization, and writing — original draft; MIG: conceptualization, funding acquisition, and research; LER: supervision, validation. All authors reviewed and approved the final version of the manuscript.

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Plant growth promoting bacteria as a tool to mitigate salt stress in crops: A review

Bacterias promotoras de crecimiento vegetal como una herramienta para mitigar el estrés salino en cultivos: una revisión

Juan Pablo Hernández$^{1, 2*}$, Edgar Palacios-Ortega$^{3, 4}$, and Helber Enrique Balaguera-López^{5}

ABSTRACT

Salinity is a factor that negatively affects the physiology of most plants, even placing food security at risk when it affects plants grown for food. This review provides an overview of the use of plant growth-promoting bacteria (PGPB) as a strategy for enhancing crop growth under salt stress, aiming to provide a sustainable solution for this environmental problem. Salinity causes morphophysiological and biochemical alterations in plants due to osmotic and ionic stress. Plants have different response mechanisms that allow them to survive and, in some cases, tolerate salinity. Various mitigation strategies have been evaluated, such as the use of plant hormones, fertilizers, nanofertilizers, silicon, antioxidants, tolerant genotypes, and inoculation with microorganisms, among others. Among the organisms used for inoculation, PGPB are of particular interest. PGPB, with the capacity to tolerate salinity conditions, can enhance germination, seedling vigor, root and shoot growth, and chlorophyll content in plants, in addition to other positive impacts. The mechanisms of action of PGPBs have been extensively studied and used to improve the quality of commercial crops and to produce bioinoculants. The study of these microorganisms is ongoing; more knowledge is needed on the mechanisms of action of the bacteria, the mechanisms of colonization, and the genes involved in the mechanisms of promotion and colonization. Additionally, it is necessary to expand knowledge of the most efficient ways to use these organisms in crops of commercial and environmental interest.

Key words: PGP bacteria, inoculation, biofertilizers, salinity, halotolerant rhizobacteria, abiotic stress.

RESUMEN

La salinidad es un factor que afecta negativamente la fisiología de la mayoría de las plantas, llegando incluso a poner en riesgo la seguridad alimentaria cuando afecta a las plantas cultivadas para producir alimentos. Esta revisión provee una descripción general del uso de las bacterias promotoras del crecimiento vegetal (BPCV) como una estrategia para mitigar el estrés salino en plantas, con una mirada para proveer una solución sustentable a este problema ambiental. La salinidad provoca alteraciones morfofisiológicas y bioquímicas en plantas debido al estrés osmótico e iónico. Las plantas tienen diferentes mecanismos de respuesta que les permiten sobrevivir y en algunos casos tolerar la salinidad. Se han evaluado diferentes estrategias de mitigación, como el uso de hormonas vegetales, fertilizantes, nanofertilizantes, silicio, antioxidantes, genotipos tolerantes e inoculación con microorganismos, entre otros. Entre los microorganismos utilizados para la inoculación, son de particular interés las BPCV. Las BPCV con capacidad de tolerar condiciones de salinidad pueden mejorar los procesos de germinación, el vigor de las plántulas, el crecimiento de raíces y brotes y el contenido de clorofila en las plantas, además de otros impactos positivos. Los mecanismos de acción de las BPCV han sido ampliamente estudiados y utilizados para mejorar la calidad de los cultivos comerciales y para la producción de bioinoculantes. El estudio de estos microorganismos está en curso. Se necesita más conocimiento sobre los mecanismos de acción de las bacterias, los mecanismos de colonización y los genes involucrados en los mecanismos de promoción y colonización. Además, es necesario ampliar el conocimiento sobre la forma más eficiente de utilizar estos organismos en cultivos de interés comercial y ambiental.

Palabras clave: BPCV, inoculación, biofertilizantes, salinidad, rizobacterias halotolerantes, estrés abiótico.

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[1] Dirección de Investigación y Posgrado, Universidad Rosario Castellanos, Ciudad de México (Mexico).
[2] The Bashan Institute of Science, Auburn, AL (USA).
[3] Biology Research Laboratory, Biology Research Group, Biology Program, Faculty of Science, Universidad El Bosque, Bogotá (Colombia).
[4] Doctorado en Ciencias Ambientales y Sostenibilidad, Universidad Jorge Tadeo Lozano, Bogotá (Colombia).
[5] Universidad Nacional de Colombia, Facultad de Ciencias Agrarias, Departamento de Agronomía, Bogotá (Colombia).
* Corresponding author: pablo@bashanfoundation.org

Introduction

The decrease in crop yield and quality worldwide is due to biotic and abiotic stress. Salinity is among the primary factors that induce abiotic stress in plants. It is considered one of the most significant environmental problems in the world, posing a threat to food security (Ibrahimova *et al.*, 2021) and jeopardizing the achievement of the Sustainable Development Goals. Statistics indicate that saline soils represent more than 6% of the total land area and approximately 20% of the total cultivated area globally; the latter percentage could increase to 50% by 2050 (Bullaín Galardis *et al.*, 2022). With significant economic loss, Qadir *et al.* (2014) estimated that salt-affected irrigated lands result in annual income losses of approximately USD 27.3 billion (adjusted for inflation) due to reductions in crop yields.

Salinity is considered an excess of soluble salts present in the soil (Ansari *et al.*, 2022). This excess is due to natural causes in areas with arid and semi-arid soils, as well as anthropogenic factors (Bullaín Galardis *et al.*, 2022), including poor irrigation practices in agricultural areas and increased evaporation during drought due to climate change (Van Zelm *et al.*, 2020).

Plants under salinity conditions present morphophysiological and biochemical alterations due to osmotic and ionic stress (Safdar *et al.*, 2019). The production of reactive oxygen species causes membrane damage, a decrease in photosynthesis, nutrient imbalance, changes in hormone concentrations, enzyme inactivation, and metabolic dysfunction (Fu & Yang, 2023; Negrão *et al.*, 2017) resulting in decreased growth and crop production.

There are halophytic species that can tolerate high levels of salinity. But most cultivable plants are glycophytes, which are very sensitive to salt stress. These plants present various mechanisms at the physiological, biochemical, and molecular levels in response to stress (Acosta-Motos *et al.*, 2017; Ismail & Horie, 2017). However, in severe cases of salinity stress, defense mechanisms are compromised, and plants suffer the effects of stress (Nigam *et al.*, 2022). Nonetheless, several management strategies can help mitigate salinity damage, including genetic improvement, the use of organic and inorganic amendments, fertilization management, the presence of mycorrhizae, the application of antioxidant substances, and growth regulators, among others. Additionally, the use of plant growth-promoting bacteria (PGPB) is a promising strategy.

PGPBs have been evaluated for mitigating different biotic and abiotic stresses. The role of PGPB in inducing tolerance to salt stress through the provision of various nutrients, conservation of a high K^+/Na^+ ratio, increased osmolyte accumulation, increased photosynthetic rates and biomass, and the activity of antioxidant enzymes have been reported in different crops (Bhise & Dandge, 2019; Kushwaha *et al.*, 2020; Nigam *et al.*, 2022). These include leguminous (Khan & Basha, 2015), lettuce (Fasciglione *et al.*, 2015), wheat (Ramadoss *et al.*, 2013) among others. The issue of salt stress in crops represents a significant challenge for global agricultural production. Various approaches have been explored to mitigate its effects, including the use of PGPB. However, it is essential to establish a clear connection between salt stress and the functionality of these bacteria, allowing for a better understanding of their mechanisms of action and their applicability in different agricultural contexts. This review provides an overview of the use of PGPB as an alleviation strategy for crop plants under salt stress, deepening the understanding mechanism of the mechanism at the molecular, biochemical, and physiological levels to provide a sustainable and practical solution for this environmental problem. Aspects related to the main bacterial strains, inoculants, and biotechnology processes are included.

Effects of salt stress on plant physiology

The adverse effects of salt stress in reducing plant growth and productivity have two leading causes according to Ismail and Horie (2017). The first of these is osmotic stress, which reduces water uptake by the roots, resulting in effects similar to those caused by water deficit. The second cause is the direct accumulation of salts, which leads to ionic toxicity, resulting in a nutritional imbalance and decreased transport. This, in turn, alters the metabolic processes, primarily at the level of photosynthesis (Ismail & Horie, 2017; Munns & Tester, 2008).

Salinity affects leaf area expansion, photosynthetic machinery, and related traits through stomatal closure, decreased chlorophyll content, and altered chloroplast structure. This, in turn, affects the light reactions, primarily the quantum efficiency of photosystem II and CO_2 assimilation (Castillejo-Morales *et al.*, 2021; Khalil *et al.*, 2022; Pan *et al.*, 2021). Salinity is reported to induce the denaturation of membrane proteins involved in photosynthesis (Bahmani *et al.*, 2015) and the degradation of chlorophyll molecules through the elevation of enzyme activity related to chlorophyll degradation.

Salt stress disrupts key physiological processes in plants, impairing enzyme activity, increasing photorespiration, and triggering the accumulation of reactive oxygen species (ROS), which leads to oxidative stress (Fu & Yang, 2023).

This oxidative damage manifests as lipid peroxidation, membrane instability, and protein degradation (Bose *et al.*, 2014; Habib *et al.*, 2016). Additionally, salt stress weakens cellular integrity by reducing antioxidant defenses and disrupting osmotic balance (Bose *et al.*, 2014; Fu & Yang, 2023). It also interferes with nitrogen metabolism by inhibiting essential enzymes (Ashraf *et al.*, 2018). It hampers growth by reducing seed germination, root development, and biomass accumulation (Zhang *et al.*, 2019). Furthermore, salt stress alters hormonal balance, decreasing the levels of auxins, cytokinins, and gibberellins while increasing the levels of abscisic acid (ABA) (Zulfiqar & Ashraf, 2021). Collectively, these effects significantly reduce crop productivity (Ansari *et al.*, 2022).

Plant salt-response mechanisms

The most important mechanisms that plants have for enhancing their tolerance to salt stress are enzymatic and non-enzymatic detoxification mechanisms, which efficiently sweep up reactive oxygen species (ROS) and involve changes in the cell wall (Mbarki *et al.*, 2018). Enzymatic mechanisms include superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), dehydroascorbate reductase (DHAR), monodehydroascorbate reductase (MDHAR), and glutathione reductase (GR) (Fu & Yang, 2023; Laus *et al.*, 2021). The primary non-enzymatic water-soluble antioxidants include ascorbate (AsA), reduced glutathione (GSH), and phenols, as well as lipid-soluble compounds such as tocopherols and carotenoids (Apel & Hirt, 2004; Hasanuzzaman *et al.*, 2021). An increase in the antioxidant activity of the plants under salinity could be a good indicator of salt tolerance (Abbas *et al.*, 2022; Nigam *et al.*, 2022).

Another mechanism to cope with salinity stress is osmotic regulation. Ibrahimova *et al.* (2021) mention that several compounds called osmoprotectants are involved in the osmotic regulation of plants, such as sucrose, sorbitol, mannitol, glycerol, arabinitol, pinitol (carbohydrates), proteins, glutamate, aspartate, glycine, proline (nitrogen compounds), malate, and oxalate (organic compounds) (Gupta & Huang, 2014). These compounds maintain a lower water potential within the cells, preventing dehydration and protecting cell structure and biomolecules. Nigam *et al.* (2022) reported that the induction of osmoprotectants is strongly related to improved salt tolerance.

The synthesis and accumulation of stress proteins are another important mechanism for salt tolerance. Some of these proteins include Ca-binding proteins of the plasma membrane involved in signal transduction, such as annexin (Parihar *et al.*, 2015), cytochrome b6-f, ATP synthase subunit c, germin-like proteins, S-adenosylmethionine synthase protein (Kamal *et al.*, 2012), and aquaporins. Other essential proteins include the Heat Shock Proteins (HSP) family, acting as a chaperon; jacalin lectin, which is involved in protein-saccharide interactions and signal transduction; and osmotic, one of the proteins associated with osmotic stress (Ibrahimova *et al.*, 2021).

At the molecular level, plants can respond to or tolerate salt stress by activating enzymes and transcription factors. Kinases and phosphatases play a crucial role in stress signal transduction, modifying proteins to trigger adaptive responses. Among the most critical regulators are Heat Shock Factors (HSFs), which activate heat shock proteins (HSPs) that protect cellular structures, stabilize enzymes, and prevent protein denaturation in response to stress. Additionally, CBF/DREB (C-repeat-binding factor/Dehydration-Responsive Element-Binding Protein). This family of transcription factors binds to drought- and cold-responsive elements in DNA, enhancing the expression of genes that improve osmotic adjustment and antioxidant defense; ABF/ABRE (ABA-Responsive Element Binding Factor/ABA-Responsive Element, when abscisic acid, (ABA) levels rise under stress, these factors bind to ABA-responsive promoter elements, activating genes involved in stomatal closure, ion homeostasis, and stress tolerance (Bahmani *et al.*, 2015; Zhang *et al.*, 2013). The response also includes osmo-sensors (such as ATHK1), phospholipid-cleaving enzymes, and the critical secondary messengers such as Ca^{2+}, PtdOH, ROS, metabolic phosphatases, Ca-dependent protein kinases, serine/threonine protein kinase, mitogen-activated protein kinase cascades, two-component histidine kinases, and Ca/calmodulin-activated serine/threonine-specific protein phosphatases (Bahmani *et al.*, 2015). As salinity tolerance is controlled by the interaction of several genes, it encompasses numerous physiological and biochemical processes that interact with one another to resist salt stress at the molecular, cellular, and whole plant levels (Acharya *et al.*, 2022; Bahmani *et al.*, 2015; Ismail *et al.*, 2007). The authors include an extracellular salt sensor, monocation-induced [Ca^{2+}]. It increases 1 (MOCA1), as well as Glycosyl inositol phosphorylceramide (GIPC), the salt overly sensitive (SOS) pathway (Acharya *et al.*, 2022; Chen *et al.*, 2025; Zhu, 2001).

NaCl is the most common salt causing soil salinity. In this regard, essential mechanisms that have been reported include membrane Na^+ transport systems functioning in avoidance of Na^+ toxicity, Na^+ efflux from roots to the rhizosphere, Na^+ sequestration in vacuoles, loading and unloading at the xylem, ROS signaling, essential Na^+ transport systems for plant salt tolerance, channels for Na^+ influx into roots, channels and transporters that affect K^+ homeostasis and Cl^- transport and homeostasis (Ismail & Horie, 2017). It is possible to increase gene expression of *NHX1* (Na^+/H^+ antiporter), which regulates Na^+ sequestration in vacuoles to maintain osmotic balance (Chen *et al*., 2025; Zhu, 2001).

Van Zelm *et al.* (2020) report that ABA is the primary plant regulator responsible for signaling salt and osmotic stress in guard cells and root tissues, regulating the process of growth and development. The same authors indicated that, by targeting and phosphorylating downstream components, both ABA-independent sucrose non-fermenting 1–related protein kinase 2 (SnRK 2) and ABA-dependent SnRKs have essential roles in transcriptional regulation and post-transcriptional regulation during a salt stress response. A graphical summary of saline stress in plant physiology is shown in Figure 1.

Salt stress mitigation alternatives

Several strategies have been evaluated to mitigate salt stress, some of which are summarized in Table 1. Various types of nanoparticles and nanofertilizers have shown promising results so far in managing salt stress; for more details, we recommend consulting the review by Zulfiqar and Ashraf (2021). Regarding mineral nutrition, the use of silicon has garnered attention as a beneficial element that confers tolerance to different types of stress, including salinity stress, mainly by increasing antioxidant capacity (El-Serafy *et al*., 2021). The beneficial effect of potassium under salinity stress is also promising, primarily because its ion plays a crucial role in various processes, including enzymatic

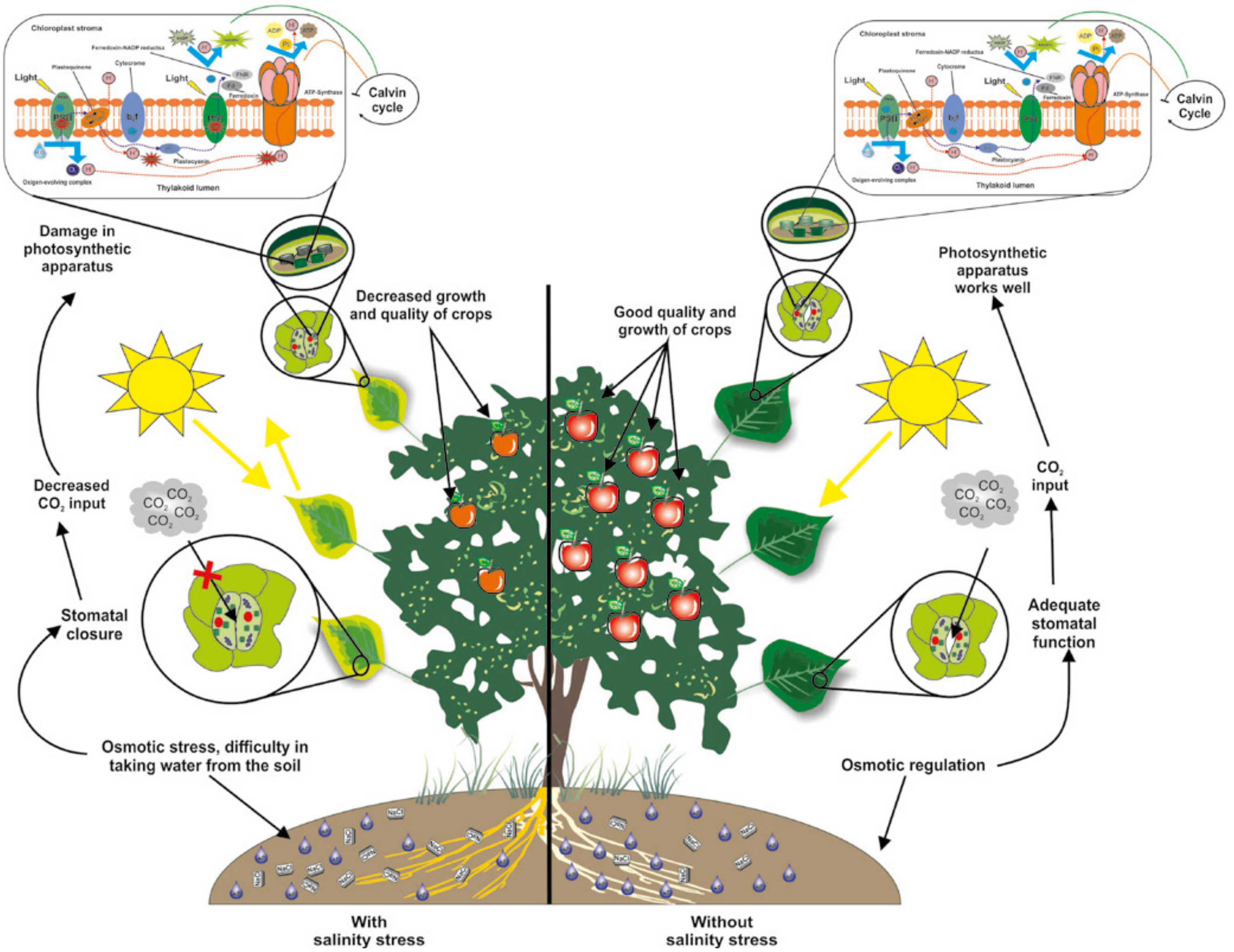

FIGURE 1. Effect of saline stress on the plant physiological processes.

activation, protein synthesis, photosynthesis, stomatal regulation, cation-anion balance, energy transmission, and osmoregulation (Abbas *et al.*, 2022).

Some studies have indicated that salt tolerance can be conferred to various plant species with exogenous applications of signaling molecules such as nitric oxide and hydrogen peroxide, protectants such as glycine-betaine and proline, and trace elements such as zinc and cobalt (Altaf *et al.*, 2020; Brengi *et al.*, 2022; Negrão *et al.*, 2017; Parihar *et al.*, 2015). Others have reported this effect through plant regulators, mainly abscisic acid, jasmonic acid, brassinosteroids, salicylic acid, and melatonin (Assaf *et al.*, 2022; Brengi *et al.*, 2022). Through genetic improvement, it is also possible to efficiently reduce the effects of salinity. For example, in potato plants, the overexpression of the *StCYS1* gene results in the accumulation of more proline and chlorophyll, thereby enhancing the plant's resistance to high salinity. The transgenic approach facilitates the overexpression of several vital genes, including *DREB, MYB, COMT, SOS, PKE,* and *NHX*, which confers salt stress tolerance (Ibrahimova *et al.*, 2021; Zhang *et al.*, 2013). In alfalfa, overexpression of an ABA-inducible homeodomain-leucine zipper I gene *MsHB7* confers salt stress (Li *et al.*, 2022).

Managing sustainable crop production under soil salinity conditions will be a significant challenge in the coming years (Nigam *et al.*, 2022). The use of microorganisms is a more sustainable alternative to address this challenge. Recently, the role of PGPB in inducing salt stress tolerance through various mechanisms has been reported, with auspicious results for agricultural production (Ali *et al.*, 2022; Kushwaha *et al.*, 2020; Nigam *et al.*, 2022; Sun *et al.*, 2025). In this regard, Bullaín *et al.* (2022) report that the ECM fungus *Scleroderma bermudense* improved salt tolerance in seagrape seedlings. The inhibitory effect of NaCl stress on maize seed germination and seedling growth was mitigated by *Bacillus subtilis* HS5B5 (Bullaín Galardis *et al.*, 2022). Sun *et al.* (2025) found that *Glutamicibacter endophyticus* J2-5-19 resists salt stress by expelling sodium ions and taking up potassium ions through Na^+/H^+ antiporters, and K^+ uptake proteins while also accumulating compatible solutes such as betaine, proline, and trehalose. Therefore, it has the potential for the development of microbial inoculants.

Plant growth-promoting bacteria

Plant growth-promoting bacteria (PGPB) are defined as free-living or endophytic bacteria that can directly or indirectly affect optimal biotic conditions for plants or mitigate

TABLE 1. Some mitigation strategies for salt stress in plants.

Salt stress mitigation alternative	Crop	Response	References
Nanoparticles	Pea (*Pisum saivum* L.)	Stimulated cyclic electron transport around photosystem I, thus protecting its photochemical activity.	(Mohamed *et al*., 2017)
Silicon	Sweet pea (*Lathyrus odoratus*)	Improved photosynthetic pigments, antioxidant activity, and phenolic compounds.	(El-Serafy *et al*., 2021)
Potassium	Wheat (*Triticum aestivum*)	Improved stomatal conductance, photosynthetic pigments, and antioxidant enzymes.	(Abbas *et al*., 2022)
Melatonin	Tomato (*Solanum lycopersicum*)	Improved redox homeostasis by the antioxidant system.	(Altaf *et al*., 2020)
Melatonin and Cobalt	Cucumber (*Cucumis sativus* L.)	Both treatments increased protein content, essential nutrient content, and catalase (CAT) activity.	(Brengi *et al*., 2022)
Salicylic acid	Barley (*Hordeum vulgare*)	Increased the content of calcium, iron, magnesium, and potassium while lowering the concentrations of sodium and malondialdehyde and electrolyte leakage.	(El-Esawi *et al*., 2017)
Abscisic acid	Tomato (*Solanum lycopersicum*)	Increased antioxidant capacity, proline content, and decreased stomatal conductance, ROS, and MDA.	(Hu *et al*., 2021)
Overexpression of *StCYS1* gene	Potato (*Solanum tuberosum* L.) plant	Transgenic plants accumulated more proline and chlorophyll, significantly increasing their resistance to high salinity.	(Liu *et al*., 2020)
Humic acid	Sorghum (*Sorghum bicolor* L.)	Improved water relations, stomatal conductance, and activation of the antioxidant enzymes (CAT, POD, and SOD).	(Ali *et al*., 2019; Hatami *et al*., 2018)
Mycorrhizal	Seagrape (*Coccoloba uvifera* L.)	Beneficial effects on the photosynthetic and transpiration rates, chlorophyll fluorescence and content, stomatal conductance, and water status.	(Bullaín Galardis *et al*., 2022)
Salicylic acid and PGPB (*Stenotrophomonas* sp.)	Spinach (*Spinacia oleracea* L.) and soybean (*Glycine max*)	Improve plant growth and yield, enhanced relative water contents, accumulated osmolytes, and increased enzymatic and non-enzymatic antioxidants.	(Nigam *et al*., 2022)

the effects of abiotic stress. These bacteria establish various relationships with plants that can be beneficial or harmful. These relationships can be symbiotic or non-symbiotic and are useful when regulatory substances are produced that enhance the plant's growth but detrimental when they are pathogenic (Di Benedetto *et al*., 2017). There are various mechanisms by which these relationships can directly affect the metabolism of plants, providing them substances necessary for their growth. These include the following: (1) nitrogen fixation, these bacteria are called diazotrophic bacteria and can fix atmospheric nitrogen and convert it to ammonium using a nitrogenase enzyme, a mechanism known as biological nitrogen fixation (BNF) (Pankievicz *et al*., 2021); (2) phosphate solubilization, bacteria solubilize phosphorus through the production of organic acid that reduce soil pH, converting them to more soluble forms that can be absorbed by plants, with the most common soluble form of P capable of absorption by plants being $H_2PO_4^-$ (Billah *et al*., 2019); and (3) production of plant hormones (Bashan & de Bashan, 2005), bacteria produce molecules that can affect plant development such auxins (indole-3-acetic acid (IAA)), abscisic acid (ABA) (Çakmakçı *et al*., 2020), gibberellins (Nett *et al*., 2017), and cytokinins. On the other hand, some mechanisms indirectly prevent the effects of phytopathogenic microorganisms by producing substances that inhibit microbial growth, alter the plant's metabolism, and increase resistance to infection (Bashan & de Bashan, 2005).

PGPBs have been studied for their mechanisms of action and potential applications. Most studies have focused on their ability to enhance the growth and productivity of commercial crops (Souza *et al*., 2015). Additionally, PGPBs have been explored for their role in ecological restoration, particularly in reforestation and rehabilitation of degraded soils (Bashan *et al*., 2012; Lopez *et al*., 2012). Beyond agriculture, PGPBs have been utilized in environmental biotechnology, such as wastewater treatment, when combined with microalgae (Choix *et al*., 2014; Glick, 2012; Palacios *et al*., 2014). They are also key components in the development of biofertilizers and bioinoculants, offering sustainable alternatives to chemical inputs.

Many bacteria have been identified and used commercially as plant growth promoters, including the genera *Bacillus, Agrobacterium, Azotobacter, Azospirillum, Serratia, Streptomyces,* and *Pseudomonas,* among others.

At the molecular level, various studies have been conducted to identify the genes that are involved in the mechanisms of action of PGPB and their interaction with plants. Jijón-Moreno *et al*. (2015) investigated the *ipdC*, *hisC1*, and *hisC2* genes, which are involved in the production of indole acetic acid in *Azospirillum brasilense.* Wisniewski-Dye *et al*. (2012) sequenced the complete genome of the *A. brasilense* CBG497. Romero *et al*. (2014) performed pyro sequencing of the 16S rRNA gene to identify the composition of endophytic bacteria present in tomato leaves. Finally, Bruto *et al*. (2014) studied 23 genes that provide 8 essential benefits for plants, finding that they can be associated and selected depending on the plant's habitat. They suggest that the PGPR condition is likely due to the accumulation of genes that contribute to the beneficial functions of plants associated with the bacteria associated with the roots.

One of the bacteria that has been most extensively studied for its ability to promote plant growth is *A. brasilense*. This was rediscovered by Döbereiner *et al*. in the 1970s and has since become a model bacterium for studying plant-growth bacteria (PGPBs). We recommend the article written by Cassán *et al*. (2020), which discusses the scientific impact and agricultural applications of this bacterium, as complementary literature on the use of *A. brasilense* as a model.

PGBP alleviates salt stress

To improve plant growth under saline stress conditions and achieve sustainable crop production, it is necessary to enhance salt stress tolerance in crops. Rhizobacteria can improve plant growth and crop productivity of the plants they colonize. *Bacillus* strains induce plant resistance to salt stress and produce various plant hormones that enhance growth (Rajendran *et al*., 2008).

PGPR, with the capacity to tolerate salinity conditions, can improve various processes in plants, enhancing germination, seedling vigor, root and shoot growth, and chlorophyll content, among other aspects (Bal *et al*., 2013; Sarkar *et al*., 2018). Ge and Zhang (2019) and Nigam *et al*. (2022) reported increased growth under salt stress after applying PGPB *Rhodopseudomonas palustris* and *Stenotrophomonas* sp., respectively. Increases in biomass due to PGPB can be attributed to the production and regulation of various plant regulators, such as auxins, which control gene expression by activating a family of transcription factors that perform distinct regulatory functions in plant cells (Numan *et al*., 2018).

PGPB (*Stenotrophomonas* sp.) evaluated in spinach and soybean plants induced K^+ and Ca^{++} uptake and reduced Na^+ uptake, even at a higher level compared to when salicylic acid was implicated. However, a higher Na^+/K^+ ratio under salt stress suggests that this defense mechanism is not highly influenced by the exogenous application of protectants (Nigam *et al.*, 2022).

Some authors have suggested that PGPB is an effective regulator of photosynthesis due to its favorable influence on leaf structure, chloroplasts, and photosynthetic pigments (Barickman *et al.*, 2014; Khan & Singh, 2008; Nigam *et al.*, 2022; Salazar-Garcia *et al.*, 2022). PGPB inoculation appears to enhance photosynthetic pigment synthesis by increasing the uptake of nitrogen, potassium, and phosphorus (Nigam *et al.*, 2022).

Some PGPR produce antioxidant enzymes such as superoxide dismutase (SOD), peroxidase (POD), or catalase (CAT), which detoxify reactive oxygen species (ROS) in plants during salt stress (Han & Lee, 2005; Shultana *et al.*, 2021). Increased antioxidant enzyme activities and upregulation of ROS pathway genes (CAT, APX, GR, and DHAR) were observed in PGPR-inoculated okra plants under salinity stress (Habib *et al.*, 2016). However, PGPR can also produce compatible solutes such as proline, an abundant osmolyte in plants, which accumulates in response to osmotic stress induced by factors like drought and salinity (Fazal & Bano, 2016; Galinski & Trüper, 1994; Landa-faz *et al.*, 2021; Sun *et al.*, 2025). For this reason, PGPR can induce changes in total protein, IAA concentration, total sugar, and ethylene in plants, a process that may enhance tolerance to abiotic stress (Sarkar *et al.*, 2018; Upadhyay *et al.*, 2011; Yang *et al.*, 2009) through induced systemic tolerance (Yang *et al.*, 2009). On the other hand, *Enterobacter* sp. P23 was shown to promote rice seedling growth under salt stress by decreasing reactive oxygen species (ROS) and stress-induced ethylene; the latter process has been linked to 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity (Sarkar *et al.*, 2018). ACC deaminase-containing PGPR can enhance the stress tolerance of plants by synthesizing plant hormones, facilitating mineral solubilization, increasing nutrient uptake, increasing leaf area, and improving chlorophyll and soluble protein content, as well as antioxidant enzyme activities (Dobbelaere *et al.*, 2003). Saravanakumar and Samiyappan (2007) found that the ACC deaminase-containing *P. fluorescens* strain TDK1 increased the vigor index of groundnut seedlings using 120 mM of salt stress.

Bacterial exopolysaccharide (EPS) can also help mitigate salinity stress by reducing the content of Na^+ available for plant uptake (Upadhyay *et al.*, 2011). Bacterial polysaccharides are macromolecules that include peptidoglycan, lipopolysaccharides, and exopolysaccharides. These acid water-soluble acids play a role in host-pathogen interactions and serve as structural components of the cell wall, such as peptidoglycan. These compounds function as biologically active substances, promoting bacterial and plant growth while facilitating surface adhesion and preventing desiccation (Shultana *et al.*, 2022).

Inoculants

The term "inoculant" can refer to a bioformulation or biofertilizer and is defined as a matrix that contains one or more microorganisms, is easy to use, can improve the growth of plants (Arora *et al.*, 2010), and acts as a system to control pests and diseases (Preininger *et al.*, 2018). The oldest microorganism used as an inoculant is rhizobia, which can colonize the roots of plants and form nodules (Santos *et al.*, 2019). The commercialization of inoculants began at the end of the seventies in India, where it is produced at a large scale today (Yadav & Chandra, 2014). The first patent of inoculant was registered in the USA in 1896 (Santos *et al.*, 2019). The use of bacterial inoculants has been widely studied and is gaining strength over time in many developed countries (Glick, 2012).

The use of PGPBs in agriculture and soil restoration suggests that alternatives should be sought that allow the inoculation of the bacteria in plants and, above all, guarantee the effectiveness of the inoculum at the time of application, limit the effect of endogenous bacteria that can compete with the inoculant and determine the consortia that can be used (Bashan *et al.*, 2002). On the other hand, the physical characteristics of the inoculant must be considered, such as whether it is liquid or dry and whether the organisms used are latent or not, as well as the timing of when the bacteria are released from the inoculant. In this regard, several methods are available for performing the inoculation. In direct methods, bacteria are incorporated directly into the plants, either physically or through application to the root or the leaves. Meanwhile, indirect methods involve a matrix in which the bacteria are embedded in various inner matrices, allowing them to be released gradually over time. This serves as a protection system against endogenous bacteria, allowing the number of bacteria added to the plant or the ground to be controlled. We suggest reading the review by Bashan *et al.* (2014), which discusses technological perspectives and inoculant formulations. Here, we present various methods for applying inoculants (Fig. 2).

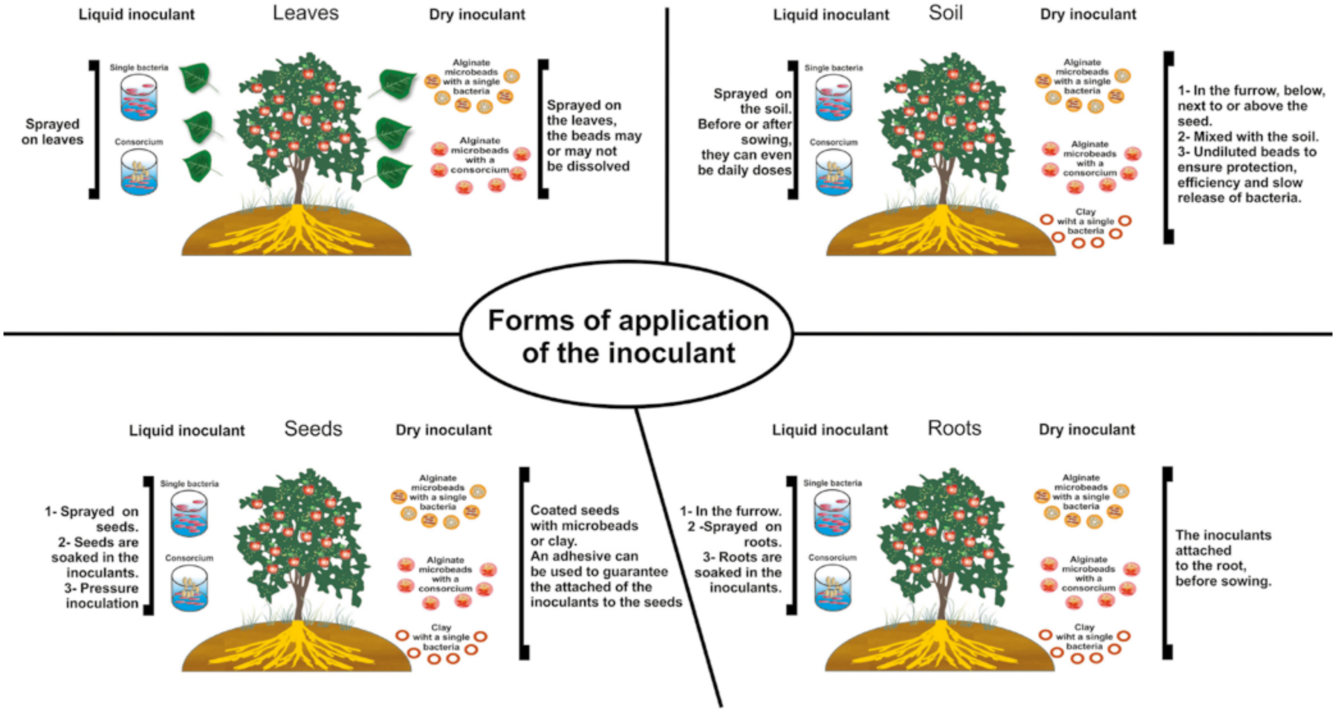

FIGURE 2. Forms of applications of the inoculant in plants.

Recent studies into PGPB have made significant progress, opening new opportunities for the formulation and creation of new inoculants or biofertilizers. These can be used not only for agricultural production but also to mitigate the effects of climate change through forest recovery of different ecosystems (Jack *et al*., 2021). However, despite the advances made in PGPB, the application of these microorganisms for crop improvement continues to be delayed and not widely explored (Baez-Rogelio *et al*., 2017).

To produce inoculants, several factors must be considered. First, the effectiveness of the bacteria and the type of technology to be used in making the inoculant must be known. Secondly, the type of application that will be made to the crop must be identified. Inoculants can be liquid or dry. Liquid inoculants are efficient in some respects; however, their shelf life is very short, and it is generally challenging to control the population of microorganisms. These inoculants are used in the rhizosphere, where they are added to the soil, allowing the roots to interact with the inoculant and then sowing the plant. Alternatively, the seed can even be inoculated by including it in the inoculant, through pressure changes to ensure that the bacteria enter the seed or by spraying in the foliar zone (Bashan *et al*., 2014; Preininger *et al*., 2018). Dry inoculants are typically produced within an inert matrix, which provides them some advantages over liquid inoculants. In general, matrices are used that provide the controlled release of microorganisms, guaranteeing their viability (Albareda *et al*., 2008; Covarrubias *et al*., 2012) and enabling the inclusion of one or more organisms without producing secondary effects on plants or the environment. Several commercial products available on the market vary in shape, composition, and application methods. They can be found in powder, liquid, and granular form. Most commercial inoculant products are peat-based because of their ease of use and their success in supporting rhizobium growth (Buntić *et al*., 2019). Several studies have been conducted to compare the efficiency of liquid inoculants and solid inoculants. Some of the results are presented in Table 2.

TABLE 2. Different types of inoculants and microorganism tests.

Type of Inoculant	Microorganisms	Crops	Effects	References
Encapsulation in alginate, skimmed milk for release	*Azospirillum brasilense*	Wheat and tomato plants	The dry weight of leaves and roots of wheat and tomato seedlings after 21 d was higher for those grown with the inoculant than without	(Bashan *et al*., 2002)
Liquid and peats	*Sinorhizobium* (Ensifer) *fredii* SMH12 or *Bradyrhizobium japonicum* USDA110	Soybean	Both types of inoculants used presented a similar seed yield	(Albareda *et al*., 2008)
Liquid and zeolite	*Bacillus subtilis, B. megaterium, A. chroococcum,* and *A. vinelandii*		All bacteria growth after 28 d of preparation of the inoculants	(Hindersah *et al*., 2021)
Liquid supplemented with agar, alginate, calcium chloride, glycerol, and ferric chloride	*Sinorhizobium meliloti* L3Si	Alfalfa seed	Bacteria can grow in liquid with supplements. Alfalfa can grow with the inoculant	(Buntic *et al*., 2019)
Liquid with exopolysaccharides	*Bradyrhizobium elkanii,* B. *japonicum*, *Bradyrhizobium viridifuturi symbiovar tropici*	Cowpea and soybean	Exopolysaccharides increase the viability of *Bradyrhizobium*, enhancing both plant growth and the viability of all inoculants	(Farias *et al*., 2022)
Liquid	*Enterobacter cloacae*	*Zea mays*	Enhancing maize growth and biomass	(Ali *et al*., 2022)

Conclusions

Saline stress is one of the significant challenges in agricultural production. This is due to the intensive use of chemical fertilizers, the overexploitation of soils, monoculture practices, and the water contamination by urban settlements, which continue to grow. Saline stress affects physiological processes in plants, including the photosynthetic machinery, and decreases the closing capacity of stomata, as well as the assimilation of CO_2.

However, plants have enzymatic and non-enzymatic mechanisms to control the effects of salinity. One of these is osmoregulation or the accumulation of proteins. However, the plant efforts to mitigate the impact of salt are very low due to its excess in the soil, so they are unable to balance the input of salt. In this regard, an alternative that has gained importance is the use of PGPBs. These halotolerant microorganisms can promote, stimulate, and protect plant growth. The use of these microorganisms as a fertilization system will allow crops to increase their production and reduce the use of chemical fertilizers, which contaminate soils and bodies of water. They will also be efficient tools for the conservation and restoration of forest areas, contributing to the preservation of biodiversity and adaptation to climate change.

PGPB has emerged as a valuable tool for the mitigation of salt stress in plants, particularly in crop production systems. Several studies have demonstrated the beneficial effects of PGPB in enhancing plant growth and tolerance to salinity stress. On the other hand, PGPB can enhance plant growth and development, even under saline conditions. They promote root and shoot growth, increase chlorophyll content, and enhance nutrient uptake, leading to improved crop productivity. PGPB can enhance the salt tolerance of crops through various mechanisms, producing and releasing plant hormones (such as auxins, cytokinins, and gibberellins) that stimulate plant growth and aid in mitigating salt stress mitigation. PGPB also produces organic acids and enzymes that improve nutrient availability and ion uptake, thereby reducing the toxic effects of salt.

Salt stress disrupts osmotic balance and ion homeostasis in plants. However, the use of PGPB can help in osmotic adjustment by synthesizing osmoprotectants (*e.g.*, proline) that maintain cellular water potential. They also facilitate the uptake of essentialions (*e.g.*, potassium) while excluding or reducing the uptake of toxic ions (*e.g.*, sodium), thus maintaining ion balance within the plant.

PGPB induces systemic resistance in plants, making them more resistant to salt stress and other biotic stresses. They activate the production of defense-related compounds, such as phytohormones, enzymes, and secondary metabolites, which help plants defend against pathogens and other stressors.

PGPB forms biofilms on the root surface and establishes a strong rhizosphere competence. This enables them to colonize the plant roots effectively, compete with pathogens, and provide a continuous supply of growth-promoting substances to the plants.

The effectiveness of PGPB in mitigating salt stress may vary among different crop species. Some crops exhibit a higher degree of response to PGPB inoculation, while others may show more modest effects. It is crucial to select PGPB strains that are well-adapted to the target crop and its specific environmental conditions.

The use of PGPB as a tool to mitigate salt stress aligns with sustainable agricultural practices. PGPB can reduce the need for chemical fertilizers and pesticides, thereby minimizing environmental pollution. They also contribute to the overall health and biodiversity of the soil ecosystem.

Given the above, PGPB offers excellent potential as a tool to mitigate salt stress in crops. Their ability to enhance plant growth, improve salt tolerance, and induce systemic resistance makes them valuable allies in sustainable crop production systems, especially in saline environments. Further research is needed to explore the specific mechanisms of PGPB action and optimize their application for different crops and agroecosystems.

The review highlights the potential of plant growth-promoting bacteria as a biotechnological tool to mitigate salt stress in crops. However, it is necessary to strengthen research in aspects such as the selection of specific strains, compatibility with different agricultural systems, and the assessment of their long-term impact. This will enable their practical implementation in crop improvement programs and environmental sustainability initiatives.

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Conflict of interest statement

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

Author's contributions

Conceptualization: JPH, EAPO, and HEBL; writing—original draft preparation: JPH, EAPO, and HEBL; writing—review and editing: JPH, EAPO, and HEBL. This research received no external funding. All authors have read and approved the final version of the manuscript.

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Hormones mitigate salt stress in tomato (*Solanum lycopersicum* L.) plants during vegetative growth

Las hormonas mitigan el estrés salino en plantas de tomate (*Solanum lycopersicum* L.) durante el crecimiento vegetativo

Hernán David Ruiz Berrio[1*] and Zulma Catherine Cardenal-Rubio[1]

ABSTRACT

Tomato is one of the most important vegetables in Colombia. This crop is sensitive to salinity, so high salt concentrations in the soil can negatively affect its growth and development; tolerance levels vary among cultivars. A viable strategy used in other cultivated species is the application of phytohormones that help plants acclimate to variable environments. Since few studies report the influence of growth regulators that alleviate this type of stress in tomatoes, this research aimed to determine the effect of different naphthaleneacetic acid and gibberellic acid doses on some physiological and growth parameters in tomato plants subjected to saline stress. We implemented a randomized design with a 2 × 6 factorial scheme. The first factor corresponded to salinity with two levels (0 and 40 mM), and the second factor involved the individual application of hormones (auxins – naphthaleneacetic acid (NAA) and gibberellins – gibberellic acid (ProGibb SP®)) at doses of 50, 75, and 100 µM each, for a total of 12 treatments with six replicates. We evaluated growth variables such as plant height, number of leaves, leaf area, fresh biomass of the aerial part and roots, as well as physiological variables of chlorophyll content and stomatal conductance (g_s). The application of gibberellins at 100 µM demonstrated the ability to mitigate the deleterious effects of salinity on some growth parameters in tomato plants by improving leaf expansion and aerial fresh biomass.

Key words: plant hormones, naphthaleneacetic acid, gibberellic acid, abiotic stress.

RESUMEN

El tomate es una de las hortalizas más importantes en Colombia. Este cultivo es sensible a la salinidad, por lo que las altas concentraciones de sal en el suelo pueden afectar negativamente su crecimiento y desarrollo, con niveles de tolerancia que varían entre cultivares. Una estrategia viable en otras especies cultivadas es la aplicación de fitohormonas, las cuales permiten a las plantas aclimatarse a entornos variables. En este sentido y considerando que pocos estudios reportan la influencia de reguladores de crecimiento en el alivio de este tipo de estrés en tomate, la presente investigación buscó determinar el efecto de diferentes dosis de ácido naftalenacético y ácido giberélico sobre algunos parámetros fisiológicos y de crecimiento en plantas de tomate sometidas a estrés salino. Se realizó un diseño completamente al azar con un esquema factorial 2 × 6. El primer factor correspondió a la salinidad con dos niveles (0 y 40 mM) y el segundo factor a la aplicación individual de hormonas (auxinas – ácido naftalenacético (NAA) y giberelinas - ácido giberélico (ProGibb SP®) en dosis de 50, 75 y 100 µM cada una, para un total de 12 tratamientos con 6 repeticiones. Se evaluaron variables de crecimiento como altura, número de hojas, área foliar, biomasa fresca de parte área y raíz, y variables fisiológicas como contenido de clorofila y conductancia estomática (g_s). La aplicación de giberelinas en dosis de 100 µM demostró que puede mitigar el efecto deletéreo de la salinidad en algunos parámetros de crecimiento en plantas de tomate, mejorando la expansión foliar y la biomasa fresca de la parte aérea.

Palabras clave: hormonas vegetales, ácido naftalenacético, ácido giberélico, estrés abiótico.

Introduction

Salinity is the result of natural and anthropogenic processes, the anthropogenic mainly associated with inadequate irrigation and excessive chemical fertilization (Sing *et al*., 2016). Agricultural soil salinity limits the productivity of various crops worldwide. About 34 million ha are affected by salinity problems and annual loss in agricultural productivity caused by salinization amounts to US$ 31 million (FAO, 2024). In Colombia, 46% of the continental and insular land area shows some degree of susceptibility to salinity (SIAC, 2025).

The accumulation of salts affects plant growth and development by inhibiting water absorption, reducing nutrient

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[1] Grupo de Manejo Biológico de Cultivos-GMBC, Facultad de Ciencias Agropecuarias, Universidad Pedagógica y Tecnológica de Colombia, Tunja (Colombia).

[*] Corresponding author: hernan.ruiz@uptc.edu.co

availability, and generating phytotoxicity. Osmotic imbalance in the soil due to overfertilization contributes to the accumulation of Na^+ and Cl^- ions in the plants, leading to physiological and biochemical disorders. At the photosynthetic level, biochemical and photochemical limitations occur. An increase in the intracellular concentration of Na^+ promotes a decrease in chlorophyll content, negatively affecting electron transport chains during the photochemical phase of photosynthesis (Fita *et al*., 2017; Taiz *et al*., 2017).

Salinity affects plant water uptake, inducing water stress and water availability for physiological processes; this condition limits CO_2 uptake of the leaf mesophyll due to reduced stomatal conductance in response to salinity. Consequently, biochemical limitations arise from a reduction in the carboxylation rates of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) and the inhibition of enzymes such as ribulose-5-phosphate kinase and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Acosta-Motos *et al*., 2017).

In addition to these effects, oxidative stress impacts plant photosynthetic metabolism. The increase in reactive oxygen species (ROS) oxidizes molecules such as lipids, chlorophyll, and proteins essential for maintaining cellular homeostasis (Taïbi *et al*., 2016). From the plant defense perspective, there are several mechanisms that enable coping with this type of stress. The first is the activation of the plant antioxidant system. Enzymes such as superoxide dismutase, catalase, peroxidase, and ascorbate peroxidase, among others, mitigate cellular oxidative damage caused by the increase in ROS (Kashyap *et al*., 2021).

Another defense mechanism during stress is related to the role of plant hormones. These growth regulators for development, and nutrient allocation are essential for modulating the physiological responses that lead to salinity tolerance (Fahad *et al*., 2015). Evidence of this can be seen in fluctuations of endogenous plant hormone levels like auxins that in conjunction with nitric oxide cooperate in different ways to mitigate abiotic stresses like salinity and heavy metals (Ahmad, 2024).

Different studies have shown that exogenous application of phytohormones increases plant tolerance to salinity stress (Iqbal *et al*., 2012; Javid *et al*., 2011); for example, the use of naphthaleneacetic acid (NAA) increases chlorophyll content, plant height, leaf area, spike length, and the number and weight of grains in oat plants (Abed Jeber & Khaeim, 2019; Jahan *et al*., 2019). Similarly, exogenous applications of gibberellic acid (GA_3) in maize plants under saline stress improve growth, reduce oxidative stress, increase the activity of antioxidant enzymes, and enhance potassium concentration (Shahzad *et al*., 2021). Additionally, the interaction between arbuscular mycorrhizal fungi (AMF) and exogenous applications of gibberellic acid (GA_3) improve tomato growth under salinity conditions (Khalloufia *et al*., 2017).

The tomato crop (*Solanum lycopersicum* L.) can be significantly affected by the excessive accumulation of salt in the soil; this impacts plant growth, physiology, and, ultimately, yield (Zhang & Sonnewald, 2017). Recognized as the most important fresh vegetable in the human diet and rich in vitamins, carbohydrates, proteins, and antioxidants such as lycopene, β-carotene, and lutein (Heuvelink, 2018; Singh *et al*., 2016), tomato cultivation is considered one of the most important vegetables globally (even though botanically it is actually a fruit), with China being the largest producer in the world (FAOSTAT, 2019). Meanwhile, Colombia has approximately 7,135 ha planted under greenhouse conditions in the Colombian high tropics, with an average yield of 25.18 t ha^{-1} (MinAgricultura, 2024).

Considering that few studies report the influence of growth regulators on alleviating this type of stress in tomatoes, our research aimed to determine the effect of different exogenous doses of naphthaleneacetic acid and gibberellic acid on the physiological characteristics of growth and development in tomato plants subjected to salinity stress.

Materials and methods

Location

The experiment was conducted in the greenhouse of the Universidad Pedagógica y Tecnológica de Colombia (UPTC), Tunja, Boyacá, Colombia (5°33'8.255" N; 73°21'21.164" W), altitude of 2,735 m a.s.l., and a maximum air temperature of 42.1°C.

Experimental design

A completely randomized design was used with a 2 x 6 factorial scheme. The first factor was salinity with two levels (0 and 40 mM), and the second factor was the individual application of hormones (auxins – naphthaleneacetic acid (NAA) (Quimicompany®) and gibberellins (GA_3) – gibberellic acid (ProGibb SP®, Bayer) at doses of 50, 75, and 100 μM for each with a total of 12 treatments (Tab. 1) with 6 replicates, with 72 experimental units, each corresponding to one tomato plant.

TABLE 1. Description of treatments.

Treatment	Dose of NaCl, mM	Hormone	Dose, μM
1	40	NAA	50
2	40	NAA	75
3	40	NAA	100
4	0	NAA	50
5	0	NAA	75
6	0	NAA	100
7	40	GA	50
8	40	GA	75
9	40	GA	100
10	0	GA	50
11	0	GA	75
12	0	GA	100

Application of treatments

We planted tomato var. Chonto seedlings at growth stage 1 (code 13–103 - third true leaf of the unfolded main stem) (Meier, 2001) in bags with a capacity of 1 L filled with soil from the La María experimental farm at the Universidad Pedagógica y Tecnológica de Colombia (UPTC). The soil used had a clay texture with a particle size distribution of 48.2% clay, 36.16% silt, and 15.64% sand, analyzed using the Bouyoucos method at the Soil Laboratory of the Universidad Pedagógica y Tecnológica de Colombia. The soil chemical properties were as follows: pH 5.7, organic matter (3.22%) determined by the Walkley-Black method, and phosphorus 97.37 mg kg^{-1} measured using Bray II – Colorimetry. Base saturation was 10.33 cmol kg^{-1} soil, potassium: 0.75 cmol kg^{-1} soil, calcium: 6.88 cmol kg^{-1} soil, magnesium: 2.35 cmol kg^{-1} soil determined with NH_4Ac extraction and atomic absorption spectroscopy. The cation exchange capacity (CEC) was 26.20 cmol kg^{-1} soil, while the electrical conductivity was 0.31 dS m^{-1}, and base saturation percentage was 38.66%.

The seedlings were watered and fertilized with Nutriponic® (Walco S.A.S.) 1 cc L^{-1} every 3 d for 15 d before starting the application of 100 ml d^{-1} of water with NaCl. Application was performed once a week. On the first day of saline application, the doses of the two phytohormones were applied using manual sprayers with 25 ml applied per plant at the established doses as a single application.

Measurement of variables

After applying the hormone treatments, we measured the variables at 20 d and 30 d. The variables included the following: (1) relative chlorophyll content from the middle-third leaves of the plant using the SPAD 502-Plus chlorophyll meter (Konica Minolta®, USA), (2) stomatal conductance (mmol H_2O m^{-2} s^{-1}) measured by taking three measurements per plant with the SC-1 Porometer in the middle-third leaves between 9:00 am and 10:00 am, (3) plant height measured with a tape measure from the base of the stem on the soil surface to the apical bud, (4) stem diameter measured with a caliper, (5) leaf count, and (6) leaf area.

For the determination of fresh weights, we separated the plants into roots and aerial parts (stems + leaves) and weighed them using an Acculab VIC 612 electronic scale with a precision of 0.01 g. We placed the samples in paper bags with a 10 kg capacity properly labeled, according to the treatment and replicate.

Statistical analysis

We analyzed the data in the following manner: for variance homogeneity we used the Bartlett test, normality using the Shapiro-Wilk test, subsequently evaluated using an analysis of variance (ANOVA) with a significance of ($P \leq 0.05$). The analyzed significant differences using a mean comparison test (the Tukey´s test, $P \leq 0.05$) for the factors of salt concentration, hormone types, and doses. We performed all analyses using the ‘agricolae’ package of the statistical software R Core Team (2022).

Results and discussion

Growth variables

The application of 40 mM NaCl to the soil affected ($P \leq 0.05$) the height of the tomato plants compared to the treatment without salinity: stressed plants showed a 14% decrease in growth (Fig. 1). Plant growth is affected by exposure to salinity; one of the first symptoms is a reduction in cell elongation, which decreases plant growth, and reduces photosynthetic rate. This damage is produced due to programmed cell death (Iqbal, 2014). Soil salinity decreases the soil water potential, preventing proper water absorption by the plants; this in turn limits metabolic reactions, impedes nutrient transport, and hinders gas exchange, thus affecting photosynthetic rates, leading to a reduction in growth (Martínez Villavicencio *et al.*, 2011).

Although the tomato is a moderately salt-tolerant species (Saldaña *et al.*, 2017), the evaluated NaCl dose had a deleterious effect on plant height. However, studies like those of Murillo-Amador *et al.* (2017) report that only NaCl concentrations greater than 100 mM show significant variations in parameters such as stomatal conductance and water relative content (WRC), important for plant growth. Rodríguez *et al.* (1997) indicate that hydraulic conductivity in plants subjected to salinity depends on the exposure time to the stressful conditions, soil type, and temperature, since the latter factor affects water flow. The same authors indicate that hydraulic conductivity in plants also depends on other hydraulic signals perceived by the roots, which communicate the soil's water status to the rest of the plant.

Several researchers have debated the possible involvement of plant hormones such as abscisic acid in this signaling (Kishor *et al.,* 2022; Taiz *et al*., 2017). However, other studies report that auxins and gibberellins may participate in the hormonal balance that allows the plant to seek growth strategies according to the soil water status (Gornals, 2015; Omena-Garcia, 2019).

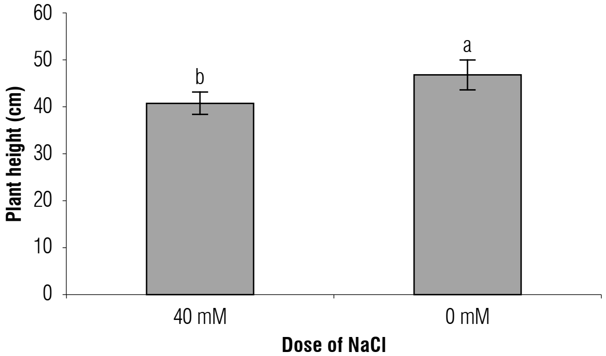

FIGURE 1. Height of tomato plants (*Solanum lycopersicum* L.) watered with 40 mM and 0 mM NaCl solution for 20 d. Different letters indicate significant differences between treatment means according to the Tukey's test ($P \leq 0.05$); bars represent the standard error, n=6.

According to our data, the plant height showed significant statistical differences ($P \leq 0.05$) for the interaction of factors, dose, and type of hormones used for day 20 and day 30 post-application (Fig. 2A and 2B). These results agreed with the findings of Taiz *et al.* (2017), who indicated that plant height is related to several environmental factors and hormonal regulation.

The application of gibberellins increased plant height at all doses in our experiment, compared to auxin applications, especially at higher doses (75 and 100 µM). The exogenous application of gibberellins increases endogenous levels, thus inducing the growth and expansion of new shoots (Bidadi *et al*., 2010).

Gibberellins act as mobile molecules that can cross the plasma membrane for cell-to-cell transport (Gao *et al*., 2017), which explains their effectiveness through exogenous applications, and the GA signal is perceived by the soluble receptor protein (GID1). Additionally, GAs promotes plant growth and development by facilitating the degradation of DELLA proteins, a family of nuclear growth repressors (Gao *et al*., 2017); this could explain the results obtained in our research.

It is important to highlight studies such as Fu and Harberd (2003) and Oh *et al.* (2014) that show that there is synergy between auxins and gibberellins for cell elongation. Once the auxin movement increases, it destabilizes DELLA proteins that are recognized as transcriptional regulators responsible for repressing responses to GA (Boccaccini *et al.,* 2014).

In general, the number of leaves affected by the type of hormones were as follow: gibberellins promoted the highest leaf formation; auxins promoted greater root formation (Fig. 3A and B). De Smet *et al.* (2010) mention that auxins regulate a wide range of morphological responses and play an important role in the initiation of lateral roots, while gibberellins are recognized for their role in cell elongation,

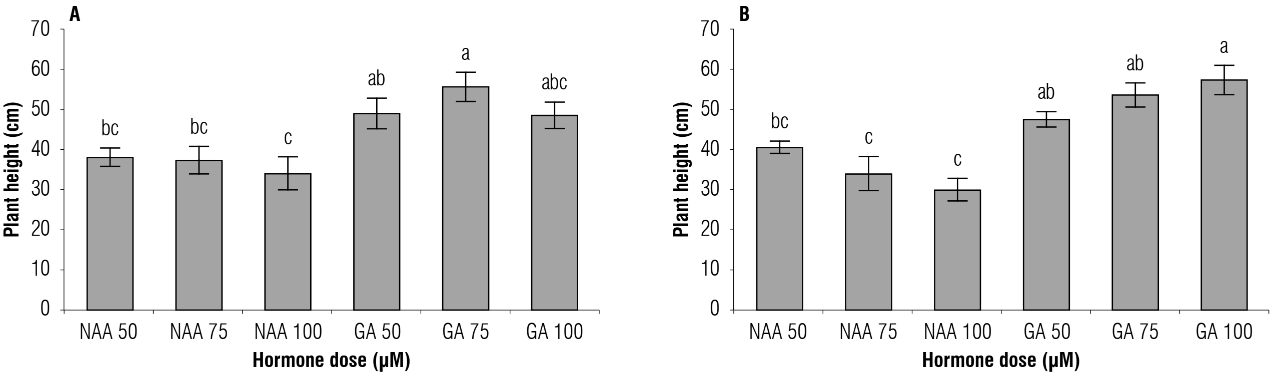

FIGURE 2. Height of tomato plants (*Solanum lycopersicum* L.) treated with 50, 75 or 100 µM of auxins – naphthaleneacetic acid (NAA) and gibberellins – gibberellic acid (GA_3) for 20 d (A) and 30 d (B). Different letters indicate significant differences between treatment means according to the Tukey's test ($P \leq 0.05$); the bars represent the standard error; n=6.

induction of floral and fruit development in some species, apical dominance, leaf expansion, and internode elongation. However, studies such as Fu and Harberd (2003) and Tong *et al.* (2014) demonstrate that, in tobacco and rice plants, stem elongation is coordinately regulated by auxins and gibberellins and brassinosteroids, and that gibberellin-induced root elongation requires auxins because their activity in cellular expansion, as well as in tissue differentiation, overlaps.

The fresh root weight had statistically significant differences related to the factor of dose and hormones used (Fig. 3C). Doses of 50 and 75 µM of naphthaleneacetic acid favored root growth in tomato plants compared to the same doses of gibberellic acid (Fig. 3C). Other studies show that high concentrations of auxins in plants, such as *Arabidopsis thaliana*, generate shorter roots but with more root hairs that increase the root volume and, consequently, the final root weight (Casanova-Sáez & Voß, 2019).

Research conducted on tomatoes with exogenous applications of gibberellins shows that the endogenous increase in GA after exogenous applications inhibits root growth (Bidadi *et al.*, 2010), similar to what we found in the present study. These results can be explained because the roots present high contents of GA, and when exogenous applications are made. The GA reach such high concentrations that they inhibit root growth (Hedden & Sponsel, 2015).

Regarding the number of leaves and the amount of fresh biomass accumulated in the aerial part for the salinity-hormones-dose interaction, we found that gibberellins at the 100 µM dose mitigated the detrimental effect of salinity on these two growth parameters compared to auxins (Fig. 3).

Salinity stress induces the synthesis of abscisic acid, leading to stomatal closure, which decreases water uptake, causing photoinhibition and oxidative stress (Silva *et al.*, 2022). The main effect of these physiological changes is the inhibition of cell expansion. Similar results are reported by Feng *et al.* (2023) in rice plants subjected to saline stress and by Li *et al.* (2022) in tomato plants.

For this reason, the exogenous application of GA can help mitigate these effects since gibberellins are involved in tolerance to biotic stress, since they are implicated in various physiological and metabolic changes: for example, in grapevine (*Vitis vinifera*) GA_3 sprays are applied shortly

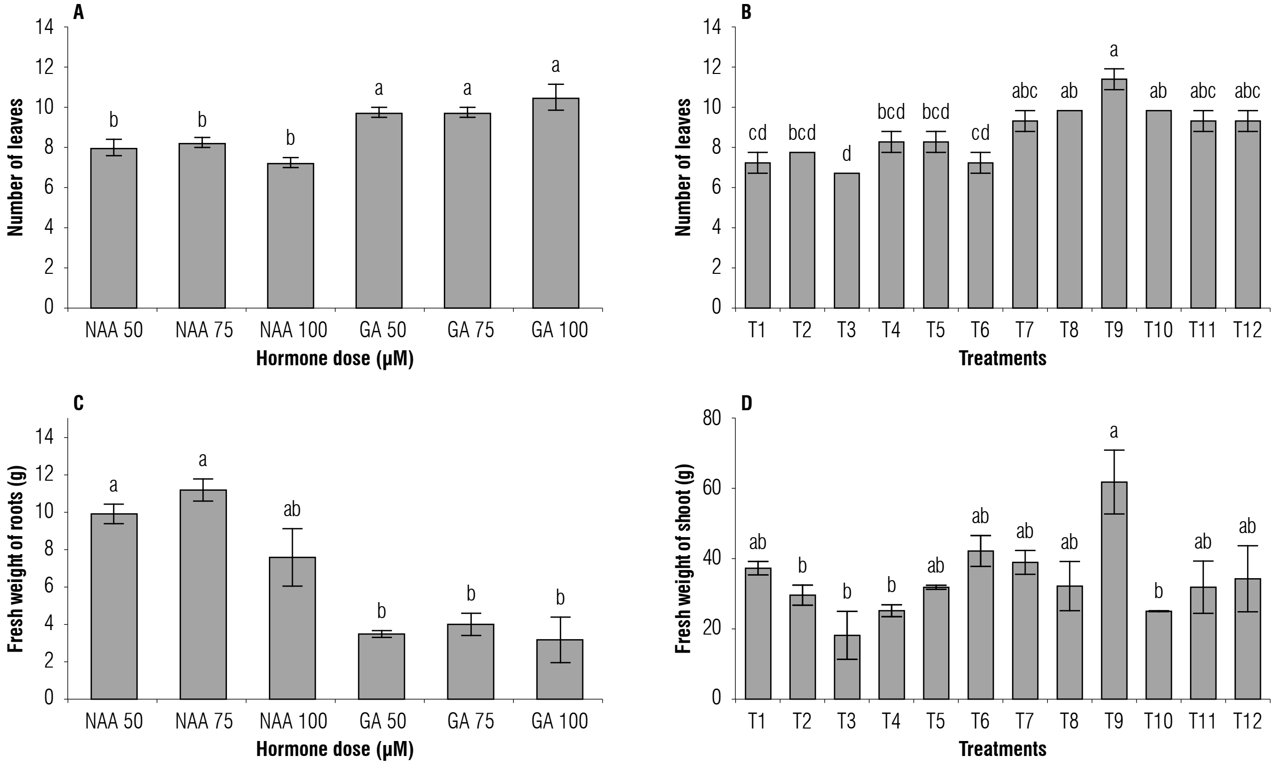

FIGURE 3. Number of leaves of tomato plants (*Solanum lycopersicum* L.) treated with 50, 75 or 100 µM of auxins – naphthaleneacetic acid (NAA) and gibberellins – gibberellic acid (GA) (A and C) and fresh weights of roots and shoots of plants grown in soil under two concentrations of NaCl (B and D) for 30 d. Treatment descriptions (T1-T12) are as in Table 1. Different letters indicate significant differences between treatment means according to the Tukey's test ($P \leq 0.05$); the bars represent the standard error, n=6.

before veraison to regulate fruit development and improve stress tolerance (Murcia *et al.*, 2017). Regarding osmotic stress caused by water deficit and/or salinity stress, GA levels are directly involved in tolerance in several plant species (Zawaski & Busov, 2014).

Gibberellins stimulate various physiological processes, including stem growth by elongation. The site of action of gibberellins is the intercalary meristem located at the base of the internode (Taiz *et al.*, 2017). While GA promotes internode elongation, the increase in the number of leaves observed in tomato plants may be associated with an overall enhancement of shoot development rather than a direct effect on leaf initiation.

Tomato plants whose leaf areas were subjected to salt stress presented significant statistical differences between treatments, with plants under stress showing a larger leaf area. Since the study did not include absolute control, we determined that the increase in this variable corresponds to the application of hormones that have a beneficial effect, even when the plant is under stress (Fig. 4A). Additionally, the interaction of hormonal doses of auxins and gibberellins showed a significant statistical difference for the gibberellin treatment at 100 µM (Fig. 4B). Gibberellic acid regulates leaf cell expansion primarily through the *SiDREB* gene, which is activated by abscisic acid (ABA) and decreases leaf expansion. Due to the GA/ABA antagonistic regulation under osmotic stress, this gene is suppressed, leading to greater leaf expansion and plant growth (Li *et al.*, 2012).

Chlorophyll contents (SPAD units) showed significant statistical differences for the hormone-dose interaction (Fig. 5A), with higher values in the 75 and 100 µM auxin doses compared to all gibberellin doses studied. Gibberellins caused an increase in plant height that leads to a nitrogen "dilution effect", in contrast to plants with auxins that did not show an increase in plant height.

The hormone-salinity-dose interaction also showed significant statistical differences (Fig. 5B). The treatment with salinity and 100 µM gibberellins presented the lowest

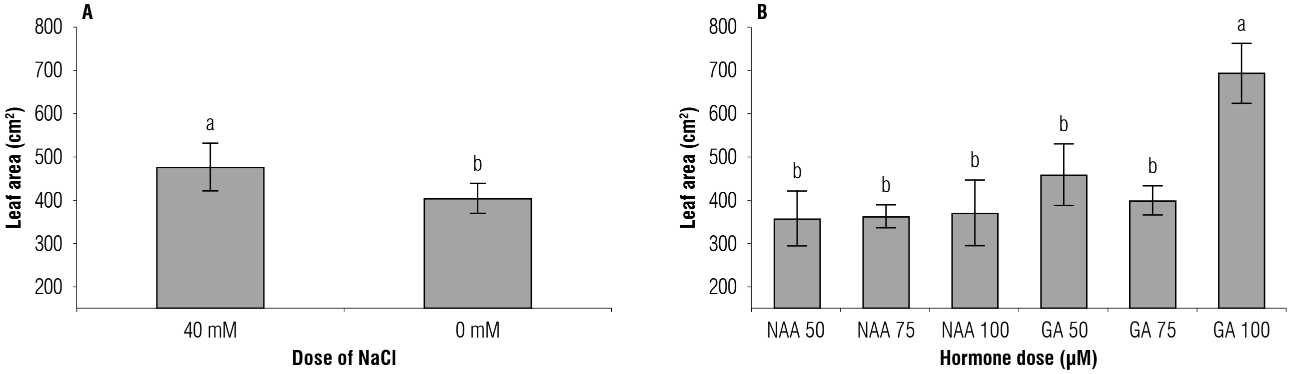

FIGURE 4. Leaf area of tomato plants (*Solanum lycopersicum* L.) grown in soil treated with two concentrations of NaCl (A) and with applications of 50, 75, or 100 µM of auxins – naphthaleneacetic acid (NAA) and gibberellins – gibberellic acid (GA) (B) for 30 d. Different letters indicate significant differences between treatment means according to the Tukey's test ($P \leq 0.05$); bars represent the standard error, n=6.

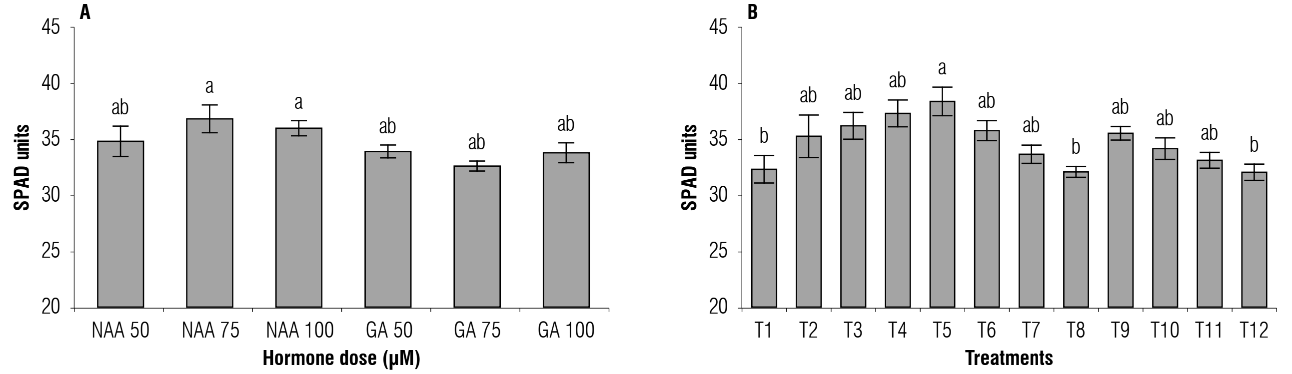

FIGURE 5. Relative chlorophyll content (SPAD units) in leaves of tomato plants (*Solanum lycopersicum* L.) treated with 50, 75, or 100 µM of auxins – naphthaleneacetic acid (NAA) and gibberellins - gibberellic acid (GA) (A) and grown under two concentrations of NaCl (B) for 30 d. Treatment description (T1-T12) as in Table 1. Different letters indicate significant differences between treatment means according to the Tukey´s test ($P \leq 0.05$); the bars represent the standard error, n=6.

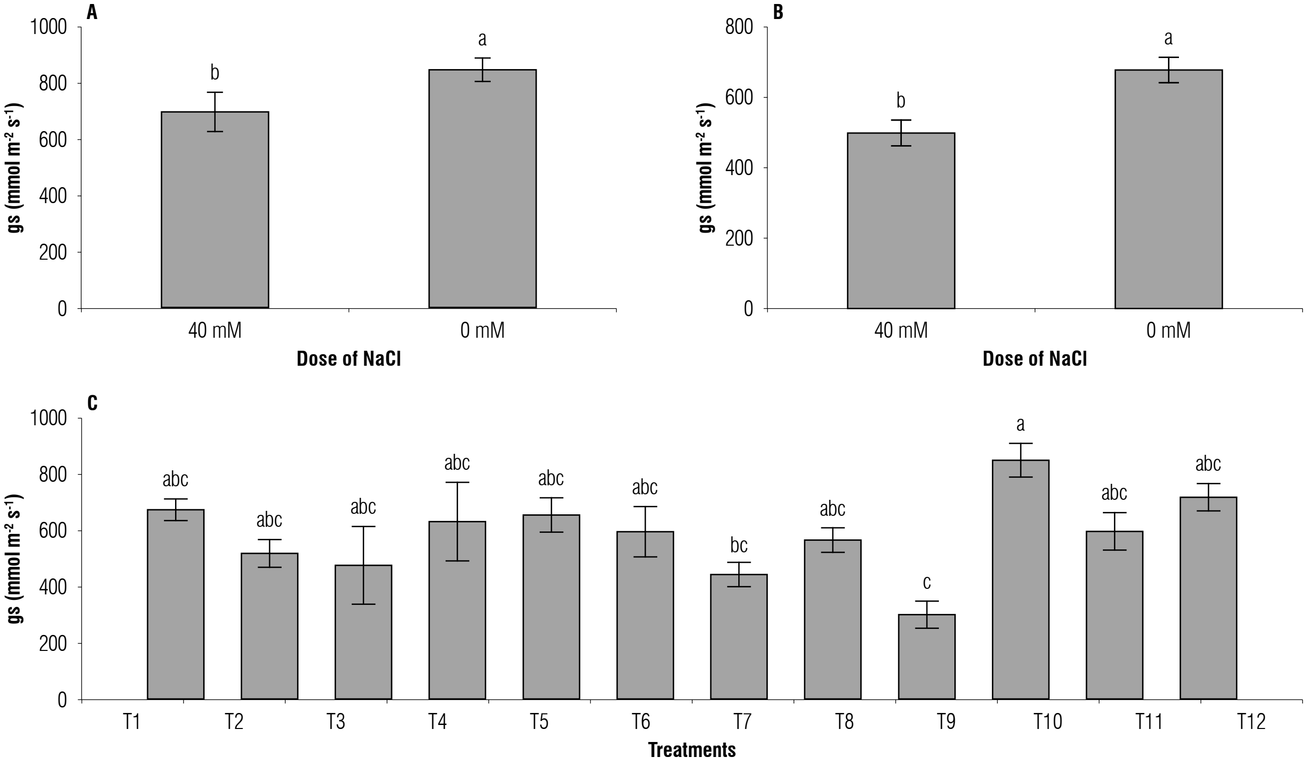

FIGURE 6. Stomatal conductance (g_s) in leaves of tomato plants (*Solanum lycopersicum* L.) grown in soil under two concentrations of NaCl at 20 d after application (A) and at 30 d (B), and g^s of tomato plants subjected to salinity and treated with 50, 75 or 100 µM of auxins – naphthaleneacetic acid (NAA) and gibberellins – gibberellic acid (GA) (C). Treatment description (T1-T12) as in Table 1. Different letters indicate significant differences between treatment means according to the Tukey's test ($P \leq 0.05$); the bars represent the standard error, n=6.

SPAD values. Total nitrogen content in the leaves can be estimated indirectly through SPAD units, which measure leaf chlorophyll content as an indicator of nitrogen status (Anjum *et al.*, 2013). These results are similar to those of Florina *et al.* (2013), who find that salt stress affects chlorophyll content in tomato plants subjected to 200 and 400 mM NaCl solutions, decreasing chlorophyll concentration and SPAD values as salt concentration in the soil solution increases.

Stomatal conductance (g_s) presented significant differences in tomato plants subjected to salt stress (Fig. 6A and B). Plants exposed to 40 mM NaCl in the soil solution showed lower values than plants not subjected to salt stress at both evaluation times. These results are similar to those of Li *et al.* (2022), who evaluate the effect of sodium nitroprusside (SNP) on tomato plants subjected to 100 mM NaCl and find a decrease in plant growth and gas exchange, similar to the findings of the present study.

The application of GA at 75 µM to plants cultivated in soil with 40 mM NaCl (Fig. 6) resulted in growth values similar to those of plants that were not subjected to stress (*e.g.*, 0 mM NaCl, 75 µM GA). Therefore, we suggest that the application of this hormone under stress conditions may help maintain gas exchange. In tomato, DELLA proteins promote stomatal closure mediated by ABA (Nir *et al.*, 2017), and the authors postulate that this could be due to the antagonistic function of gibberellin. Recent studies find that endogenous GA levels control stomatal opening in steady-state conditions in *Arabidopsis* through the GID1 receptors in a mechanism involving the degradation of DELLA proteins (Sukiran *et al.*, 2020). However, future research should evaluate parameters such as transpiration, net photosynthesis, and water use efficiency.

Conclusions

The use of gibberellins is a viable option to reduce the negative effect of salinity and improve osmotic stress tolerance in tomato crops when concentrations of 40 mM NaCl are present in the soil solution. The use of this phytohormone improves stomatal conductance, bringing stressed plants closer to the g_s values observed in plants without saline stress. However, auxins at concentrations of 50 and 75 µM NAA promote root growth and fresh weight of roots in plants not subjected to saline stress, making it a viable option to improve nutrient absorption in commercial crops.

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Conflict of interest statement

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

Author's contributions

ZCCR and HDRB designed the conceptual approach and objectives; ZCCR and HDRB carried out the field and laboratory experiments and elaborated visual representations of the data and results; ZCCR and HDRB designed and developed the research methodology, including methods of data collection, equipment and verified the accuracy and reliability of the research results through a validation process; ZCCR and HDRB wrote the initial draft. All authors participated in the critical review and approval of the final version of the manuscript.

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Effect of indole-3-butyric acid and gibberellic acid on rooting and growth of arracacha propagules

Efecto del ácido indol-3-butírico y ácido giberélico en el enraizamiento y crecimiento de propágulos de arracacha

**Maria Gladis Rosero-Alpala[1]*, Jaime Lozano-Fernández[1],
Carlos Enrique Velásquez-Arroyo[1], and Johanna Paola Garnica Montaña[2]**

ABSTRACT

Ex situ conservation of Andean roots requires asexual propagation to maintain germplasm genetic integrity. This study evaluated the effects of phytohormones on adventitious root formation and growth in low-rooting arracacha (*Arracacia xanthorrhiza*) propagules (accession CUI15010083) from Colombia Germplasm Banks for Food and Agriculture. Indole-3-butyric acid (IBA; 7.5, 15, 1000 or 3000 mg L^{-1}) and gibberellic acid (GA; 50 or 100 mg L^{-1}) were applied at varying immersion times (1, 30 min for IBA; 5 min for GA), with a water-only control. Propagation was assessed across three phases: 1) water-based rooting, 2) acclimatization in soil, and 3) field transplant, measuring vigor, plant height, leaf length, and fresh/dry biomass. High IBA concentrations (1000-3000 mg L^{-1}; 1 min immersion) induced propagule mortality. Lower IBA doses (≤15 mg L^{-1}; 30 min immersion) enhanced root quality, producing propagules with intermediate root category scores, though statistically comparable to controls. GA treatments (≤100 mg L^{-1}; 5 min immersion) showed no biomass differences relative to controls. Low-concentration IBA stimulated early rooting, improving adventitious root development, survival, and subsequent growth. GA primarily promoted cell elongation, confirming its role as a vegetative growth enhancer.

Key words: *Arracacia xanthorrhiza* Bancr., auxins, gibberellins, growth promoters, adventitious roots.

RESUMEN

La conservación *ex situ* de raíces andinas requiere de propagación asexual para preservar la integridad genética del germoplasma. Este estudio evaluó el efecto de fitohormonas en el enraizamiento y el crecimiento de propágulos de arracacha (*Arracacia xanthorrhiza*; accesión CUI15010083) con baja capacidad de formación de raíces adventicias, proveniente de los Bancos de Germoplasma para la Alimentación y la Agricultura de Colombia. Se aplicó ácido indol-3-butírico (AIB; 7,5, 15, 1000 o 3000 mg L^{-1}) y ácido giberélico (AG; 50 o 100 mg L^{-1}) en distintos tiempos de inmersión (1 y 30 min para AIB; 5 min para AG), junto con un testigo con agua. La propagación se analizó en tres etapas: 1) enraizamiento en agua, 2) aclimatación en suelo, y 3) trasplante a campo; evaluando vigor, altura de planta, longitud de hojas, y biomasa fresca/seca. Las dosis altas de AIB (1000-3000 mg L^{-1}; 1 min de exposición) causaron mortalidad en los propágulos. Dosis bajas de AIB (≤15 mg L^{-1}; 30 min) mejoraron la calidad del enraizamiento, con mayor cantidad de raíces en categorías intermedias, aunque sin diferencias estadísticas frente al testigo. Tratamientos con AG (≤100 mg L^{-1}; 5 min) no mostraron variaciones en biomasa respecto al testigo. El AIB en bajas concentraciones estimuló el enraizamiento temprano, facilitando la formación de raíces adventicias, la supervivencia y el crecimiento. El AG confirmó su rol como promotor del crecimiento vegetal, principalmente en la elongación celular.

Palabras clave: *Arracacia xanthorrhiza* Bancr., auxinas, giberelinas, promotores del crecimiento, raíces adventicias.

Introduction

Arracacha (*Arracacia xanthorrhiza* Bancr., Apiaceae), also called “racacha” or “white carrot”, is a socioeconomically important crop in Colombian and Andean agriculture due to its high local food value (Heywood, 2014). Colombian production rose from 49,587 to 105,408 t between 2009 and 2020, highlighting its significance for the country´s agriculture (MADR, 2022). The edible storage root, with varied colors of epidermis and pulp (Rincón Rueda *et al*., 2021), is valued for its culinary versatility, easily digestible starches, and nutrient richness (Pacheco *et al*., 2020).

For asexual propagation to renew accessions in Agrosavia Germplasm Banks for Food and Agriculture (BGAA), variable rooting potential (RP) has been observed. Materials

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[1] Corporación Colombiana de Investigación Agropecuaria (Agrosavia), Centro de investigación La Selva, Rionegro, Antioquia (Colombia).
[2] Corporación Colombiana de Investigación Agropecuaria (Agrosavia), Centro de investigación Nataima, Espinal, Tolima (Colombia).
* Corresponding author: mroseroa@agrosavia.co

with low RP pose challenges for growth and survival and risk the conservation of genetic resources (Rosero Alpala *et al.*, 2023). To address this, propagules are pre-rooted to ensure nearly 100% field establishment (Santos & Carmo, 1998). Auxins are commonly used to stimulate high-quality root growth, enhancing plant uniformity and agricultural productivity (Pinto-Acero *et al.*, 2012).

Indole-3-butyric acid (IBA), a synthetic auxin insoluble in water, is highly effective in promoting rooting in plant species (Castrillón *et al.*, 2008). However, its low mobility and susceptibility to degradation by light or microbial enzymes limit its use (Othman & Leskovar, 2022). IBA has proven effective in inducing adventitious roots in tree seedlings; optimal doses range from 2000 to 3000 mg L^{-1} (Román Clemente, 2014). Prolonged exposure to IBA can lead to excessive callus formation that hinders rooting, as in *Prunus* sp. rootstocks (Justamante *et al.*, 2022).

Gibberellins, like gibberellic acid (GA), are endogenous tetracyclic diterpenoid compounds that promote plant growth and development (Othman & Leskovar, 2022; Palma Soto *et al.*, 2022; Peng *et al.*, 2020). They enhance physiological processes such as gas exchange and source-sink relationships (Othman & Leskovar, 2022) and interact with environmental factors like light and temperature to stimulate seed germination and growth across phenological stages (Alcántara Cortés *et al.*, 2019). GA induces stem elongation by stimulating apex cells, increasing cell wall plasticity, so that taller stems show higher gibberellin activity. However, excessive GA can lead to problems like hypertrophy, chlorosis, root rot, and inhibited secondary root formation, and these negatively impacting plant health and growth (Bashyal, 2018; Hernández Rodríguez *et al.*, 2024).

Several factors, such as dosage, variety sensitivity, and plant conditions, are crucial when using rooting and growth promoters. This study aimed to evaluate the rooting and growth response of an arracacha accession from the BGAA with low rooting potential, that was treated with auxins and gibberellins at varying concentrations and exposure times.

Materials and methods

The study was carried out during the years 2019 and 2020 at the La Selva Research Center in Rionegro, Antioquia, Colombia, located at 6°07’52.7” N, 75°24’51.9” W and an altitude of 2100 m a.s.l. The average temperature of the area is 16°C with a relative air humidity of 74.8%. The life zone is classified as low montane humid forest (bh-MB) (Holdridge, 1978).

Plant material

Propagules from the BGAA accession (CUI) BGVCOL 15010083 collected from 9-month-old mother plants were washed and cut to 1.5-2 cm lengths from the petiole insertion above the third cormel ring. They were disinfected by soaking in a chlorine dioxide (ClO_2) solution (2 ml L^{-1}) for 15 min. This accession exhibits low rhizogenic potential, marked by excessive callus formation and limited root development (Rosero Alpala *et al.*, 2023).

Experimental design and data analysis

The treatments were established from the two hormones used: indole-3-butyric acid with four application doses, gibberellic acid in two concentrations, and the control (water), for a total of seven treatments with four replicates, under a completely randomized experimental design. One experimental unit (EU) is a container with a volume of 354.9 ml containing a propagule, for a total of 28 EUs. The immersion time was adjusted according to the technical data sheet of each product (Tab. 1).

To evaluate the relationship between the ordinal qualitative variables, Kendall’s Tau-b test was utilized for the number of equal response levels, and Kendall’s Tau-c test for the number of different levels. The V-Cramer test was used to compare ordinal and dichotomous qualitative variables

TABLE 1. Description of treatments with indole-3-butyric acid (IBA) and gibberellic acid (GA) applied to arracacha propagules.

Treatment	Hormone type - Active ingredient	Commercial presentation	Hormone concentration (mg L^{-1})	Immersion time (min)
IBA _ 7.5	Auxin - IBA	Suspension 3000 mg L^{-1}	7.5	30
IBA _ 15.0			15.0	30
IBA _ 1000		Powder 99 %	1000	1
IBA _ 3000			3000	1
GA _ 50	Gibberellin - GA	Powder 1 g 10 g^{-1}	50	5
GA _ 100			100	5
Control	Water	-	0	30

or between dichotomous ones. A cluster analysis was performed at each stage, employing the most important variables for grouping the treatments. Qualitative variables were analyzed with cross tables to study their associations.

Descriptive analyses were conducted on quantitative variables to assess normality (Shapiro-Wilk), homoscedasticity (Levene), and residual independence (Durbin-Watson) at $\alpha=0.05$. Variables meeting these assumptions were analyzed using parametric tests, with Duncan test for mean comparisons. Nonparametric methods, such as Kruskal-Wallis, were applied otherwise: T3-Dunnett for variables with normality but not homogeneity of variances, and Kruskal-Wallis with Bonferroni correction for those failing all assumptions. Analyses were performed using SAS v. 9.4.

Variables of rooting and growth

During the development of the experiment, three stages were considered in the vegetative propagation process: A) Rooting in water; B) Hardening in soil; and C) Plant growth in field, where the yield components and the harvest of storage roots were evaluated. The variables evaluated in the stages of the vegetative propagation process of arracacha propagules treated with growth promoters are shown in Table 2.

Water turbidity was assessed through visual observation, based on the presence or absence of yellow coloration in the water. Root abundance was determined using the arracacha (*Arracacia xanthorrhiza)* rooting variable scale proposed by Rosero Alpala *et al.* (2023). Basal tissue texture was evaluated manually via tactile analysis, with softening levels classified into four categories: null (0), low (1), medium (2), and high (3). Adventitious root length was measured as the length of the longest root per propagule. Plant vigor was assessed based on leaf physical quality and developmental stage, categorized into three levels: low (1 = short stature, limited leaf development), medium (2 = intermediate stature, moderate development), and high (3 = tall stature, robust leaf development). Canopy diameter was calculated by averaging two perpendicular measurements taken in north-south and east-west orientations. Fresh and dry weights of the crown and storage root (Blas *et al.*, 2008) were quantified using a Fenix-plus analytical balance (Lexus brand). Total yield was expressed in grams per square meter (g m^{-2}).

TABLE 2. Variables evaluated in the vegetative propagation process stages of arracacha propagules treated with indole-3-butyric acid and gibberellic acid.

Stage	Variable	Variable type	Scale/Unit
A. Rooting in water (0-22 DAS)	Survival	Qualitative dichotomous	Yes (1), No (0)
	Presence of leaves		
	Water turbidity		
	Root abundance	Qualitative ordinal	Null (0), low (1), medium (2), high (3)
	Softening of basal tissues		
B. Plant hardening in soil (23-49 DAS)	Survival	Qualitative dichotomous	Yes (1), no (0)
	Number of leaves	Quantitative	Number
	Plant height		cm
	Root length		mm
	Plant vigor	Qualitative ordinal	Low (1), medium (2), high (3)
C. Plant growth in field (50-365 DAS)	Number of leaves	Quantitative	Number
	Plant height		cm
	Canopy diameter		cm
	Leaf length		cm
	Petiole length		cm
	Colletotrichum incidence		%
	Leaf fresh weight		g
	"Crown" fresh weight (Blas *et al*., 2008)		g
	Storage root fresh weight		g
	Leaf dry matter		g
	"Crown" dry matter		g
	Storage root dry matter		g

DAS: days after sowing.

A) Rooting in water

The propagules, in a mesh house, at an average ambient temperature of 18°C, were prepared and rooted in water (Rosero Alpala *et al.*, 2023). They were immersed in hormone solutions for the times recommended by the respective technical data sheet (Tab. 1). To promote the healing of the basal tissues, they were placed at rest and without water for 3 d in aluminum containers with a capacity of 750 ml. Every 3 d, the water was changed for 22 d. In the three stages, the variables presented in Table 2 were measured.

B) Plant hardening in soil

The propagules continued in mesh houses and planted in 236.6 ml plastic containers filled with loamy soil characterized with pH 5.6, 17.2% organic matter, 113.7 mg L^{-1} phosphorus (P), 8.12 mg L^{-1} sulfur (SO$_4^{2-}$), 0.17 mg L^{-1} boron (B), 13.13 cmol kg^{-1} calcium (Ca), 2.54 cmol kg^{-1} magnesium (Mg), 0.53 cmol kg^{-1} potassium (K), and 16.3 cmol kg^{-1} effective cation exchange capacity (ECEC). The soil was disinfected with chlorine dioxide (ClO$_2$) at 50 ml L^{-1}. To control soil phytopathogens, a biological fungicide containing *Bacillus* sp., *Trichoderma harzianum*, and *Trichoderma asperellum* was applied three times at 1 ml L^{-1}. Seedlings were watered twice weekly, and aphids were controlled 12 d after sowing using an abamectin-based insecticide at 0.5 ml per 500 ml of water.

C) Plant growth in field

At 50 days after sowing (DAS), propagules were transplanted into open field with 0.4 m spacing between plants and 1.0 m between rows. The soil was pre-treated and disinfected as described earlier. Agronomic management included preventive pest control, targeting beetle larvae ("chizas") with chlorpyrifos insecticide (0.5 ml L^{-1}). Fertilization was applied in three stages: i) at transplant, 2 g/plant of a compound fertilizer (10% N, 30% P$_2$O$_5$, 10% K$_2$O); ii) at 75 DAS, 15 g/plant of a mix containing 8 g of the same compound fertilizer, 3 g KCl, and 4 g of another fertilizer (8% N, 5% P$_2$O$_5$, 18% Ca, 6% Mg, 1.6% S, 0.14% Cu, 1% B, 2.5% Zn); and iii) at 225 DAS, 10 g/plant of a mix with 2 g of the compound fertilizer and 8 g KCl. Hilling was done at 90 DAS, and weeds were controlled every 30 d for the first six months. The entire plant was harvested at 365 d for storage roots yield evaluation.

Results and discussion

A) Rooting in water

At 22 d, root abundance, basal tissue softening, presence of leaves, and water turbidity were evaluated. Kendall's Tau-c showed no significant relationship between phytohormone treatments and root abundance (0.041, P=0.835), basal tissue softening (0.395, P=0.002), or presence of leaves (V-Cramer=0.443, P=0.358). A significant medium relationship was found with water turbidity (V-Cramer=0.72A, P=0.012). Water turbidity showed significant medium associations with basal tissue softening (V-Cramer=0.7626, P=0.002) and root abundance (0.675, P=0.005). All treated propagules in all treatments survived this stage.

Cross-table analysis showed three groups: the first group with the following treatments IBA_7.5, IBA_15, GA_100; the control showed no turbidity or basal tissue softening, promoting root development. In contrast, a second group, those treated with IBA_3000 for 1 min exhibited high basal tissue softening (25%) and no root generation. Propagules treated with GA_50 for 5 min showed 25% with and 75% without basal tissue softening, resulting in low root generation. The third group was more marked in the stage of hardening in soil. Overall, increased basal tissue softening reduced rooting, and turbid water indicated propagule deterioration in hormone-treated groups (Fig. 1A, 1B and 1C).

The delay in root formation is probably due to the formation of a callus, in those treatments with prolonged exposure times, as observed in cuttings of Flordaguard peach *Prunus persica* L. (Justamante *et al.*, 2022). On the other hand, short exposures from 3 s to 2 min to concentrations higher than 500 mg L^{-1} of IBA in woody species, such as peach, favored root development (Kaur, 2017; Natarajan *et al.*, 2023).

B) Plant hardening in soil

At 28 DAS, propagule survival showed a high and significant association with the treatments used (V-Cramer= 0.849, P=0.003). All propagules reached 100% survival, except those treated with IBA_3000, which had a survival rate of only 25% (Fig. 2).

Plant vigor lacked normality and homogeneity of variance (Shapiro-Wilk and Levene, P<0.05), with a significant treatment effect (Kendall's Tau-c=0.451, P=0.003). High vigor predominated in propagules treated with GA, IBA, and the control, except for those treated with 1000 mg L^{-1} and 3000 mg L^{-1} of IBA, which showed low to medium vigor, though differences were not statistically significant (Marginal homogeneity test, P=0.313). A low, inverse association was found between IBA concentrations and vigor (Kendall's Tau-c, r(17) = -0.457, P=0.002, Fig. 2).

Seedling height showed normality (Shapiro-Wilk, P=0.709) but not homoscedasticity (P=0.004) with no significant differences between treatments (P=0.765) and a coefficient of

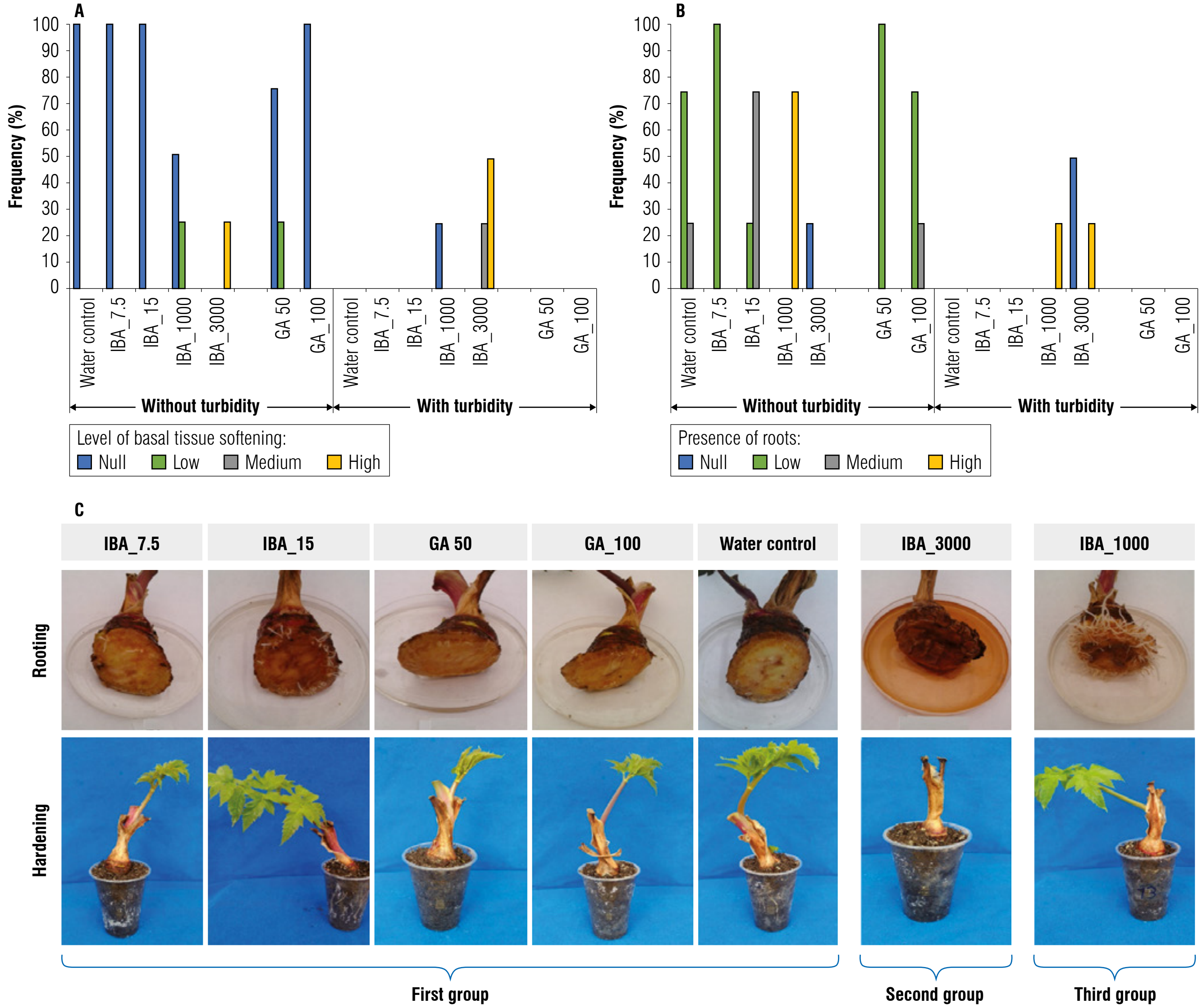

FIGURE 1. Frequencies of the level of basal tissue softening (A), presence of roots (B) and cluster characteristics for the rooting stage (C) in arracacha propagules at the rooting stage treated with indole butyric acid (IBA) and gibberellic acid (GA) in the presence and absence of water turbidity.

variation (CV) of 21.1%. Root length and leaf number did not meet assumptions ($P<0.05$). Trend curves indicated that height, root length, and leaf number decreased with IBA doses near 1000 mg L^{-1}, which also caused 69% chlorosis, and made up the third grouping of the treatments (Fig. 1B and 3). In contrast, *Passiflora nitida* cuttings treated with 1000 mg L^{-1} IBA for 5 s showed optimal survival, rooting, and callus formation (Vale *et al*., 2020).

On the other hand, the variables increased with IBA concentrations lower than 15 mg L^{-1} and imbibition for 1 min and higher than 2000 mg L^{-1} with exposure for 30 min. In the treatments with GA, the height increased with concentrations up to 100 mg L^{-1}, imbibed for 5 min without presenting significant differences with the control ($P>0.05$), while the root length and the number of leaves decreased (Fig. 3). These results could be explained by the effect found in the excessive formation of calluses caused by IBA concentrations that are much lower than those applied in this work. In a work where *Prunus* cuttings were treated with 0.9 mg L^{-1} with constant incubations of 24 h, the prolonged exposure caused the excessive formation of calluses that limited root development. This work concluded that the short incubation with IBA is sufficient to trigger root initiation (Justamante *et al*., 2022).

In *Arracacia* sp., 7.5 ml L^{-1} of IBA and GA applied for 30 min increased root number and length, confirming their

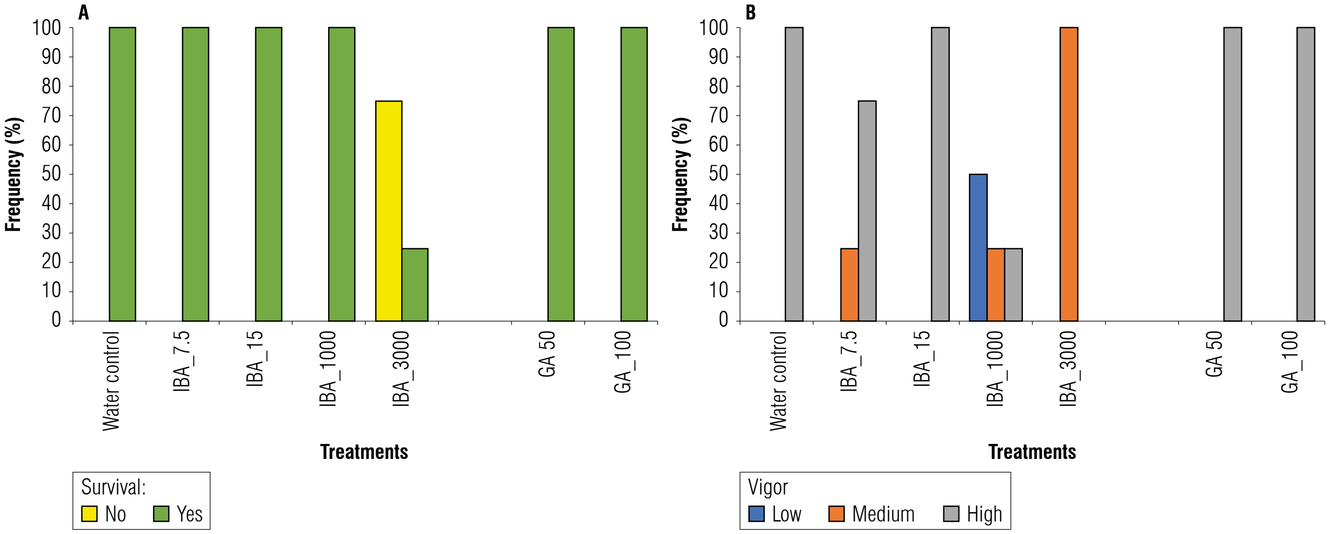

FIGURE 2. Frequencies of survival (A) and vigor (B) of arracacha propagules at the hardening stage treated with indole-3-butyric acid (IBA) and gibberellic acid (GA).

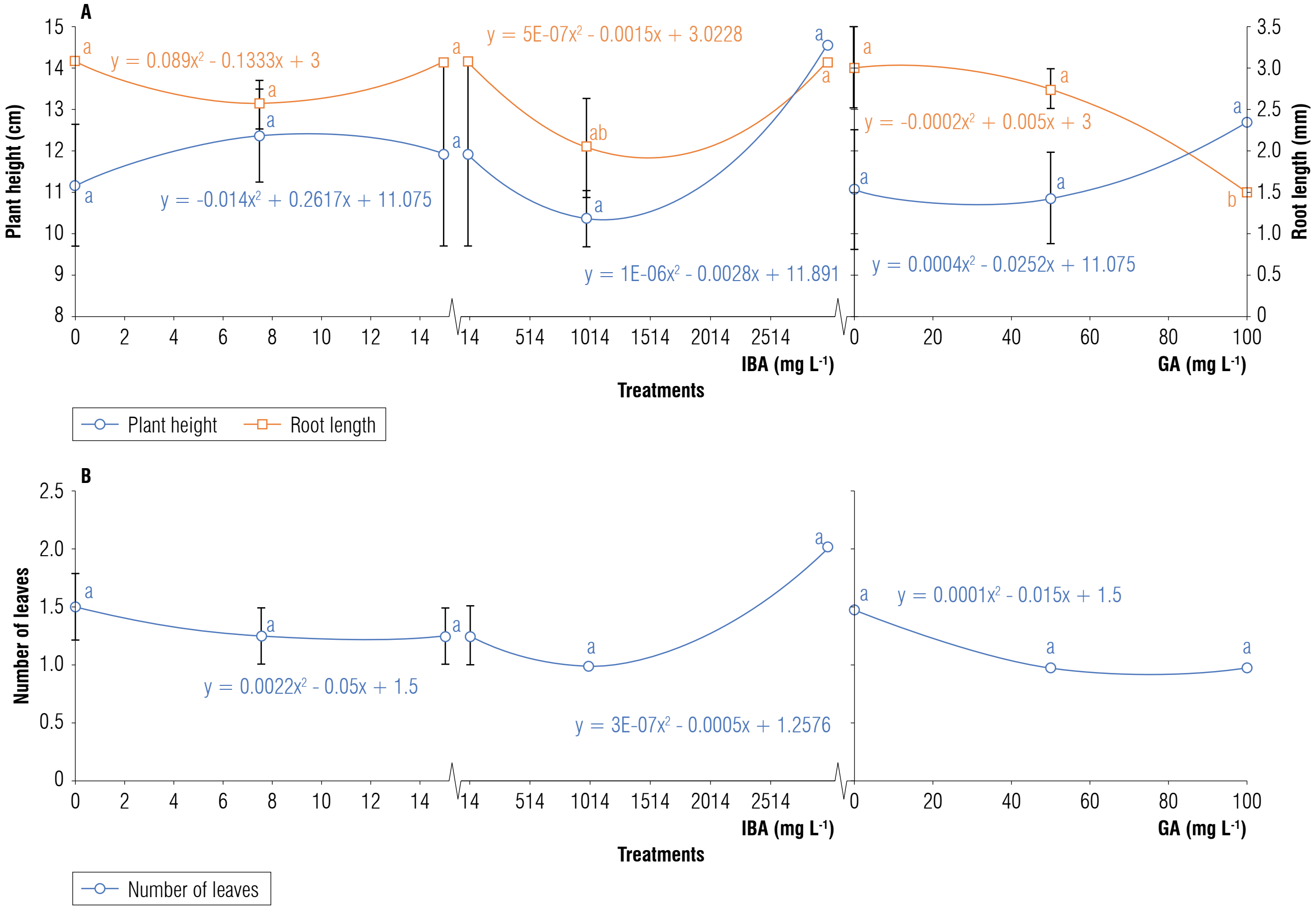

FIGURE 3. Effect of different doses of indole-3-butyric acid (IBA) and gibberellic acid (GA) applied on arracacha propagules on height, root length (A), and number of leaves (B) during hardening in soil. Averages of a series with the same lowercase letter do not show significant differences (For height: T3-Dunnett, for root length and leaf number: Kruskal-Wallis with homogeneous subsets based on asymptotic and Bonferroni significances, Wallis with homogeneous subsets based on asymptotic and Bonferroni significances, >0.05). Mean with standard error (SE) bars with n=4 for treatments.

effectiveness as rooting stimulators (Reghin *et al*., 2000). Higher doses of 25 and 50 mg L^{-1} of IBA in *Gossypium barbadense* L. cuttings showed moderate rooting (Rojas-Idrogo *et al*., 2013), but lower doses in potato (*Solanum tuberosum* L.) shoots treated with 1.0 mg L^{-1} of IBA registered efficient root production (Sharde *et al*., 2024). Moreover, rooting was obtained in *Viola odorata* L. using leaves as propagules and treated with 1.5 mg L^{-1} of IBA (Vilas Haralkar & Raosaheb Biradar, 2020). These publications demonstrate the significant effect of a range of IBA concentrations used in different species in short exposure times.

C) Plant growth in field

At this stage, no significant differences ($P>0.05$) were found between treatments and the evaluated variables. Plant height, canopy, leaf, and petiole lengths met statistical assumptions. Seedlings treated with IBA_1000 and IBA_3000 for 1 min did not survive. *Colletotrichum* incidence was low, averaging 5% in GA_100-treated seedlings soaked for 5 min. Similar results were observed in arracacha propagules treated with 500 mg L^{-1} IBA for 20 s, achieving 95% rooting, though no differences from the control (hormone-free) were noted, and higher doses proved phytotoxic (Câmara, 1992).

At 365 DAS, variables such as fresh weight and dry matter of leaves, crown, and storage roots did not meet normality, and crown dry matter lacked homoscedasticity ($P<0.05$). Treatment effects showed no significant differences based on ANOVA and Kruskal-Wallis tests ($P>0.05$). Height, canopy diameter, and lengths of leaves, and petioles varied with hormone type: IBA-treated plants showed a slight increase at 7.5 mg L^{-1}, declining at lower or higher doses, while GA-treated plants exhibited reduced height and length with increasing doses, though not statistically different from the control (Fig. 4).

The results coincide with those found in potato, where treatments were applied with GA doses of 20 mg L^{-1}, lower than those used in the present trial, obtaining the highest

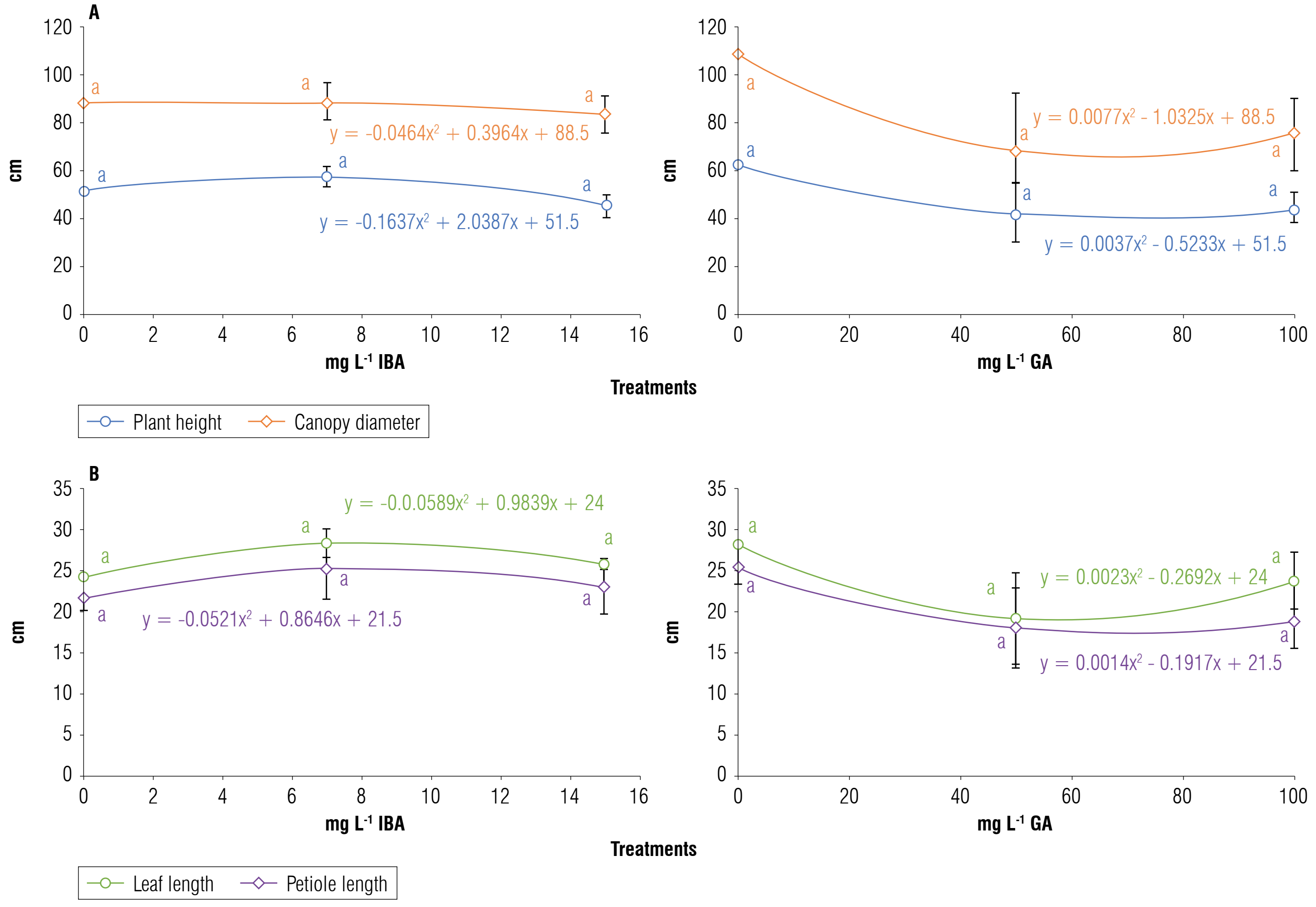

FIGURE 4. Behavior of plant height, canopy diameter (A), and leaf and petiole length (B) during the transplant to the field stage of propagules treated with different doses and exposure times of indole-3-butyric acid (IBA) and gibberellic acid (GA). Averages of a series with the same letter do not show significant statistical differences (Duncan, $P>0.05$). Mean standard error (SE) bars with n=4 for treatments.

root length and leaf area values, while concentrations higher than 300 mg L^{-1} inhibited their development (Almanza Merchán *et al*., 2015). It is possible that, given the primary function of GA, which acts on stems elongation, its action on cell growth is not as strong (Almanza Merchán *et al*., 2015). The responses of different doses to stimulate root development were not significant concerning the control treatment with only water.

The effect on stem elongation was evident in tomato seeds evaluated with gibberellins concentrations that inhibited root growth to a certain degree and where the growth response was produced by low concentrations of gibberellin, together with a longer imbibition time (Balaguera-López *et al*., 2009). Plants from seeds treated with 300 mg L^{-1} for 36 h showed the highest increases in leaf area, fresh leaf, rootstock mass, root length, and vigor. The lowest response occurred with the 18 h imbibition treatment and 600 mg L^{-1} of GA. They also found that the GA concentrations evaluated (300, 600, and 900 mg L^{-1}) inhibited root growth to a certain degree and that the growth response was due to low GA concentrations and a longer imbibition time (36 h) (Balaguera-López *et al*., 2009).

The fresh weight of the leaf, crown, and storage root showed the same tendency when increasing the dose of the GA and IBA promoters; the correlations were positive and higher than Pearson r=0.89. Leaf and crown height tended to decrease when increasing the dose, while in IBA, the weight of the storage root increased in plants treated with IBA_7.5 exposed for 30 min but decreased in IBA_15 with the same imbibition time without presenting statistical differences (P>0.05, Fig. 5A). In cuttings of herbaceous species such as *Mentha spicata* L. immersed for 0, 10, and 20 min in a solution of 500 mg L^{-1} of IBA, the number of roots increased by prolonging the immersion time to 20 min (Ferraz *et al*., 2018).

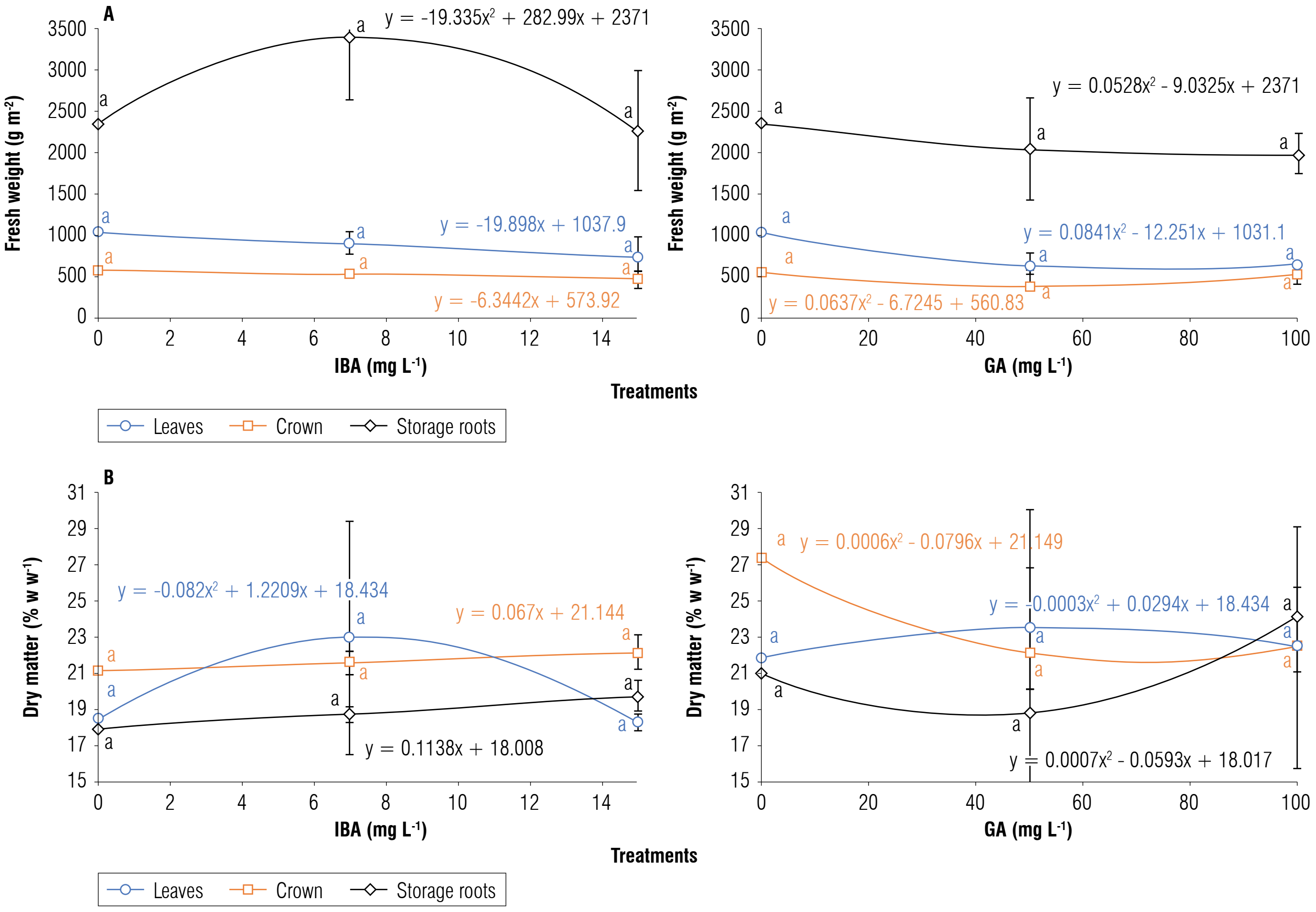

FIGURE 5. Response of fresh weight per m^2 (A) and dry matter (B) of leaves, crown, and storage roots during harvest in plants treated with different doses of indole-3-butyric acid (IBA) and gibberellic acid (GA). Averages with the same letter do not present significant statistical differences (Duncan, P>0.05 for fresh weight and Kruskal-Wallis with homogeneous subsets based on asymptotic significances and Bonferroni, P>0.05 for dry matter). The bars of the averages represent the standard error (SE) with n=4 for treatments.

Dry matter changed according to the hormone used, although there were no significant differences ($P>0.05$). With the GA treatments, irregular trends were registered in the storage root. The dry matter of leaves and storage roots decreased in plants treated with GA_50 and increased slightly with doses of GA_100. In plants treated with IBA, the dry matter of crown and storage roots had a slight increase with increasing doses, but in leaves it increased up to IBA_7.5 and decreased with a dose of IBA_15 without presenting statistical differences ($P>0.05$, Fig. 5B).

Conclusions

Treatment with indole-3-butyric acid (IBA) at low concentrations and varying exposure times stimulated early rooting of arracacha propagules; this facilitated adventitious root formation, survival and growth.

The primary role of gibberellic acid as a plant growth promoter was confirmed, particularly its involvement in cell elongation. However, since its most pronounced effects are observed through exogenous applications, such as foliar spraying, no significant differences in biomass production were detected between the treated plants and the control group.

It is important to continue researching the sensitivity of the concentrations of the IBA and GA promoters and the exposure times in non-woody species, such as arracacha, to improve the response in root and biomass development.

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Conflict of interest statement

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

Author's contributions

MGRA contributed to the study of conception and design. MGRA and CEVA performed the material preparation and data collection. JLF performed the analysis. MGRA, CEVA, JLF and JPGM wrote the first draft of the manuscript, and all the authors commented on the previous versions of the manuscript. All authors have read and approved the final version of the manuscript.

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Biological control of *Sclerotium rolfsii* Sacc. in *Stevia rebaudiana* using native isolates of *Trichoderma* spp. from Paraguay

Control biológico de *Sclerotium rolfsii* Sacc. en *Stevia rebaudiana* usando aislamientos nativos de *Trichoderma* spp. de Paraguay

Andrés D. Sanabria-Velázquez[1], Hassan K. Bittar-Vega[2], Gloria Montiel[3], Federico Cantero[3], Rosanna Britos[3], Cynthia Ortiz[3], and Humberto J. Sarubbi-Orué[2]

ABSTRACT

Stevia, *Stevia rebaudiana* [(Bertoni) Bertoni], is cultivated in Paraguay as a natural, non-nutritive sweetener, but yields are reduced because of wilting and plant death caused by the fungal pathogen *Sclerotium rolfsii*. This study aimed to evaluate the native fungi *Trichoderma* spp. isolates, individually and in mixtures, for controlling *S. rolfsii*. As a first step, ten *Trichoderma* isolates from agricultural soils of Paraguay were screened *in vitro* using dual culture tests against *S. rolfsii* to identify the most effective isolates. This preliminary phase allowed screening to select the most promising candidates before conducting *in planta* experiments under more realistic conditions. A greenhouse experiment with seven treatments and four replicates was carried out to assess their efficacy in controlling *S. rolfsii* in stevia. Treatments included commercial *Trichoderma viride,* three native isolates (3KH and TCAS, *T. asperellum*; MS28, *T. erinaceum*), a mixture of these isolates, a fungicide (azoxystrobin + cyproconazole), and a control. Stevia plants of the variety "Katupyry" were preventively treated with *Trichoderma* (1×10^7 spores ml^{-1}) twice before *S. rolfsii* inoculation. Fungicide and *Trichoderma* treatments were applied to the plant base and substrate after inoculation, followed by two weekly applications. Disease incidence and the area under the disease progress curve (AUDPC) were evaluated. The AUDPC for TCAS (7.67) was significantly lower than that in the control (13.27), the mixture (13.77), and the commercial *T. viride* (15.58), but not significantly different from the 3KH (9.15) or MS28 (10.47). Fungicide-treated plants had the lowest AUDPC (1.25). These results suggest that the *Trichoderma* isolate TCAS effectively manages *S. rolfsii* under greenhouse conditions and has potential for use in both organic and conventional stevia production.

Key words: sweeteners, stevia, biocontrol, mycology.

RESUMEN

Estevia, *Stevia rebaudiana* [(Bertoni) Bertoni], se cultiva en Paraguay como edulcorante natural no nutritivo, pero los rendimientos se ven afectados por el marchitamiento y la muerte de plantas causados por el hongo patógeno *Sclerotium rolfsii*. Este estudio tuvo como objetivo evaluar aislados nativos del hongo *Trichoderma* spp., individualmente y en mezcla, para el manejo de *S. rolfsii*. Como primer paso, se evaluaron *in vitro* diez aislamientos de *Trichoderma* provenientes de suelos agrícolas de Paraguay mediante pruebas de cultivo dual contra *S. rolfsii* para identificar los aislamientos más efectivos. Esta fase preliminar permitió filtrar y seleccionar los candidatos más prometedores antes de realizar experimentos *in planta* en condiciones más realistas. Un experimento en invernadero con siete tratamientos y cuatro repeticiones evaluó su eficacia. Los tratamientos incluyeron *Trichoderma viride* comercial, tres aislados nativos (3KH y TCAS, *T. asperellum*; MS28, *T. erinaceum*), una mezcla de estos aislados, un fungicida (azoxystrobin + cyproconazole) y un control. Las plantas de estevia de la variedad "Katupyry" fueron tratadas preventivamente con *Trichoderma* ($1x10^7$ esporas ml^{-1}) dos veces antes de la inoculación con *S. rolfsii*. Los tratamientos con fungicida y *Trichoderma* se aplicaron al sustrato y en la base de las plantas después de la inoculación, seguidos de dos aplicaciones semanales. Se evaluó la incidencia de la enfermedad y el área bajo la curva de progreso de la enfermedad (AUDPC). El AUDPC para TCAS (7,67) fue significativamente menor que en el control (13,27), en la mezcla de *Trichoderma* (13,77) y en el *T. viride* comercial (15,58), pero no difirió significativamente del 3KH (9,15) o MS28 (10,47). Las plantas tratadas con fungicida tuvieron el AUDPC más bajo (1,25). Los resultados sugieren que el aislado TCAS de *Trichoderma* maneja eficazmente a *S. rolfsii* en condiciones de invernadero y tiene potencial para su uso en la producción orgánica y convencional de estevia.

Palabras clave: edulcorantes, estevia, biocontrol, micología.

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[1] Department of Plant Pathology, The Ohio State University, Wooster, OH (USA).
[2] Facultad de Ciencias Agrarias, Universidad Nacional de Asunción, San Lorenzo, Central (Paraguay).
[3] Hernando Bertoni Research Center, Paraguayan Institute of Agricultural Technology (IPTA), Caacupé, Central (Paraguay).
[*] Corresponding author: adsanabr@ncsu.edu

Introduction

Stevia (*Stevia rebaudiana* [(Bertoni) Bertoni], also known as "ka'a he'ê" in the Guaraní language, is a plant grown to produce non-caloric sweeteners (Britos & Jongdae, 2016). It is a semi-perennial plant that grows naturally in the mountains of Mbaracayú and Amambay, primarily in Paraguay (Bogado-Villalba *et al*., 2021). Stevia is currently widely cultivated in many countries worldwide, such as Argentina, Colombia, Japan, Singapore, Taiwan, South Korea, China, and the USA (Ismail *et al*., 2020).

Stevia production can be significantly affected by plant pathogens such as the fungus *Sclerotium rolfsii* Saac., the causal agent of stevia "stem rot" and also known as "white silk" disease (and informally "enfermedad del hongo blanco") in Paraguay (Britos & Jongdae, 2016). This disease causes significant losses since it causes the wilting and death of the plant and is difficult to control. The fungus can survive several years in the soil because of resistance structures called sclerotia (Koehler & Shew, 2014). This disease can be controlled by synthetic chemicals such as quinone outside inhibitors (QoI) fungicides (Koehler & Shew, 2017; Sanabria-Velazquez *et al*., 2023). However, due to the high demand for stevia free of chemical pesticides in international markets (Ismail *et al*., 2020), the application of a biological control agent that exhibits antagonism to *Sclerotium rolfsii* seems a promising alternative for managing stevia stem rot (Correa *et al*., 2007).

Biological control with antagonistic fungi is a promising option for the sustainable management of this disease since this technology is directly involved with the selection and reintroduction of an antagonist. A biological control agent can be established in the same ecological niche of the pathogen, and it can protect the root system of the stevia plant, avoiding greater disease intensity (Bull *et al*., 1991; Hoitink & Boehm, 1999; Mazzola, 2004; Panth *et al*., 2020).

One of the most studied and efficient antagonists in several pathosystems is the fungus *Trichoderma* spp. (Cai & Druzhinina, 2021; Holmes *et al*., 2004; Samuels, 2006). Trichoderma, as a biological control agent, can exert mechanisms of direct and indirect action on the pathogen through the production of volatile chemicals and antibiotics (Louzada *et al*., 2016), secretions of toxic enzymes (Galante *et al*., 1998), by penetration of hyphae (Sanabria Velázquez & Grabowski, 2016), competition for oxygen and nutrients as well as for space in the soil (Benítez *et al*., 2004) and as an inducer of resistance modifying the physiology of the plant (Rivera-Méndez *et al*., 2020). Native isolates of *Trichoderma* spp. are usually more effective than imported ones. These native isolates are better adapted to the local environment and can improve effectiveness in the edaphoclimatic soil conditions where they are introduced (Mukherjee *et al*., 2014; Sanabria Velázquez, 2020; Sanabria-Velázquez *et al*., 2023). Therefore, native *Trichoderma* isolates can help reduce stem rot incidence, promote plant growth, and increase stevia yields.

The main objective of this research was to select and evaluate native isolates of *Trichoderma* spp. for the management of *Sclerotium rolfsii* in stevia production. To optimize the identification of effective *Trichoderma* spp. isolates, a two-phase strategy was implemented. First, *in vitro* tests were carried out to evaluate the antagonistic capacity of each isolate against *S. rolfsii*. Subsequently, the isolates that demonstrated greater efficacy in these preliminary tests were subjected to *in planta* evaluations to validate their performance under more representative culture conditions. The specific objectives were to: i) evaluate *in vitro* the antagonism exerted by native isolates of *Trichoderma* spp. on *S. rolfsii* using the dual culture technique, and ii) assess the most promising native *Trichoderma* spp. isolates against *S. rolfsii in planta* to confirm their efficacy under greenhouse conditions.

Materials and methods

We carried out the experiments in the laboratory and greenhouses of the Olericultural Crops Research Program (PICO) of the Hernando Bertoni Research Center (CIHB) at the Paraguayan Institute of Agricultural Technology (IPTA), Cordillera, Paraguay (25°23'25.73" S; 57°11'98" W). The experimental period was between January 2015 and March 2018.

We collected samples from various stevia and horticultural production farms in Paraguay. To isolate the *Trichoderma* spp., we adapted the soil dilution method described by Fernández (1993). This method involved mixing 3 g of soil with 100 cc of sterile distilled water and shaking the mixture. Then, 1 cc of the suspension was placed in a sterilized Petri dish under a laminar flow chamber. We added PDA (Potato-Dextrose-Agar) + Oxytetracycline culture medium and gently shook the dish; and we incubated the plates at 28°C for 5 d in darkness. We labeled the pure cultures of *Trichoderma* spp. with an arbitrary nomenclature (Tab. 1).

The fungus *S. rolfsii* was previously isolated from symptomatic plants of the stevia experimental plot of the Ka'a he'ê and Medicinal Plants Research Program-CIHB,

TABLE 1. Georeferenced soil samples for obtaining *Trichoderma* spp. isolates.

Sample name	*Trichoderma* spp. isolates	Location	Coordinates
1KH	TKC14-01	Cordillera	S 25.3883413; W 57.1869743;
3KH	TKC14-03	Cordillera	S 25.38807153; W 57.18603803
1 PICO 4	TFC14-04	Cordillera	S 25.38756; W 57.18980
1 PICO 6.1	TFC14-05	Cordillera	S 25.38763; W 57.18969
1 PICO 6.2	TFC14-06	Cordillera	S 25.38765 W 57.18960
TQUI	TFCe14-09	Central	S 25.33297699 W 57.35262201
TCAS	TFCe14-10	Central	S 25.32913 W 57.362126
K6	TKI14-11	Itapúa	S 26. 67623 W 57.08865
T9	TKI14-12	Itapúa	----------------------------
MS28	TSESP14-15	San Pedro	S 24.148507 W 56.641762
C2 (Foreign sample)	TFLE-07	Ecuador	----------------------------

Cordillera, Paraguay. We washed the roots collected from stevia plants with running water and cut them into 1 to 2-cm pieces, disinfesting them with a 70% alcohol solution and a 2% sodium hypochlorite solution, followed by triple rinsing in sterile distilled water. The plant tissue was dried and transferred to Petri dishes with a PDA culture medium under a laminar flow chamber. Subsequently, they were incubated at 28°C for 8 d. Pure cultures were maintained at 4°C until they were used in the experiments.

In vitro antagonism of *Trichoderma* spp. against *Sclerotium rolfsii*

We used the dual culture method to select the isolates of *Trichoderma* spp. with antagonistic capacity against *S. rolfsii*. The test consisted of facing a 9 cm Petri dish with PDA culture medium, two discs 5 mm in diameter with culture medium, and mycelium of *Trichoderma* spp. and *S. rolfsii,* separated by 7 cm from each other and incubated at 28°C until *S. rolfsii* treatment filled the plate. We evaluated the antagonistic activity of *Trichoderma* spp. isolates by quantifying the mycelial growth and production of S. *rolfsii* sclerotia in dual cultures. We employed a completely randomized design with 11 treatments, 10 being *Trichoderma* isolates, each confronted with *S. rolfsii*, and a control consisting of a disc with culture medium and mycelium of the pathogen without opposition to antagonists. Each treatment received five replicates, resulting in 55 experimental units (EU) consisting of one Petri dish each.

In planta antagonism of *Trichoderma* spp. against *Sclerotium rolfsii*

For the experiments, we used 90-d-old stevia seedlings of the cultivar "Katupyry", developed through genetic selection programs aimed at enhancing agronomic performance and disease resistance. This cultivar was selected for its adaptability to local environmental conditions, high steviol glycoside content, and its recognized tolerance to biotic and abiotic stresses, making it an ideal candidate for evaluating biological control strategies against *Sclerotium rolfsii*. The seedlings were provided by the Ka'a he'ê and Medicinal Plants Research Program-CIHB and were propagated through cuttings treated with indole butyric acid at a rate of 2 g L^{-1} of water. They were then transplanted into black polyethylene pots (60 µm, 20 cm x 20 cm) containing a sterilized substrate composed of sand, white sand, and humus in a 3:2:1 ratio. Substrate sterilization was performed using wood fire, maintaining a temperature of 250°C for 6 h to minimize microbial contamination. The stevia cuttings were transplanted on September 2017 and remained in polyethylene pots throughout the experiment.

The experimental design was completely randomized, with seven treatments and four replicates. The treatments consisted of treating the stevia plants with a *Trichoderma viride* isolate formulated as a soluble powder (TRIFESOL 1000 WP®, Biocultures, Ibagué, Colombia) with 1x conidia per gram. We used three native isolates of *Trichoderma* spp. (*T. asperellum* 3KH*, T. asperellum* TCAS, *and T. erinaceum* MS28) a mixture of the three native strains of *Trichoderma* spp., a synthetic fungicide azoxystrobin 200 g L^{-1} + cyproconazole 80 g L^{-1} (Priori Xtra® Syngenta, France) applied in a dose of approximately 5 ml L^{-1} water, and a control inoculated with the pathogen (Tab. 2). For the mixture, we combined individual suspensions of the native strains with concentrations of 10^7×spores ml^{-1} in equal proportions and diluted until the concentration of the mixture was equal to 10^7×spores ml^{-1}. The experimental unit (EU) consisted of nine pots with one plant each, using 252 stevia plants.

TABLE 2. Description of treatments for the control of *Sclerotium rolfsii* in stevia.

Treatment	Description	Dose	Application
1	*Trichoderma* mix	1× spores ml^{-1}	Preventive
2	*T. asperellum* 3KH	1× spores ml^{-1}	Preventive
3	*T. asperellum* TCAS	1× spores ml^{-1}	Preventive
4	*T. erinaceum* MS28	1× spores ml^{-1}	Preventive
5	*T. viride*	1× spores ml^{-1}	Preventive
6	Azoxystrobin + cyproconazole	5 ml L^{-1}	Curative
7	Inoculated control	-	-

We carried out the preparation of the suspension of *Trichoderma* spp. following the methodology of Sanabria Velázquez (2020), described as follows: *Trichoderma* spp. isolates previously isolated from the culture of stevia were selected, and they were seeded in Petri dishes with PDA culture medium and incubated in an incubator chamber (Shimadzu Corporation, BITEC-300, Kyoto, Japan) at 25°C for 7 d. For the multiplication of *Trichoderma* spp., 1 kg of rice was used (Tio Nico®, Fine Long Glazed Rice Type 00000, Paraguay), soaked for one hour in a two-liter beaker; the water was allowed to drain for half an hour; 200 g of rice were loaded into polypropylene bags and autoclaved for 20 min at 120°C and 2 atm pressure.

For the colonization of the culture medium, 25 ml of sterilized water per plate was added; the surface was then scraped, creating a suspension of spores and hyphae. This solution was then placed in a magnetic stirrer for 20 min. To inoculate a bag of sterilized rice, 10 ml of spore suspension was taken, and 200 g of rice was dispensed in previously sterilized polypropylene bags. The bags were shaken manually to homogenize the distribution of the spore suspension and incubated at 28°C for 12 d in an incubator chamber.

To prepare *Trichoderma* spore suspension for *in planta* trials, the following steps were taken: 500 ml of sterilized distilled water was added to a polypropylene bag containing rice colonized by *Trichoderma* spp. and the mixture was stirred to extract as many conidia as possible and then poured into a beaker. To extract the rest of the spores, 500 ml of sterilized distilled water was added back into the polypropylene bag, stirred again, and poured into a beaker until 1000 ml of suspension with sterilized distilled water was obtained. Conidia were counted in the Neubauer chamber, and the concentration of conidia was adjusted to 10^7×spores ml^{-1}.

Spore suspensions of *Trichoderma* spp. were dissolved in water with a concentration of 10^7×spores ml^{-1}, and five applications were made. Each application differed from the previous one for one week. The spore suspensions in the respective pots were applied to the point of percolation. After 7 d of the second preventive application of the suspension of 10^7×spores ml^{-1} of *Trichoderma* spores coincided with the infestation with *S. rolfsii,* the chemical control (azoxystrobin + cyproconazole) was dispensed in recommended doses, applying 200 ml of the product in each pot. The suspension was applied directly to the pot's substrate and the base of the stevia plants.

Infestation with the phytopathogenic fungus *Sclerotium rolfsii*

We obtained inocula of *S. rolfsii* following the methodology of Sanabria-Velazquez *et al.* (2019) described as follows: an isolate of *S. rolfsii* previously isolated from symptomatic plants of the stevia culture was selected. This sample was seeded in Petri dishes with PDA culture medium and incubated at 25°C for 4 d. Potato slices with a diameter of approximately 5 cm and 2 cm in height were cut and autoclaved for 20 min at 120°C and 2 atm pressure. Under a laminar flow hood, discs of PDA (5 cm) colonized by *S. rolfsii* were extracted and placed on the previously sterilized potato slices, then incubated in an incubator chamber (Shimadzu Corporation, BITEC-300, Kyoto, Japan) at 28°C for 3 d.

We used a potato disc infested by *S. rolfsii* to infest a pot with stevia located 2 cm from the pot's center on the substrate surface. We treated the plants previously with two applications of *Trichoderma*. When the plants were treated with *S. rolfsii*, we applied all the treatments, including the chemical treatment described in the previous section. The surface of the plant was then covered with a polyethylene bag simulating a wet chamber. The plants were kept covered inside the greenhouse until the end of the experiment.

Evaluation of variables

Disease incidence was obtained by counts of wilted plants with symptoms of *S. rolfsii.* The incidence of the disease was recorded from 2 d after inoculation (DAI) with the pathogen until all control plants presented symptoms. Using the mean percentage of disease at each estimated date of incidence for each experimental unit, the area under the disease progression curve (AUDPC) was calculated using the formula:

$$\text{AUDPC } = \sum_{i=1}^{Ni-1} \left(\frac{y_i + y_{i+1}}{2}\right)(t_{i+1} - t_i) \qquad (1)$$

where

AUDPC = area under the disease progression curve for EU;

y_i = percentage of disease incidence at each assessment date for each EU;

t_i = each evaluation date.

The percentage of disease control for the different treatments was calculated using the following formula:

$$\text{Control} = 100\% - [\left(\frac{PC}{TC}\right) \times 100] \qquad (2)$$

where

Control: percentage control of disease progression (%);

PC: area under the progression curve of the EU disease with the highest incidence;

TC: area under the disease progression curve of each EU.

Data analysis

Analysis of variance (ANOVA) was used for data results; the means of the treatments were compared using the Fisher LSD test at a 5% probability of error. Statistical analysis was performed with the statistical package InfoStat® (Di Rienzo *et al*., 2008). For all studies, normality of distributions was checked earlier for analysis. Untransformed means and confidence intervals were reported.

Results and discussion

Table 3 shows the production of *S. rolfsii* sclerotia compared to the 10 *Trichoderma* spp. isolates and control (only the pathogen) after 72 h of incubation.

TABLE 3. Production of *Sclerotium rolfsii* sclerotia in dual culture with *Trichoderma* spp. isolates during *in vitro* antagonism tests.

Treatments	Number of sclerotia*
TCAS	0.00 C
1KH	0.00 C
3KH	0.00 C
1 PICO 4	19 B
1 PICO 6.1	33 B
1 PICO 6.2	20 B
TQUI	19 B
K6	30 B
C2	40 B
T9	124 A
Control	178 A

* Means with a common letter are not significantly different ($P > 0.05$) based on the Fisher LSD (Least significant difference) test.

Significant differences in sclerotia production were observed when *Sclerotium rolfsii* was confronted with various *Trichoderma* spp. isolates (Fig. 1). The number of sclerotia per Petri plate ranged from 0 to 178. Notably, the TCAS, 1KH, and 3KH isolates significantly inhibited sclerotia formation compared to other *Trichoderma* isolates and the control (Tab. 3). In contrast, isolates 1 PICO 4, 1 PICO 6.1, 1 PICO 6.2, K6, and C2 showed no significant differences in sclerotia production, with values ranging from 19 to 40 sclerotia per plate. The T9 isolate exhibited a low capacity to inhibit sclerotia production, yielding 124 sclerotia per plate, which was not statistically different from the control. The variability in sclerotia inhibition among different *Trichoderma* isolates underscores the complexity of their antagonistic interactions with *S. rolfsii*, suggesting that some isolates may be more effective biological control agents than others.

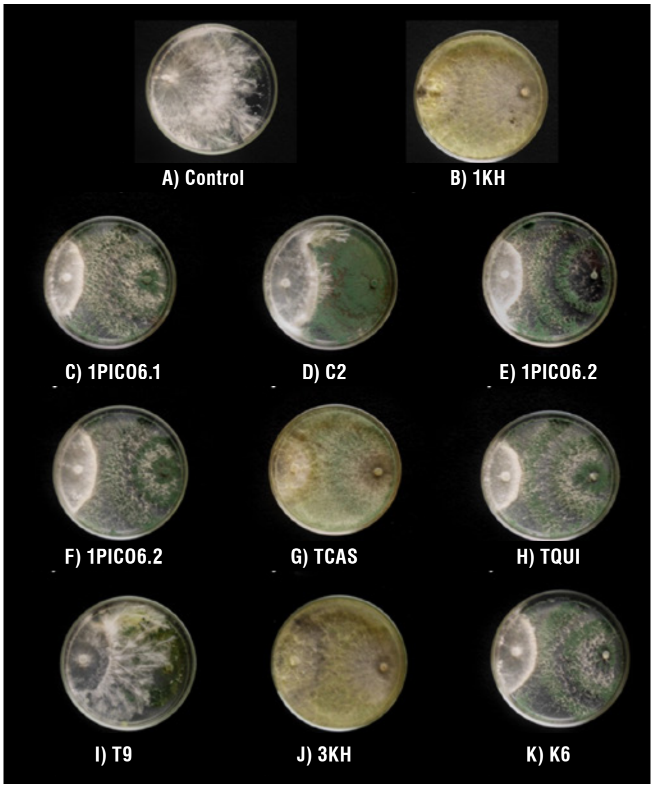

FIGURE 1. Inhibition of growth and production of sclerotia of *Sclerotium rolfsii* in dual culture: *Sclerotium rolfsii* (left) and *Trichoderma* spp. (right).

These results emphasize the importance of screening multiple isolates to identify the most effective candidates for biological control, as their performance can vary significantly, even within the same species or geographic origin. Similar levels of inhibition when selecting 12 out of 20 strains of *Trichoderma* spp. from agricultural areas in Brasilia, Brazil, were observed against *S. rolfsii*, inhibiting the pathogen in a range of 18.97 to 44.12% *in vitro* (Correa *et al*., 2007). Isolates of *Trichoderma* spp. interfere with the survival and development of sclerotia through various antagonistic mechanisms (Hoyos-Carvajal *et al*., 2008). Similarly, several studies have demonstrated the effectiveness of using *Trichoderma* spp. strains against *S. rolfsii* (Ram *et al*., 2020; Rodriguez-Paez & Jaraba-Navas, 2023).

Incidence of stem rot caused by *Sclerotium rolfsii*

Significant differences were observed between plants treated with *Trichoderma* TCAS isolate and those treated with azoxystrobin + cyproconazole in terms of reduction of wilt caused by *S. rolfsii* compared to untreated control plants (Tab. 4).

TABLE 4. Effect of different treatments with *Trichoderma* spp. and azoxystrobin + cyproconazole isolates on the area of the disease incidence progress curve (AUDPC) in *Stevia rebaudiana* plants inoculated with *Sclerotium rolfsii* under greenhouse conditions.

Treatments	AUDPC^{w} (Mean ± standard deviation)		Confidence Interval 95%^{y} (%)	
T. viride (commercial)	15.58 ±1.28	A^{x}	12.55	18.62
Trichoderma mix	13.77 ±3.73	AB	10.74	16.8
Control	13.27 ±2.36	ABC	10.24	16.3
MS28	10.47 ±2.75	BCD	7.44	13.51
3KH	9.15 ±4.40	CD	6.12	12.19
TCAS	7.67±2.91	D	4.63	10.69
Azoxystrobin + cyproconazole	1.25 ±1.73	E	-1.78	4.28

^{w}Area under disease progress curve obtained based on the progress of the incidence of the disease during 20 d after inoculation. ^{x}Means with a common letter are not significantly different ($P>0.05$) based on the Fisher LSD (Least significant difference) test. LSD=4.29. ^{y}AUDPC 95% confidence interval.

Among the *Trichoderma*-based treatments, only the TCAS isolate showed a significant difference compared to the control. Plants treated with the TCAS isolate had a disease progression area of 7.67. In contrast, those treated with the 3KH isolate had a progression area of 9.15, which was not statistically different from the disease progression observed in plants treated with the MS28 isolate (AUDPC 10.47). Plants treated with the mixture of isolates (TCAS, 3KH, and MS28) had an AUDPC of 13.77, which was significantly higher than that of plants treated solely with the TCAS isolate. Meanwhile, plants treated with the *T. viride* formulation showed a disease progression area of 15.58, which was not significantly different from the control treatment.

The biological control agent *Trichoderma* TCAS effectively reduced the progress of *S. rolfsii*-induced wilt under greenhouse conditions. It could directly attack and parasitize the sclerotia of the pathogen (Fig. 2). These results are consistent with Rawat and Tewari (2010), who studied the interaction between *T. harzianum* and *S. rolfsii* sclerotia using light and transmission electron microscopy. This research observed and confirmed this parasitism of *Trichoderma* on the sclerotia of *S. rolfsii*. The lysis and deformation of the cell wall of hyphae, the degradation and disappearance of cytoplasmic contents, and the loss of cell integrity in *S. rolfsii* sclerotia parasitized by *T. harzianum* were evident from transmission electron micrographs.

Sclerotia are the disease's primary inoculum, and reducing their viability can significantly reduce the progression of the disease. Therefore, the TCAS isolate presents excellent potential for managing stevia stem rot (Lourenço Jr *et al*., 2018). In our study, no significant differences were observed in disease suppression among *Trichoderma* isolates of the same species. However, TCAS (*T. asperellum*) presented the least progress of disease incidence per area.

Hoyos-Carvajal *et al*. (2008) demonstrated the ability of *Trichoderma to* reduce the incidence of bean wilt (*Phaseolus vulgaris* L.) caused by *S. rolfsii* and cotton wilt (*Gossypium barbadense* L.) by *Rhizoctonia solani* under nursery conditions. Their results showed that the antagonistic capacity of the isolates varied depending on the targeted pathogen. Consistent with the results of this study, they found no direct correlation between taxonomic classification and antagonistic activity. Instead, significant variation was observed within the same species, emphasizing the need for careful selection of isolates in phytopathogen control programs.

Combining native *Trichoderma* isolates in a mixture was no more effective than applying each isolate separately (Tab. 4). This may be due to incompatibility among the isolates, which could have compromised their antagonistic action against the pathogen. Similarly, Ortuño *et al*. (2013) observed an antagonistic effect between different *Trichoderma* isolates by forming a defense barrier between incompatible strains within a shared growth area through the secretion of antifungal substances.

The plants treated with the formulated *T. viride* preparation exhibited the highest disease incidence. Likely, non-native *Trichoderma* isolates do not adapt well to Paraguayan conditions. Previous work demonstrated that highly effective *Trichoderma* isolates introduced from Ecuador exhibited a low level of inhibition of *Colletotrichum* spp. during *in vitro* and field tests (Sanabria Velázquez, 2020). However, native *Trichoderma* isolates have been relatively effective against soil pathogens such as *Macrophomina phaseolina* in soybeans (Franco Ortellado & Orrego Fuente, 2013)

FIGURE 2. *Stevia rebaudiana* plants inoculated with *Sclerotium rolfsii* under greenhouse conditions. A) Wilt caused by *S. rolfsii* in the control treatment. B) Mycelium of the pathogen *S. rolfsii* growing in the control treatment. C) Healthy plants treated with *Trichoderma* suspension. D) White and green colonies corresponding to *Trichoderma* spp. parasitizing sclerotia of *S. rolfsii* in treated plants.

and *Rosellinia* spp. in macadamia (Sanabria Velázquez & Grabowski, 2016). However, despite these promising results, the absence of formulation methodologies has hindered the commercial development of products based on native *Trichoderma* strains.

Percentage control of stem rot caused by *Sclerotium rolfsii*

The azoxystrobin + cyproconazole treatment reduced disease incidence by 92.7% compared to the control (24.8%) and was significantly more effective than treatments with *Trichoderma* spp. (Tab. 5). The fungus *S. rolfsii* can rapidly infect both the roots and stems of stevia, emphasizing the importance of curative treatments with fungicides in stevia production. The most effective control of *S. rolfsii* in stevia was attained with the fungicides azoxystrobin + cyproconazole. This finding aligns with previous research, which reported that azoxystrobin was the only fungicide applied as a transplant water treatment with significantly lower AUDPC values from stevia stem rot caused by *S. rolfsii* in stevia crops in North Carolina, USA (Koehler & Shew, 2017).

In plants treated with the TCAS isolate, the area of disease progression was 7.67%, corresponding to a 55.48% reduction compared to plants with 100% incidence. The 3KH isolate achieved a 44.1% control, which was statistically similar to TCAS, although it exhibited greater variability, with a 95% confidence interval ranging from −4.53% to 92.73%. The MS28 isolate provided an average control of 38.85%, with a confidence interval between 13.29% and 64.40%. The control treatment did not show the highest incidence, and the disease control achieved with the *Trichoderma* mixture (18.27%) and the formulated *T. viride* (8.84%) treatments was 24%. However, since there were no significant statistical differences between these treatments, their effectiveness can be considered equivalent.

TABLE 5. Percentage control of the disease caused by *Sclerotium rolfsii* for different treatments with *Trichoderma* spp. and azoxystrobin + cyproconazole isolates under greenhouse conditions.

Treatments	Control (%) (Mean ± standard deviation)		Confidence Interval 95%^{y} (%)	
T. viride (commercial)	8.84 ±07.27	A^{x}	-2.72	20.41
Trichoderma mix	18.27 ±21.25	AB	-15.55	52.08
Control	24.80 ±17.49	AB	-3.03	52.62
MS28	38.85 ±16.06	BC	13.29	64.40
3KH	44.10 ±30.56	BC	-4.53	92.73
TCAS	55.48 ±16.81	C	28.73	82.23
Azoxystrobin + cyproconazole	92.7 ±10.10	D	76.63	108.77

^{x}Means with a common letter are not significantly different ($P>0.05$) based on the Fisher LSD (Least Significant Difference) test. LSD=26.03. ^{y}95% confidence interval of the percentage control of the disease caused by *Sclerotium rolfsii* under greenhouse conditions.

The effectiveness of native *Trichoderma* isolates, such as TCAS, in suppressing *Sclerotium rolfsii* highlights the importance of selecting locally adapted strains for biological control. Native isolates are more likely to be well-adapted to the environmental and soil conditions where they are applied, potentially leading to greater persistence and efficacy in the field. Their use aligns with sustainable disease management strategies, reducing reliance on synthetic fungicides and minimizing ecological disruption. The variability observed among *Trichoderma* isolates reinforces the need for comprehensive screening programs to identify the most effective strains for specific agricultural settings.

The findings of this study have practical implications for stevia producers, who face significant losses due to *S. rolfsii*. The observed 55.48% pathogen control by TCAS in greenhouse trials suggests that *Trichoderma* based biocontrol strategies can contribute to disease suppression. However, integrating additional management strategies is likely to enhance control levels further. Similar improvements have been documented in sugar beet production, where *T. harzianum* achieved up to 88% disease control when applied to *S. rolfsii*-infested soil across two growth cycles (Upadhyay & Mukhopadhyay, 1986). Additionally, combining *T. harzianum* with the fungicide PCNB significantly reduced disease incidence and increased crop yield. A more recent reference documenting the effectiveness of *T. harzianum* in controlling *S. rolfsii* in sugar beet production is the study by Ellatif *et al.* (2019). This study found that *T. harzianum* (strain KJ831197) significantly suppressed the radial growth of *S. rolfsii* with an efficiency ranging from 77.77% to 91.11% in dual culture techniques. Additionally, the combination of *T. harzianum* with β-glucanase enzymes increased disease control effectiveness and improved sucrose content in sugar beet (Ellatif *et al.*, 2019). Other studies have demonstrated that *T. asperellum* BCC1 reduced white rot-related mortality by 74% in greenhouse conditions (Rivera-Méndez *et al.*, 2020), while *T. asperellum* NVTA2 suppressed disease incidence by up to 69% and enhanced plant growth (Vinodkumar *et al.*, 2017). These findings suggest that an integrated disease management approach, combining *Trichoderma* with compatible fungicides or cultural practices, may be the most effective strategy for managing *S. rolfsii* in stevia cultivation.

Despite the promising results, this study has limitations that should be acknowledged. The trials were conducted under controlled greenhouse conditions, which may not fully represent the environmental variability encountered in field settings. Factors such as soil microbiome interactions, temperature fluctuations, and moisture levels could influence the performance of *Trichoderma* isolates in commercial stevia production. Future research should focus on validating these findings in field trials, optimizing application methods, and evaluating the long-term persistence of *Trichoderma* in soil. Additionally, exploring the potential of combining *Trichoderma* with organic amendments or other biocontrol agents could further enhance disease suppression. Understanding the mechanisms underlying the differential effectiveness of *Trichoderma* isolates will also contribute to the development of more targeted and reliable biocontrol strategies. By addressing these research gaps, the application of *Trichoderma* spp. can be optimized to provide stevia producers with a sustainable and effective solution for *S. rolfsii* management.

Conclusions

This study demonstrated the potential of native *Trichoderma* spp. isolates as effective biological control agents against *Sclerotium rolfsii*, a major pathogen affecting *Stevia rebaudiana* production in Paraguay. The two-phase evaluation strategy identified *T. asperellum* TCAS as the most effective isolate, reducing disease incidence by 55.48% under greenhouse conditions. However, applying *Trichoderma* as a mixture did not enhance control, suggesting possible antagonistic interactions among strains. The findings emphasize the advantages of using native isolates, which are better adapted to local conditions and offer a sustainable alternative to synthetic fungicides. While these results provide valuable insights for integrating *Trichoderma*-based biocontrol into stevia production systems, further field validation is needed to assess its long-term efficacy, persistence, and potential synergies with other management strategies. Optimizing application methods and formulation techniques will be crucial for maximizing the practical benefits of *Trichoderma*, reducing reliance on chemical control, and enhancing sustainable disease management in stevia cultivation.

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Conflict of interest statement

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

Author´s contributions

ADSV conceptualized and designed the study, supervised the experimental work, performed data analysis, and drafted the manuscript. HKBV assisted with the design and execution of greenhouse experiments, contributed to data collection, and revised the manuscript. GM conducted laboratory work, including the isolation and maintenance of fungal cultures, and contributed to manuscript preparation. FC performed statistical analyses, contributed to data visualization, and assisted in results interpretation and manuscript review. RB coordinated the collection of soil samples, conducted in vitro assays, and contributed to manuscript preparation. CO participated in greenhouse trials, monitored disease progression, and assisted with data recording and manuscript revision. HJSO provided technical guidance, facilitated access to research facilities, supported experimental design and execution, and reviewed the manuscript. All authors approved the final version of the manuscript.

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Citizen science and digital data for trend analysis and impact assessment of *Prodiplosis* as an emerging pest in foliage crops

Ciencia ciudadana y datos digitales para el análisis de tendencias y la evaluación del impacto de *Prodiplosis* como plaga emergente en cultivos de follaje

Laura Alejandra Valbuena-Gaona[1], Hector Julio Villamil-Martha[2],
Luz Mary Pardo-Ramirez[2], and Joaquín Guillermo Ramírez Gil[1,*]

ABSTRACT

Prodiplosis longifila is a pest of significant economic relevance, severely impacting crops like tomatoes and asparagus. Its effect on crops of ornamental foliage remains poorly documented, despite its growing importance in the agricultural sector. This study addresses our knowledge gap by implementing and validating digital tools of epidemiology (DE) and citizen science (CS) to enable a dynamic and participatory approach to pest monitoring. A trend analysis of scientific publications was conducted using web searches and social media interactions to identify topics concerning *Prodiplosis* over time, our knowledge gaps, and emerging areas of public interest. We assessed the impact of *Prodiplosis* on foliage crops, focusing on indirect effects and farmer-led management strategies shared through digital communication. Results show that digital tools such as trend monitoring on social media, web data analysis, WhatsApp group discussions, and farmer-managed digital platforms were effective for identifying the pest's distribution, significance, and control practices. DE and CS approaches revealed critical knowledge gaps concerning the biology, ecology, and management of *Prodiplosis*, particularly in ornamental crops. Field data confirmed the pest's negative impact on foliage yield and quality, with a strong dependence on chemical control methods, often applied without technical guidance. This study introduces an innovative methodology for assessing pest impacts through digital data analysis, offering practical insights for agricultural and policy decision-making. Moreover, the study highlights the potential of natural language processing as a powerful tool for synthesizing and detecting patterns in textual data and enhances the efficiency of pest surveillance and management systems.

Key words: bibliometrics, digital platforms, Google trends, social networks, spatial analysis.

RESUMEN

Prodiplosis longifila es una plaga de alta relevancia económica, que afecta gravemente cultivos como tomate y espárrago. Sin embargo, su impacto sobre cultivos de follaje ha sido poco estudiado, a pesar de su creciente importancia. Este estudio aborda dicha brecha mediante la implementación y validación de herramientas de epidemiología digital (ED) y ciencia ciudadana (CC), que permiten un enfoque participativo y dinámico para el monitoreo de esta plaga. Se realizó un análisis de tendencias basado en publicaciones científicas, búsquedas en internet e interacciones en redes sociales, con el objetivo de identificar los temas tratados, los vacíos de conocimiento y las áreas emergentes de interés. Adicionalmente, se evaluó el impacto de *Prodiplosis* en cultivos de follaje, describiendo sus efectos indirectos y las estrategias de manejo adoptadas por los agricultores a través de canales digitales. Los resultados muestran que herramientas digitales como el análisis de tendencias en redes sociales, la exploración de datos web, los grupos de WhatsApp y las plataformas digitales gestionadas por productores son eficaces para identificar la distribución, importancia y estrategias de control de *Prodiplosis*. Las metodologías de ED y CC también revelaron vacíos críticos en el conocimiento sobre la biología, ecología y manejo de esta plaga en cultivos ornamentales. El análisis de campo confirmó su impacto negativo en el rendimiento y la calidad del follaje, con una alta dependencia del control químico, usualmente sin asesoría técnica. Este estudio propone una metodología innovadora basada en datos digitales, destacando el potencial del procesamiento de lenguaje natural para fortalecer la vigilancia y gestión fitosanitaria.

Palabras clave: bibliometría, plataformas digitales, Google trends, redes sociales, análisis espacial.

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[1] Universidad Nacional de Colombia, Facultad de Ciencias Agrarias, Departamento de Agronomía, Laboratorio de Agrocomputación y Análisis epidemiológico, Center of Excellence in Scientific Computing, Bogotá (Colombia).

[2] Economía Agraria – Agropecuaria Villapard, Cachipay, Cundinamarca (Colombia).

[*] Corresponding author: jgramireg@unal.edu.co

Introduction

Digital data (DD) associated with digital epidemiology (DE) has become a crucial tool in the management of public health problems at the regional level, allowing the collection and analysis of real-time data for informed decision-making (Fulk *et al.*, 2022; Salathé, 2018). This approach takes advantage of information and communication technologies (ICT), such as social networks and web platforms, in order to monitor and respond to disease outbreaks with more speed and precision (Cervellin *et al.*, 2017; Fulk *et al.*, 2022). Through the use of data from online platforms, DE has indirectly enabled the monitoring and analysis of the spread and distribution of diseases as an alternative to traditional epidemiological surveillance tools (Ekman & Litton, 2007; Fulk *et al.*, 2022). The advantages include rapid data collection and early identification of epidemiological outbreaks. But these tools have some limitations, such as poor control over data quality, access to data, use of analytic tools, and access to and quality of the internet (Lippi & Cervellin, 2019; Park *et al.*, 2018).

As a complement to the DE, citizen science (CS) is a research approach that actively involves the public in scientific research, contributing data collection, analysis, and problem-solving (Fraisl *et al.*, 2022; Sherbinin *et al.*, 2021). This participatory model democratizes science, leveraging the collective intelligence of non-experts to address complex challenges (Sherbinin *et al.*, 2021). In the realm of public health, CS has gained significant importance, particularly in the field of DE (Carney *et al.*, 2022). DE uses multiple computational and digital tools as methods to monitor and analyze disease patterns in real time (Fulk *et al.*, 2022; Salathé, 2018). However, its success depends on engaging users who generate and share data through mobile apps, wearables, social media, and other digital platforms (Johnston *et al.*, 2023; Rosas *et al.*, 2022). This is where CS acts as a critical bridging tool, facilitating the interaction between DE and the general population.

In agricultural sciences, to our knowledge, the concept of DE and CS have not been widely explored. DE has been mainly used to monitor epidemics associated with public health in humans. One of the first reports was the study of the relationship between Google searches and the occurrence of respiratory diseases such as influenza (Ginsberg *et al.*, 2009). Meanwhile, CS had its beginnings in the social and natural sciences, especially focused on ecology, and public health (Kullenberg & Kasperowski, 2016). Its fundamentals can be adapted to pest management that allows farmers access and real-time sharing of information on the presence and population dynamics of pests in their crops. This approach could not only improve the capacity to respond to phytosanitary problems, but also it promotes collaboration and knowledge sharing and fosters the democratization of knowledge (Ryan *et al.*, 2018). In this way, the application of digital epidemiology to agriculture can lead to more efficient and sustainable pest management, reducing economic losses and promoting informed decisions for optimizing management strategies. The practical application of this concept is still in its early stages, but it can also help farmers improve their responses to phytosanitary problems (Ryan *et al.*, 2018).

Empowering individuals to contribute plant health-related data can enhance CS surveillance systems, and this improves early outbreak detection, and supports data-driven decision-making. But it is necessary to recognize and identify biases and errors in the data (Tran *et al.*, 2021). Additionally, CS surveillance systems foster public trust and awareness and encourage active participation in plant pest prevention and control. Initiatives such as participatory disease tracking apps exemplify how citizen engagement strengthens DE, transforming passive data collection into an interactive and collaborative process (Katapally *et al.*, 2021). Ultimately, the synergy between CS and DE represents a paradigm shift, where communities become integral players in the global monitoring of plant health and response.

In the floricultural industry, Colombia is the world's second-largest exporter of cut flowers, and this production is complemented by that of green foliage, which represent an essential component in ornamental horticulture (Adebayo *et al.*, 2020). Foliage is widely used in flower arranging, decoration, and landscaping because of its ability to beautify and enhance both indoor and outdoor spaces (Wei *et al.*, 2023). The constant demand for foliage is reflected in a global market of high economic value and continuous growth (Wei *et al.*, 2023).

In Colombia, green foliage took on greater importance in 2010 when the country began exporting bouquets, in which foliage and ferns played a complementary role to traditional flowers (Rojas Burgos, 2022). The most important foliage plants include Ruscus (*Ruscus aculeatus* L.) and Cocculus (*Cocculus laurifolius* L.), recognized for their potential for long vase life (Faust & Dole, 2021). The main foliage producing areas in Colombia are in the department of Cundinamarca, where they are grown in greenhouses, open field cultivation, and under shade nets.

Currently, foliage production in Colombia faces different threats, especially of phytosanitation. One of the most concerning pests is *Prodiplosis longifila* Gagné (Diptera: Cecidomyiidae) (Gagné, 1986). This pest is significant for damage to foliage, not only due to the damage it causes, but also due to the lack or inexistence of available information on its biology, ecology, population dynamics, impact, and sustainable management strategies. In the Cundinamarca highlands and particularly in the municipalities of Cachipay, Anolaima, Zipacón, and La Mesa, a considerable regional problem associated with the emergence of this emerged in recent years. The situation has generated uncertainty in the flower growing industry, since green foliage has been seriously affected by the presence of *P. longifila,* compromising the competitiveness and sustainability of these crops. One of the hosts reported for the species is the carnation (*Tagetes* sp.) (EPPO, 2017). Based on preferences for this species, it is possible that it could spread to other species of cut flowers and threaten their production. The importance of this pest lies in the fact that it is considered an invasive species in regions importing flowers from Colombia (EPPO, 2025; Goldsmith *et al*., 2013; Hernandez *et al*., 2015).

Prodiplosis longifila (commonly called *Prodiplosis* or *negrita* or *caracha*) is one of the main phytosanitary problems in the tomato (*Solanum lycopersicum* L.) and asparagus (*Asparagus officinalis* L.) crops (Geraud-Pouey *et al*., 2022; Hernandez *et al*., 2015; Castillo Valiente *et al*., 2020). This pest is a neotropical and polyphagous fly, considered to be of economic importance due to the serious damage it causes. It severely affects buds of Tahiti lime (*Citrus x latifolia* Tanaka ex Q.), (Jimenez, 2019), asparagus, various young shoots (asparagus, avocado, *Persea americana* Mill.), grapes (*Vitis vinifera* L.), leaves (tomato), inflorescences or flowers (tomato, Tahiti lime, asparagus), and vegetables (tomato), deforming them and limiting production (EPPO, 2017; Gagné, 1986; Hernandez *et al*., 2015). Adults of *P. longifila* are considered to be difficult to see in the field during the day, since they are active at dusk; females lay their eggs inside protected structures, such as in the buds of leaves and flowers, as well as under the calyx in tomato crops, or under the bracts or in flowers in asparagus (EPPO, 2017; Gagné, 1986; Hernandez *et al*., 2015). The presence of *P. longifila* has been confirmed in Colombia, Ecuador, Peru, and the USA where larvae damage buds and tender tissues of asparagus, tomato, sweet pepper (*Capsicum annuum* L.), cucumber (*Cucumis* sp.), citrus, and other crops (Geraud-Pouey *et al*., 2022; Hernandez *et al*., 2015). In tomato crops in Colombia and Ecuador, damage from this pest is reported close to 100% (Geraud-Pouey *et al*., 2022). In Peru, the economic impact on potatoes and asparagus is about 16% (Cedano & Cubas, 2012; Kroschel *et al*., 2012). In the USA, the impact of this insect reaches up to 25%, when this plague was reported in the 1990's (Pena *et al*., 1987). There is a lack of recent information on its status. Due to the severe damage caused by this pest, the use of chemical pesticides has increased significantly, resulting in the selection of populations with a loss of sensitivity to the different pesticides used (Mujica & Kroschel, 2019).

Despite the growing interest in the cultivation of foliage and the significant potential impact of *P. longifila*, limited knowledge of this pest indicates the need to investigate key aspects of its biology and ecology, and to develop management alternatives that are based on scientific evidence. This research, conceived as a baseline, aimed to employ a set of tools within the DE, CS and data analysis approaches to identify and understand relevant trends about this pest in other hosts such as tomato or asparagus, and in foliage in particular. In this sense, our work focused on three main areas: academic trends through bibliometric analysis, trends in social networks such as X (previously Twitter) and YouTube, and information available on the Web under the Google trends platform. The perceptions of foliage producers in the production area of Cachipay, Cundinamarca, Colombia were explored using specifically designed surveys and direct communications in group chats. The present research defines potential alternatives for how these tools can be a solid basis for future research and control strategies for the floriculture and foliage production industry.

Materials and methods

Localization data origin

To analyze the tendencies of the foliage producers for aspects such as damage, importance, impact, management strategies, and others of *Prodiplosis*, we worked under the supervision and participation of the Economía Agrícola-Agropecuaria Villapard, and other farms located in the municipality of Cachipay in the department of Cundinamarca, Colombia (4°44’17.13” N, 74°25’37.68” W). Environmental conditions were in greenhouses under cover as well as in open fields of Ruscus and Cocculus foliage. Environmental conditions were semi-humid temperate conditions, with air temperatures ranging from 10°C -25°C, annual accumulated rainfall between 1300 mm and 2500 mm, and a bimodal distribution with dry periods in December – February and June – August. This research includes data collected from producers between the years 2018 (the time of the first pest reports) and 2023 (when the first part of the field work for this project was completed).

Identification of insects was carried out according to a protocol developed by the company. Identification concluded after a morphological and life cycle characterization that confirmed *P. longifila* as the pest population. This process was confirmed by the Museo Entomologico-UNAB of the Universidad Nacional de Colombia, Facultad de Ciencias Agrarias, Bogota, Colombia. As a complement and under the concept of pests of economic importance, the Institutio Colombiano Agropecuario (ICA) carried out the inspection, collected samples, and supported the identification of the pest. It should be noted that part of the producers’ work team, headed by the retired entomologist Professor Dario Corredor, sent photographs to the world expert Raymond J. Gagné, who stated that it was possible that it was the species *P. longifila*, but that the genus was under revision and that he was not committed to giving an accurate opinion. For the purpose of this research, we decided to consider this pest as *P. longifila*, but it is necessary that this be confirmed by taxonomic experts in the future.

Trends and perceptions of *Prodiplosis* in foliage crops and other hosts

A) Bibliometric

We carried out a primary analysis of scientific information in different bibliographic databases: Scopus(https://www.scopus.com/search/form.uri?display=basic#basic), PubMed (https://pubmed.ncbi.nlm.nih.gov/), and Google Scholar (https://scholar.google.com/?oi=gsb00&lookup=0&hl=es), as a basis for recognizing the state-of-the-art in research concerning *P. longifila*. For this objective, the search was limited to technical papers, theses, reports, and manuscripts published in English, Spanish, and Portuguese, and whose search equation was *Prodiplosis* which is identified as a common marker for the three selected languages. Some synonyms, or common names such as *negrita*, *caracha*, *caregato* were also included. The documents were then exported to BibTeX files, where the metadata contained authors, year of publication, journal or publication medium, country, author keywords, and abstract. The databases of the three bibliographic managers were merged, eliminating repeated results, and analyzed using the library pyBibx (Pereira *et al*., 2025), modifying some forms of visualization, executed in the Google Colab user environment under the Python programming language.

B) Web search and social networks (X and YouTube)

A detailed analysis was performed using various platforms, regardless of country, host, or other factors that might affect the usage of this type of tool (internet access, age, social status, academic strata, etc.) in order to identify and understand trends from the Web and social networks related to *Prodiplosis*. The platforms used for this analysis included X (previously known as Twitter) for the period in which information could be freely accessed (2011-2022), Google trends (2004-2024) and YouTube (2010-2024). The analysis focused on collecting search and published data on these platforms, covering a specific date range that allowed free use of the available data.

Initially, the occurrences of searches over time were evaluated, and the proportions of queries associated with different search algorithms were analyzed. From this information, trend curves were generated that illustrate fluctuations in the particular topic and relative searches over time. Subsequently, the text associated with these trends (comments, information, descriptions, among others) was extracted and integrated into the analysis, including content from indirectly linked pages that allowed for a more complete understanding of the topic. For the processing and analysis of these data, deep learning analysis based on natural language processing (NLP) tools were used (described in detail in the following section). We used the free software Python as the programming language, under the Google Colab user environment, specifically utilizing the sntwitter, googleapiclient, and pytrends libraries, which facilitated the extraction and systematic analysis of the information.

For the validation of the data associated with the search or count on social networks (X and YouTube) and the Web (Google trends) related to *Prodiplosis,* a historical database was used. This database was associated with reported cases of damage and the presence of the insect *Prodiplosis* from the Colombian phytosanitary service, the Plant Health Technical Directorate of the Instituto

Colombiano Agropecuario-ICA and monitoring data of the foliage production systems in Cundinamarca (n=50). It also included the different farmers dedicated to tomato production in Colombia that carry out monitoring as part of their agronomic practices (n=35, Antioquia, Cundinamarca, Boyacá, Caldas, Risaralda, Valle, Huila, Tolima, and Santander). For each social network or in Google trends, the historical series of available data in terms of searches, views, or tweets was used and integrated with the cases reported by the ICA in terms of both damage and presence of the insects associated with different hosts such as foliage and tomatoes.

Given that digital data presents several biases, both spatial and temporal, methodological adjustments were necessary to address these issues. Spatial biases arise from factors such as internet access, the location of the farmer, the technical assistant, or the individual conducting the search. Temporal bias is also significant since farmers tend to use sources like Google, videos, fellow producers, or technical advisors as their primary sources of information. These searches are often triggered by the ¨*identification of a phytosanitary problem*¨, knowledge about it, and the perceived economic impact. To reduce spatial bias, only areas relevant to tomato and foliage production in Colombia were considered, excluding unrelated queries that, while potentially real, could not be reliably validated. Temporal bias posed a greater challenge; thus, an approach was adopted to identify the statistically closest *lag* between online search behavior and actual field data. Techniques such as time lags, cross-correlation, and Granger causality tests were employed to assess the predictive or reactive nature of search activity about field observations

For the period of analysis, a series of lags were identified, which were associated with periods between 4 and 12 months and were adjusted for the digital data. Subsequently, a Spearman correlation analysis was performed, given the discrete origin of the variables. The confidence interval was calculated using the bootstrapping resampling method, and the results were visualized. The entire process was carried out in the Colab environment, utilizing the freely available programming language Python, ensuring a robust analysis of the relationship between online activity and reported agricultural cases.

C) Producer perceptions of *Prodiplosis* in foliage crops from surveys and use of WhatsApp social chat

Two methodological tools were used for this section. First, a semi-structured survey was designed and distributed to foliage producers in the Cachipay region and surrounding municipalities, inviting them to participate voluntarily. The survey was designed to gather farmer perceptions and management practices related to *Prodiplosis* in their cropping systems. It was structured into five thematic sections, each addressing specific aspects of the issue: i) Economic Perception and Impact (R1–R7). This section focused on understanding 'farmers' perceptions of the economic importance and impact of *Prodiplosis* on their crops, including yield losses, production costs, and overall farm profitability; ii) Use of Physical Control Measures (R8–R12). These questions addressed the use and perceived effectiveness of physical barriers such as protective curtains and traps in the management of *Prodiplosis*; iii) Population Dynamics and Environmental Factors (R13–R28). This block explored 'farmers' observations regarding *Prodiplosis* population levels and their potential associations with surrounding weeds, neighboring crops, and various agronomic practices such as fertilization, irrigation, and the presence of other pests; iv) Use of Inputs and Control Strategies (R29–R48). This section collected detailed information on pest control practices, including the use of bio-inputs and synthetic chemical products. Questions covered product names, dosages, timing and frequency of application (including time of day), as well as farmer perceptions of effectiveness and control success; v) Sociocultural and Farm Context (R49–R53). The final section captured background information on farmers, such as cultural practices, land tenure trends, educational level, access to technical assistance, and farm size. For more information, review the supplementary information 1.

In parallel, a group chat was accessed on the WhatsApp social network, in which farmers of foliage from the same region participated. The purpose of this study was to complement the information obtained in the survey and observe in real-time interactions and discussions related to the production of flowers and the situation associated with *Prodiplosis* during a period of analysis between 2018 and 2022. This group provided an additional platform for the exchange of knowledge and practices, allowing for the capture of qualitative data of interest.

In both cases, it was guaranteed that the ethical regulations related to the use of personal data were complied with. Informed consent was obtained from all participants, ensuring the confidentiality of sensitive information and the anonymity of the data collected. Furthermore, they were informed that the purpose of the study was strictly academic and details of how the data would be used in the context of the research were provided. These measures ensured that participation was conducted under rigorous ethical standards aligned with best research practices.

In the first phase, the survey was implemented through the Google Forms platform. This tool contained a total of 53 questions structured to evaluate different aspects associated with foliage and *Prodiplosis*. Of these, 32 questions were closed-ended and formulated based on a Likert-type response scale (strongly disagree, disagree, neutral or neither agree nor disagree, agree, and strongly agree) (Supplementary information 1). This allowed the measurement of the intensity of respondent perceptions and attitudes regarding various specific statements, enabling the quantification of opinions and a rigorous statistical analysis of the responses (Jebb *et al*., 2021; Joshi *et al*., 2015). Additionally, nine open-ended questions were included, designed to collect data for textual analysis (described in detail in Supplementary information 1). Seven dichotomous questions were formulated, with simple yes or no response options, focusing on capturing specific decisions and behaviors related to the study (Supplementary Information 1). The remaining seven questions were aimed at collecting personal and demographic information from the respondents. Once the data were collected, they were processed and analyzed using the likert library within the R programming environment version 4.4.2 (R Core Team, 2004), as well as other descriptive statistical methods.

Natural language processing (NLP) techniques were employed to analyze textual data obtained from the analysis of social networks and producer chats (Maulud *et al*., 2021; Tripathy *et al*., 2021). For this particular case, a neural network known as Skip-gram, which is a model that has shown promising results in NLP (Xiong *et al*., 2017), was retrained and implemented. We used words obtained from information found on X and pages linked to the tweet (6438 words, 40 tweets), titles of chats between farmers (19451 words, 1300 chats) and surveys (9359 words, 25 surveys). We then proceeded to optimize the parameters of the Skip-gram network. The World 2 Vec package was used for this process and the Skipgram network was executed in Python within the Google Colab user environment.

Design of a digital platform for connecting with producers in knowledge exchange processes

As a fundamental component to unify the concepts of digital epidemiology and citizen science, this study developed two interconnected digital applications. These applications were designed to perform partially similar yet complementary functions, facilitating data acquisition, visualization, and predictive modeling in plant pest monitoring (*Prodiplosis* case).

The first tool was a mobile application developed using an open cross-platform language (such as Dart) through the Flutter framework. This app was provided to the end users (a group of farmers participating in the trial). Five main modules were included: i) user module authentication and user profile management; ii) sensor configuration module settings for data acquisition from connected sensors; iii) data processing module graphical representation of collected and processed data; iv) georeferenced interactive map module visualization of spatial disease distribution and; v) disease forecasting and recommendations module integration of predictive models and disease management recommendations. The second tool was a web application developed using Python and the Flask framework. This platform includes the same core modules as the mobile application. But three additional features were introduced: i) API (Application Programming Interface) integration module facilitating access to external APIs for enhanced data analysis; ii) interactive forecast map module providing spatially explicit disease prediction visualizations and; iii) administrative dashboard module enabling real-time monitoring of measured variables. Communication between the platforms was established via a cloud-based infrastructure, utilizing a relational database and a virtual server. This setup enabled data storage, file hosting, and seamless information exchange between the Web and mobile applications, ensuring real-time synchronization and accessibility for end users.

Results

Bibliassociated with the current state of knowledge of *Prodiplosi*s worldwide

The search of bibliographic databases yielded 957 documents of which 932 belonged to Google Scholar, 22 to Scopus, and 3 to PubMed, with 16 duplicates. The first document associated with this search dates to 1812, and no further papers were found until 1908. From 1987 onward there was a continuous increase in the number of papers published, culminating in a peak, in 2019 with 82 papers (Fig. 1A). Prior to this, 16 papers were reported. Most of these articles are associated with *P. longifila* in flowers or fruits of crops such as tomato, asparagus, chili, bell pepper, blueberry, and potato and encompass topics associated

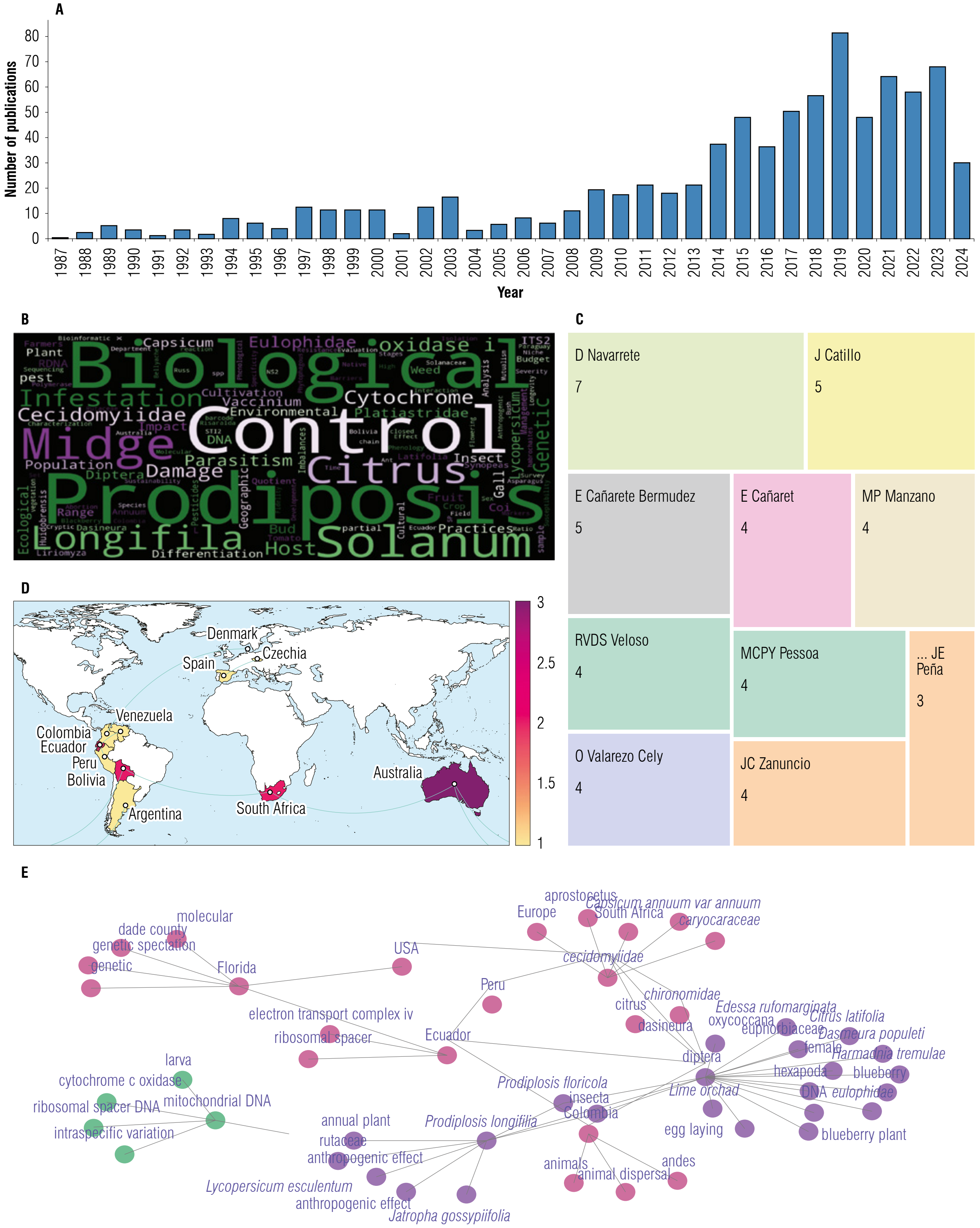


FIGURE 1. Meta-analysis of the scientific literature on *Prodiplosis*, based on a search query using the term "Prodiplosis" across the Scopus, PubMed, and Google Scholar databases. A-annual number of publications; B- most frequent terms extracted from abstracts; C-authors with the highest number of contributions; D-international collaboration network between countries; E-keyword co-occurrence clustering, illustrating thematic groupings within the literature.

with identification, distribution, damage, quality, yield, management, and genetics (Fig. 1B). Three main authors were highlighted (Fig. 1C). When analyzing the geographical relationships, 11 countries were identified, 6 of which are in Latin America (Fig. 1D), suggesting that this region is where this pest has the greatest impact.

When observing textual connectivity, 3 clusters were found. The first (green) is related to larval stages, neotropical regions, population structures, phylogenetics, quarantine measures, and molecular topics at the DNA and protein level (Fig. 1E). The second cluster (red) shows different subgups, specifically a relationship between Peru, Ecuador, and Florida, USA, with topics related to genetics, molecular biology, and the dipteran family Cecidomyiidae, associated with the host genus *Capsicum*. It also includes relationships between countries such as those involving South Africa, Europe, and the hymenopteran genus *Aprostocetus* and plants of the family Caryocaraceae. Slightly further away is the country of Colombia, which is closely tied to the Andes, animals, and to animal dispersal. Finally, cluster 3 (purple) shows three parts. The first of these is associated with *P. longifila,* which is linked to annual plants, Rutaceae, and anthropogenic effects, as well as plants such as *Jatropha gossypiifolia* L. and *S. esculentum*. The second is a bridge between the first and third groups and is associated with the class Insecta and *P. floricola*. Finally, the third subgroup is associated with Diptera, females, DNA, and some crops such as limes and blueberries (Fig. 1E).

Trends and perceptions of *Prodiplosis* using social media and web searches

The results obtained from the social network X revealed that the primary searches related to the term ¨Prodiplosis¨ were concentrated in Peru, Colombia, and, to a lesser extent, Ecuador (Fig. 2A). In both English and Spanish, the search trends showed peaks between 2016 and 2018. There has been a growing trend of Spanish-language tweets from 2016 onward, suggesting that this pest started to become a problem in Andean countries around this time (Fig. 2B). Furthermore, a word cloud analysis revealed that the most recurring terms were the species name, hosts (tomato, citrus, asparagus, among others), pest, control and life cycle, indicating that in recent years this insect has gained significant economic importance (Fig. 2C).

Similarly, web searches (Google Trends), which also include social media platforms such as YouTube, reflect findings like those of X. In this data source, *Prodiplosis* appears to be a pest of high importance in the previously mentioned Andean countries (Fig. 3A). In Colombia, the highest search volumes were associated with the departments of Cauca, Nariño, Cundinamarca, Huila, and the coffee growing region (Fig. 3A), aligning with the most productive areas for its well-known host, table tomatoes. Meanwhile, in Cundinamarca, the searches are linked to their potential impact on foliage and the threat to the floriculture sector. Temporal trends in Google searches reveal a significant peak in 2004, coinciding with reports of this pest in Peru, Ecuador, and Colombia. Furthermore, there has been a notable increase in searches since 2022, corresponding to a growing impact of this pest on host crops (Fig. 3B). Similarly, in the social network X, search terms in both English and Spanish focus on keywords such as name, control, populations, and hosts (Fig. 3C).

On YouTube, the highest number of views related to *Prodiplosis* occurred after 2019 in countries such as Peru, Colombia, and Ecuador (Fig. 4A and B). On this platform, videos related to integrated pest management, biological control, and *Prodiplosis* in tomatoes stand out, with most of the content in Spanish (Fig. 4C). Key terms frequently associated with this pest on YouTube include host plants, control methods, common and scientific names of the pest, presence in specific countries, and management practices (Fig. 4D).

Associated validation between social media searches, the Web and actual pest presence and reporting data

In the case of the validation of searches on social networks and the Web about the cases and damage to *Prodiplosis* reported by the ICA, a considerable potential for social networks and the Web is revealed to indirectly monitor phytosanitary problems in the agricultural sector (Fig. 5). According to the r metrics and the confidence interval, they indicate that the social network X, YouTube, and Google Trends presented values of 0.42, 0.51, and 0.73, respectively, based on the correlations observed within the lower and upper limits (Fig. 4A, B and C). These values suggested that, although all platforms have a degree of association with the *Prodiplosis* reports, Google Trends had the strongest relationship, indicating its greater effectiveness in reflecting actual infestation trends.

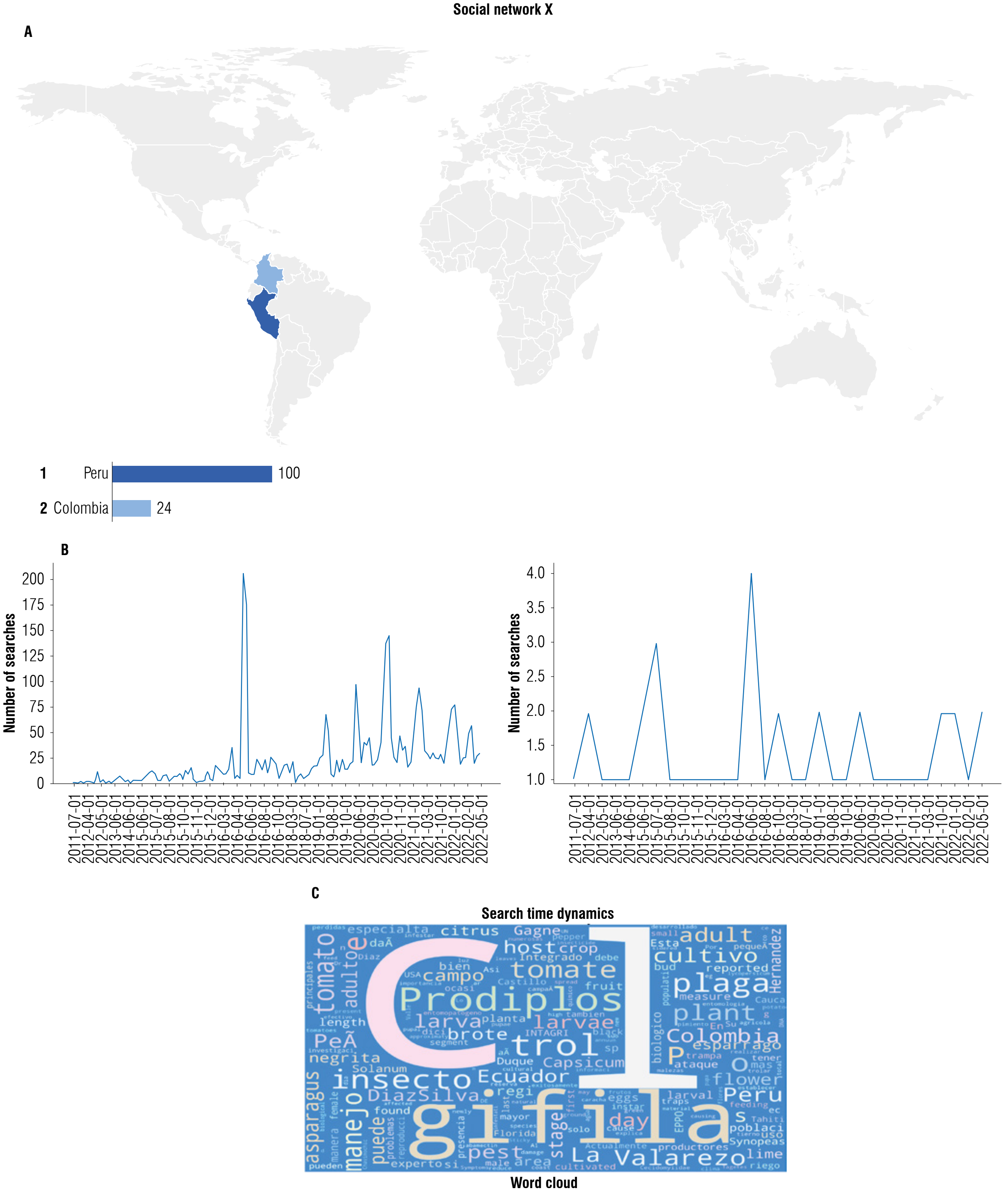
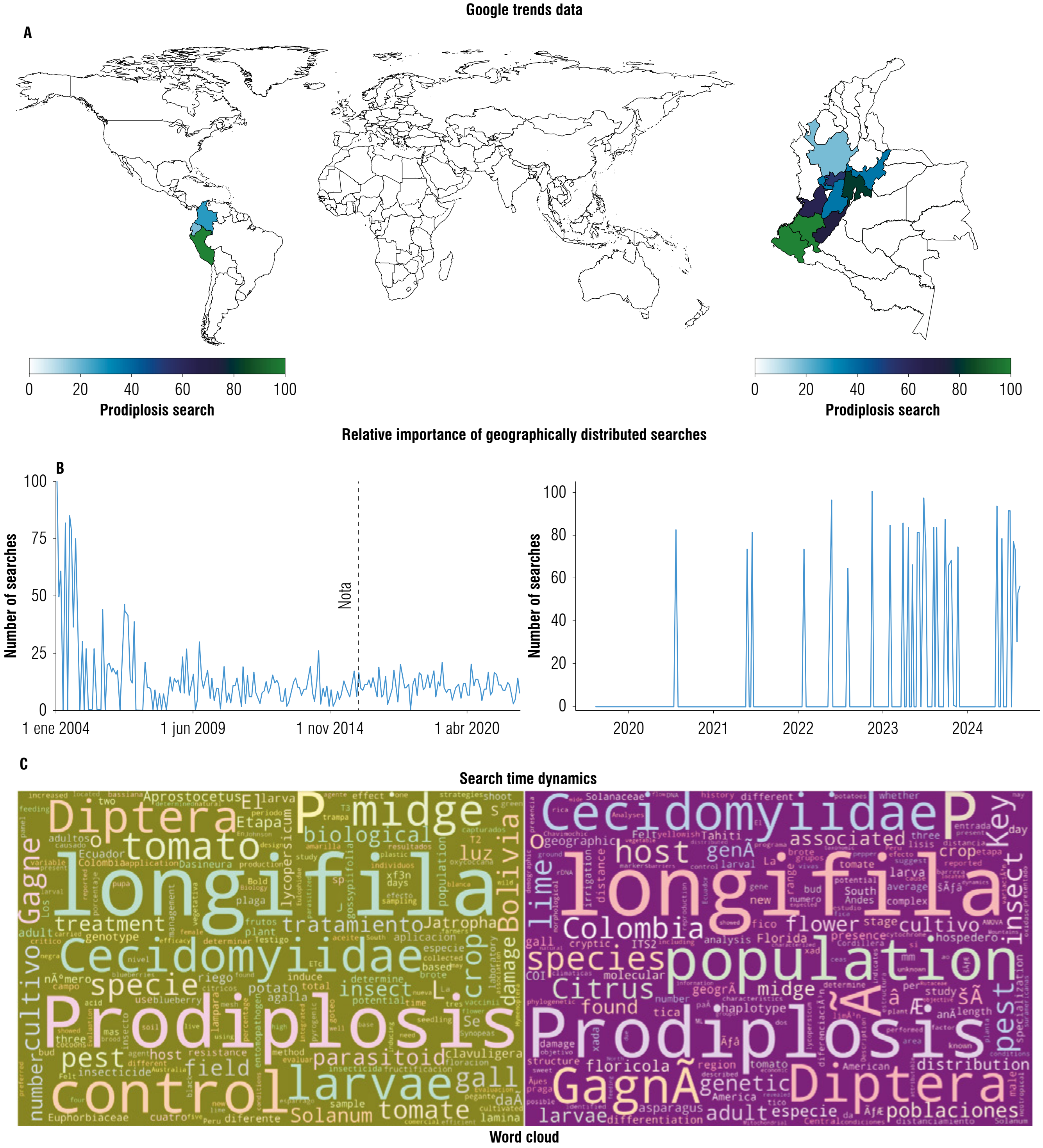


FIGURE 2. Aspects associated with trends in the social network X, the Web and YouTube related to aspects of *Prodiplosis* worldwide and in different hosts. A to C- analysis in social network X (previously Twitter). A-countries with most *Prodiplosis* publications; B-search time dynamic; C-world-cloud or related terms with *Prodiplosis*.


Google trends data

Relative importance of geographically distributed searches

Search time dynamics

Word cloud

FIGURE 3. Aspects associated with trends in the social network X, the Web and YouTube related to aspects of *Prodiplosis* worldwide and in different hosts. A to C-analysis on the Web (Google trends); A-countries where people searched about *Prodiplosis* worldwide and in Colombia (right); B-search time dynamic worldwide and in Colombia (right); C-world cloud of related terms with *Prodiplosis* searches in worldwide.

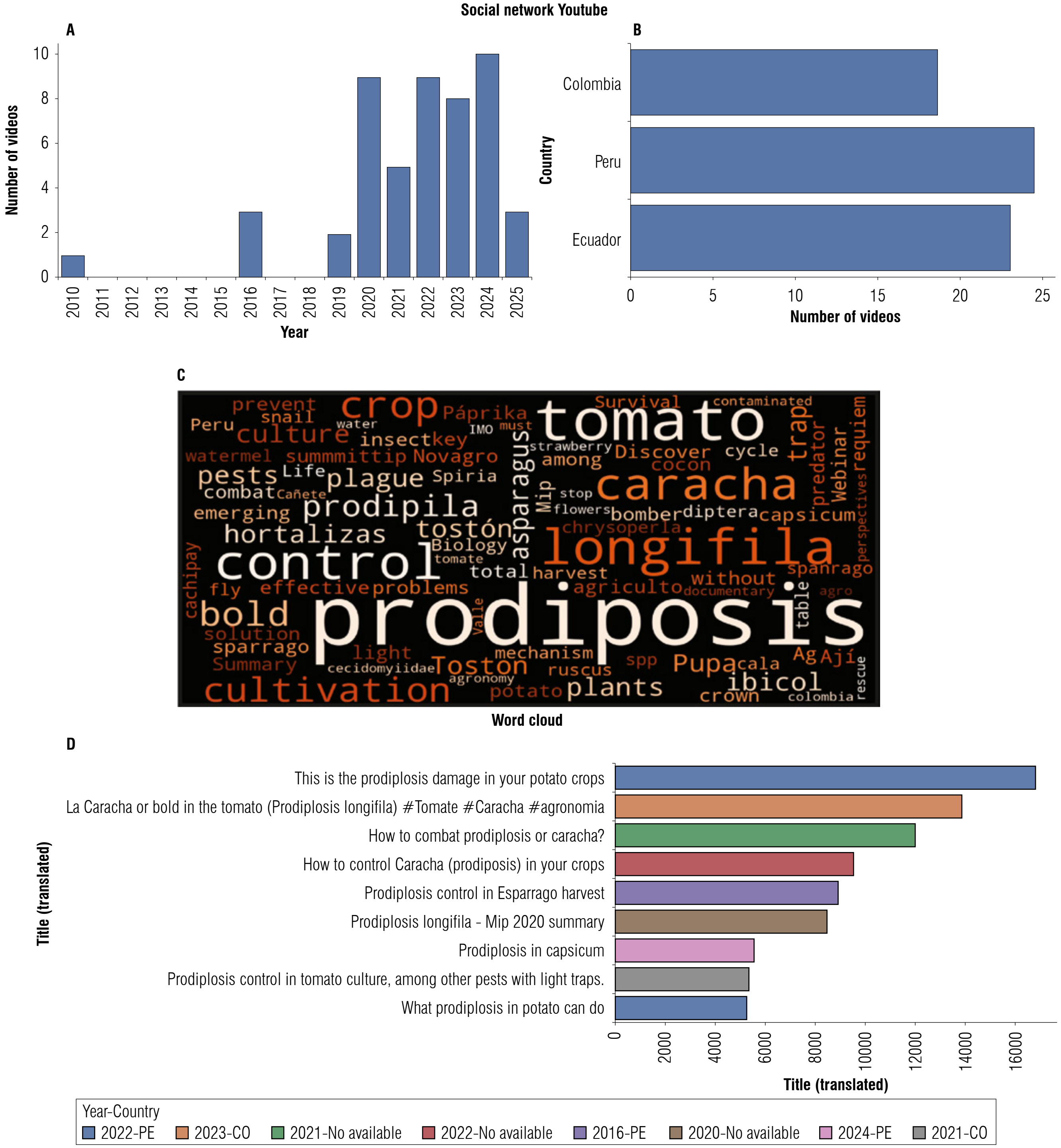


FIGURE 4. Aspects associated with trends in the social network X, the Web and YouTube related to aspects of *Prodiplosis* worldwide and in different hosts. A to D-YouTube social network analysis; A-number of videos published by year; B-countries where videos were published; C-world cloud of related terms of *Prodiplosis* videos; D-year and country of the most viewed videos, CO - Colombia, PE - Peru, EC - Ecuador.

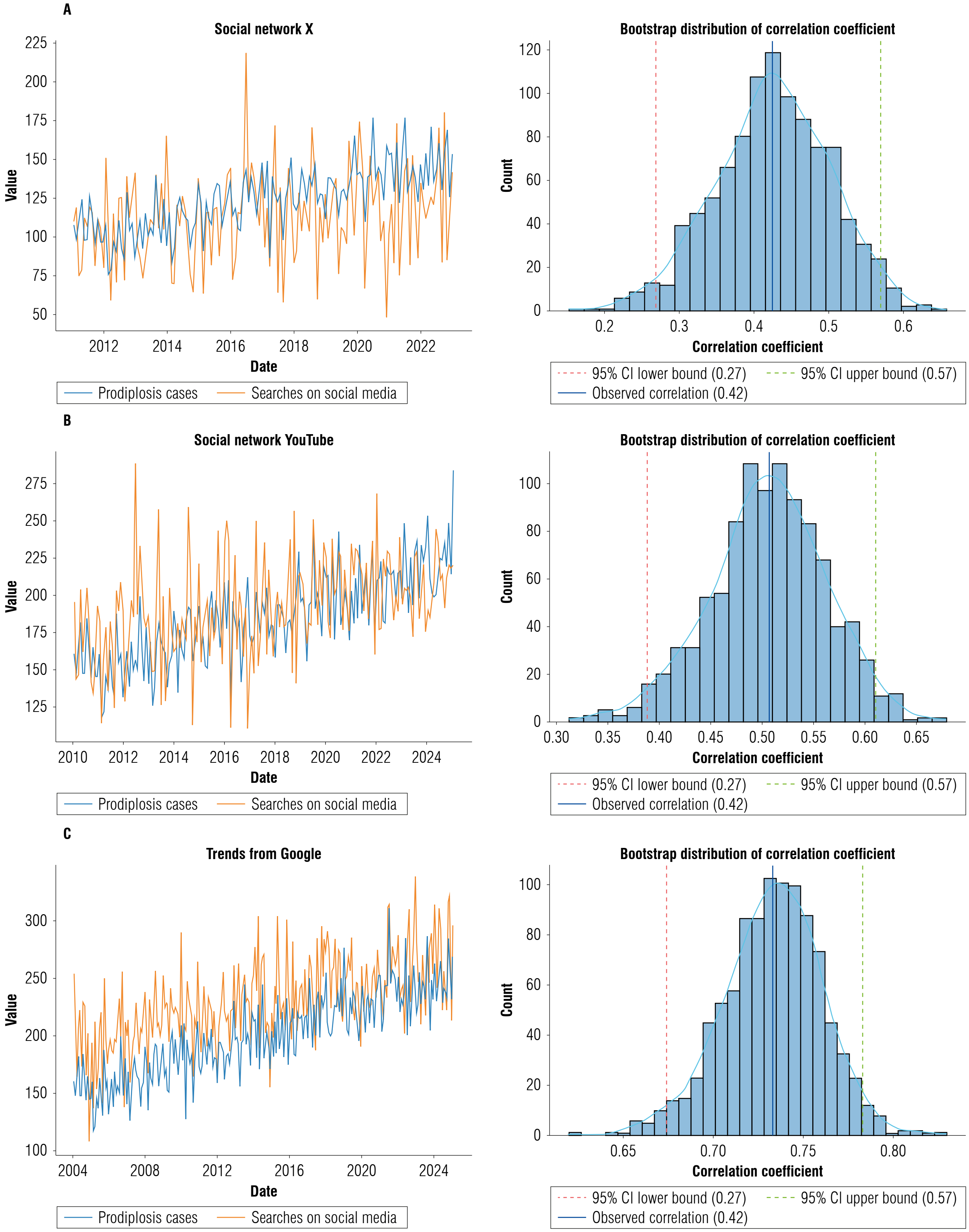


FIGURE 5. Validation of digital data sources related to *Prodiplosis* through comparison with actual reports from farmers and national phytosanitary systems in Colombia. A-analysis of posts and interactions on social network X (formerly Twitter), identifying geographic origin, frequency, and thematic content related to *Prodiplosis*; B-examination of YouTube content, including the number of views, user engagement, and the nature of videos referencing *Prodiplosis* or its damage on crops; C-trends from Google search queries, showing temporal dynamics and peaks of public interest at the national level.

Producer perceptions of the effect of *Prodiplosis* on foliage crops from surveys and the use of WhatsApp social chat

Analysis of trends observed in surveys (n = 25) revealed that more than 50% of *Ruscus* and *Cocculus* growers reported an increase in *Prodiplosis* populations since its emergence as a pest and increased production costs. However, growers still considered production to be profitable. Regarding spatial distributions, approximately 90% of the respondents confirmed that their neighbors also faced this pest and shared common weeds. This exacerbates the situation. However, only 47% perceived the management measures implemented as effective, although most agreed that the pest was related to technical shortcomings such as greenhouse design, changing climatic conditions, poor fertilization plans, and lack of monitoring tools (Fig. 6A and B). Most implemented curtains in greenhouses, with a perceived efficiency of 80%, while color (yellow and blue, which are the most abundant, not based on any evaluated criteria, but only on the recommendation of the ICA.) traps

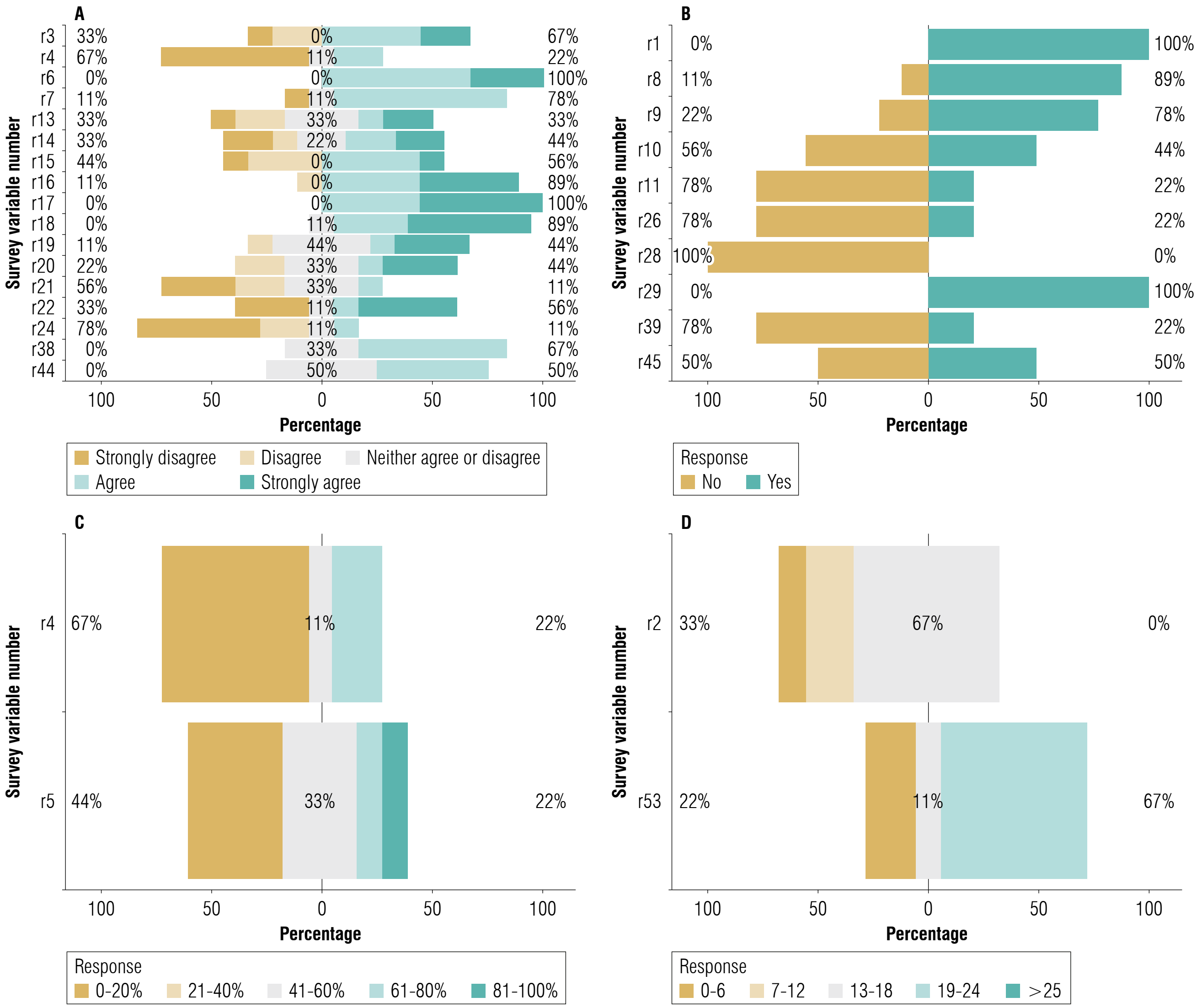


FIGURE 6. Summary of producer perceptions, management practices, and contextual variables related to *Prodiplosis*. in foliage crops from Cundinamarca, Colombia. A-general perception of *Prodiplosis*. And its relationship with *Ruscus* y *Cocculus* foliage based on the Likert scale; B-management carried out for the control of *Prodiplosis* based on dichotomous response questions; C-magnitude of the problem caused by *Prodiplosis* on the foliage crop; D-time since you consider the pest appeared in the crops (upper) based on the time you have been working (lower). *Prodiplosis* in Cachipay, Cundinamarca Colombia. r1–r7 includes economic impact of Prodiplosis, including yield losses and profitability. r8–r12 use and effectiveness of physical control methods. r13–r28 relationships between Prodiplosis populations and factors like weeds, neighboring crops, fertilization, irrigation, and other pests. r29–r48 pest management strategies, including bio-inputs and chemical controls covering product types, application practices, and perceived effectiveness. r49–r53 sociocultural and farm-related data, such as land tenure, education, technical assistance, and farm size. For more details, refer to Supplementary Information 1.

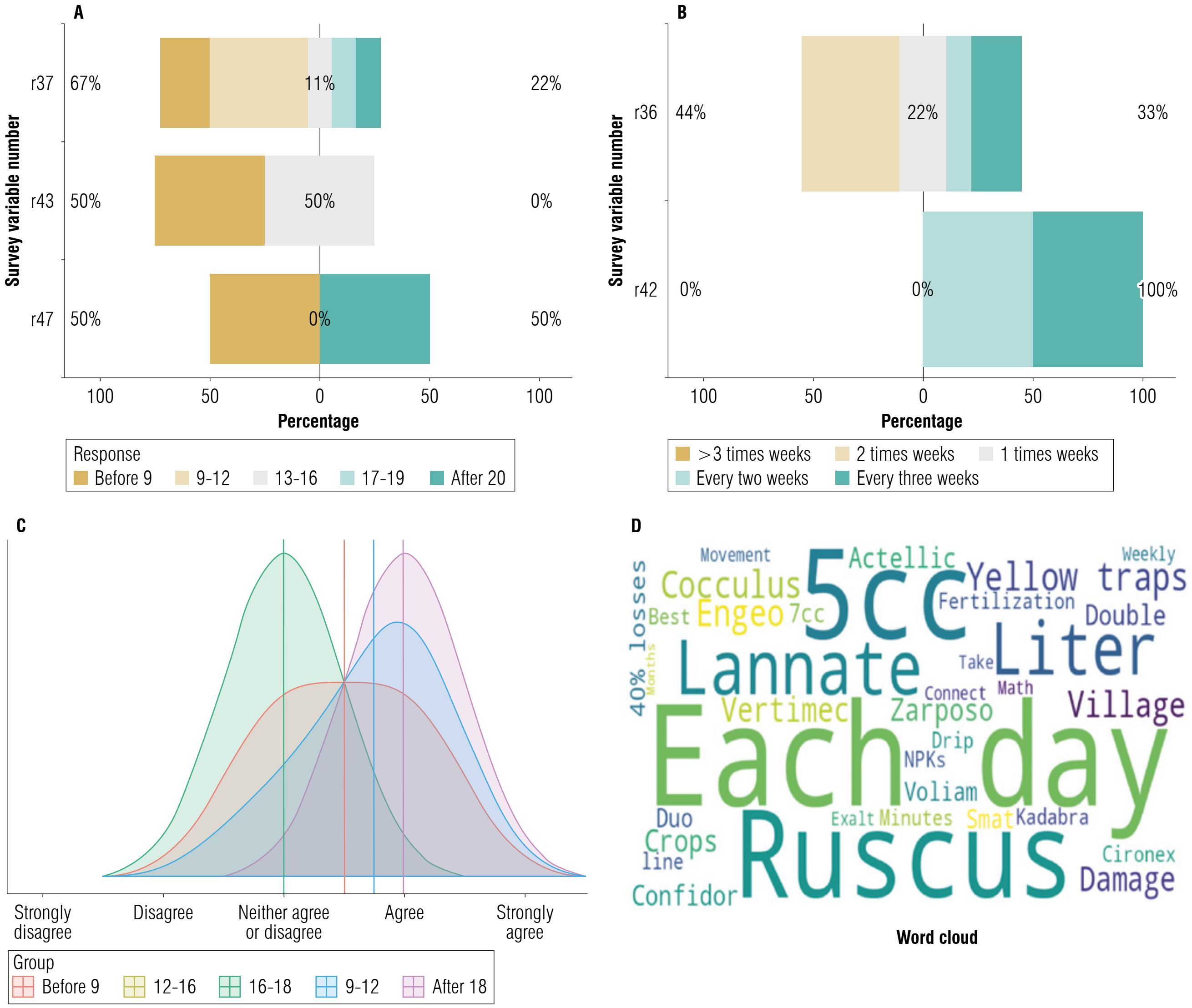


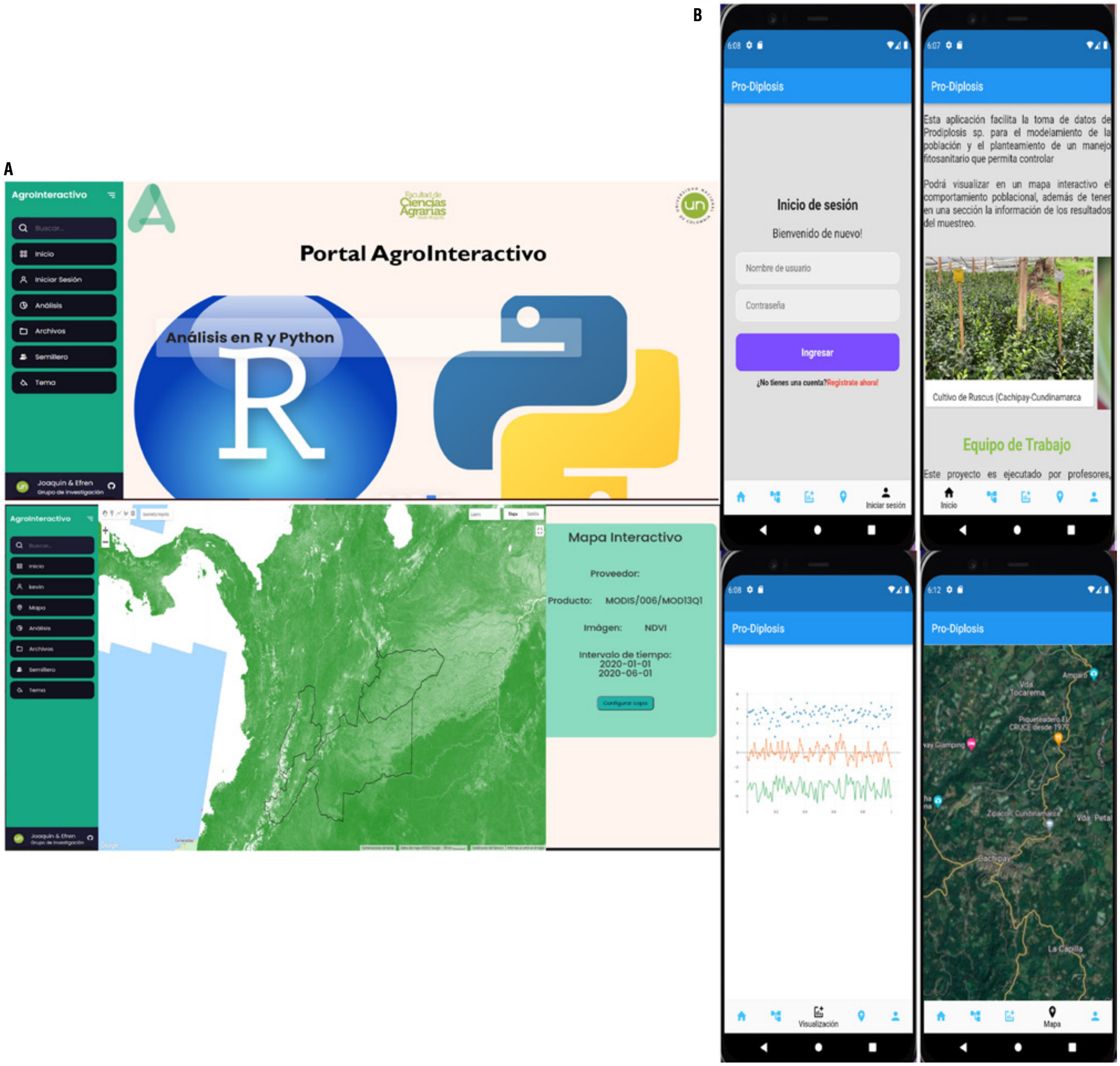
FIGURE 7. Summary of producer perceptions, management practices, and contextual variables related to *Prodiplosis*. in foliage crops from Cundinamarca, Colombia. A-time of application of chemical and biological treatments and bio inputs on the farms surveyed; B-frequency of application of chemical and biological products for the control of *Prodiplosis* in Cachipay, Cundinamarca, Colombia; C-perception of success in controlling *Prodiplosis* according to the time of application of chemicals; D-word cloud associated with the perception of producers associated with the use of the survey and WhatsApp chat in foliage producers in Cundinamarca. r29–r48 pest management strategies, including bio-inputs and chemical controls covering product types, application practices, and perceived effectiveness. For more details, refer to Supplementary Information 1.

for monitoring and biological control presented lower levels of acceptance and efficiency (Fig. 6A and B).

Regarding economic losses and affected areas, more than 50% of the respondents experienced losses between 20-40% (understood as the amount of foliage with the presence of larvae or damage that makes the foliage unsuitable for sale at market quality), although some farms showed up to 100% of the field was damaged (without loss, as they can make some process of damage removal or cosmetic appearance that improves the visual quality and can be sold, with a potential value request, depending on the degree of damage) (Fig. 6C and D). In terms of application frequency, chemical pesticides were applied more than three times a week in about 50% of farms. In contrast, biological applications were less frequent, once a month or every two weeks (Fig. 7B). Application times varied between producers, with chemical applications carried out mostly between 9 am and 12 m, and biological applications between 9 am and 4 pm (Fig.7A and C). The results associated with the use, impact and effect of the products used for the management of *Prodiplosis* suggested that the perception by farmers of success of chemical control was related to applications made after 6 pm each 2 or 3 weeks, coinciding with adult *Prodiplosis* activity (Fig. 7C and B). Most of the producers pointed out that the pest appeared since 2018 and colonized the foliage production systems according to the specific conditions of each farm, generating a high risk in the plantation, according to perceptions of damage, presence, impact, without being able to define a specific and forceful pattern (Fig. 6D). Meanwhile, the word cloud mainly was associated with the use and dosage of chemical products, to a lesser extent with aspects of traps related to color, and to a lesser extent yet with other management measures (Fig. 7D).

Design of a digital platform for connecting with producers in knowledge exchange processes

The development of two integrated digital platforms, a mobile application for field data collection and a web-based visualization and analysis interface, successfully fulfilled the objective of establishing a connection between foliage farmers and the academic community (Fig. 8). This integration served as a bridge, linking the scholarly perception of *Prodiplosis* related issues with the firsthand experiences



Esta aplicación facilita la toma de datos de Prodiplosis sp. para el modelamiento de la población y el planteamiento de un manejo fitosanitario que permita controlar

Podrá visualizar en un mapa interactivo el comportamiento poblacional, además de tener en una sección la información de los resultados del muestreo.

Este proyecto es ejecutado por profesores,

FIGURE 8. Visualization of digital Tools developed for connecting digital epidemiology and citizen science (in Spanish) in a case study on *Prodiplosis* population trends and dynamics. A- “Portal Agrointeractivo” – Web application - A data visualization and analysis platform that integrates interactive maps, predictive models, and external API connections, facilitating real-time monitoring and decision-making. B-¨Pro-Diplosis¨ – Mobile application- A field data collection tool that allows farmers and field technicians to record observations, configure sensor data acquisition, and visualize disease distribution through an interactive map.

of farmers in their fields. By facilitating this exchange, the platform enabled a more comprehensive understanding of the problem from both perspectives. Additionally, the platform provided a space for discussion and knowledge-sharing among farmers, serving as a foundation for the transmission and transfer of empirical knowledge. The system allowed real-time access to critical data related to the target problem through a bidirectional approach. Data were collected directly by farmers (citizen science), while digital data sources (digital epidemiology) extracted from various online platforms contributed to the analysis. This dual approach fostered interaction between the two data streams, enabling a validation process that enhanced the credibility and applicability of the collected information (Fig. 8).

Moreover, this study highlights the multiple challenges associated with integrating digital epidemiology and citizen science in agricultural settings. Among the most significant hurdles were data bases and the adoption of digital platforms, which impacted data quality and user engagement. These findings underscore the need for refined methodologies to mitigate biases and enhance user participation, ensuring more reliable and actionable insights in digital plant health monitoring.

Discussion

Our work demonstrated how the monitoring of web searches and social networks, bibliometric analysis, and further analysis of the information using data science tools (specifically visualization and advanced analytics elements such as the use of NLP as a deep learning algorithm) showed a high capacity to process and find patterns in textual data sources (Maulud *et al.*, 2021; Tripathy *et al.*, 2021). NLP is a valuable method for identifying empirical patterns in the behavior of pest populations, specifically at the agricultural level, under the focus of this study. These freely available tools allowed for a comprehensive view of the importance and geographical distribution of these pests. Focusing on the species *P. longifila*, we highlighted the geographic and thematic interest of the related queries, reflecting a particular interest in certain regions, possibly associated with its impact on crops of regional importance. In addition, we provided a detailed overview of search and discussion trends on digital platforms, proposing this methodology not only as an epidemiological monitoring strategy but also as a way to share information, convey messages, and develop more efficient management strategies.

Implementing a digital epidemiology and citizen science approach in agriculture could transform pest management by facilitating communication and connection between farmers and other actors such as researchers and agricultural technicians. Social networks, for example, can be used for rapid dissemination of alerts on pest outbreaks. At the same time web platforms can centralize geolocated data on infestations, facilitating the identification of patterns and trends of information (Charles-Smith *et al.*, 2015; Samaras *et al.*, 2020). Additionally, bibliometric analyses of the academic literature on specific pests can provide comprehensive overviews of existing research, guiding evidence-based management strategies and identifying knowledge gaps (Merigó & Yang, 2017).

In this sense, our work identified multiple gaps in the knowledge associated with *Prodiplosis* in foliage crops, particularly in aspects of biology, ecology, population dynamics, and management, at the level of knowledge accumulated in other hosts such as tomato, asparagus, and others. This work is also essential considering the scarcity of relevant information for green foliage crops. This lack of information aggravates the situation, given that farmers, as demonstrated in this work, are mostly focused on chemical control, very much associated with replicating what has been done in other crops with little or no success. This leads to the multiplication of errors and the abuse of chemical control.

The implementation of digital platforms successfully integrated citizen science and digital epidemiology but also revealed key challenges affecting data reliability and adoption. A significant issue was data quality control, as farmers lacked standardized protocols for measurement and monitoring. Despite the valuable practical experience possessed by farmers in the field of crop management, the study identified some common challenges associated with the morphological characterization of *Prodiplosis*. The presence of insects with similar morphology can make accurate identification difficult, even for experienced farmers, occasionally leading to misidentifications. Furthermore, the limited utilization of structured data management techniques, such as recording, storage, and retrieval of pest observations by growers, has led to the emergence of challenges in data management. Technical barriers such as poor internet access and low digital literacy further limited engagement with digital tools.

One of the primary challenges in the agricultural sector is the adoption and correct perception of technologies by farmers, who often prefer simple, low-cost, and accessible methods (Cáceres-Zambrano *et al.*, 2023; Tey & Brindal, 2012). In this context, we implemented an effective communication strategy using WhatsApp, a widely used platform in today's society with multiple advantages in communication, message learning exchange, and participatory monitoring, among others (Agnese *et al.*, 2024; Nain *et al.*, 2019). This tool facilitated communication, idea exchanges, and reporting on the impact, costs, and spread of the significant pest *Prodiplosis* in foliage crops, especially given the limited technical support available in the literature for designing integrated management strategies for this impactful pest. In addition, the provision of technical assistance from governmental or private entities is necessary to facilitate the reduction of existing gaps in identification and management.

Our approach achieved significant progress in enhancing information communication strategies and accessing real-time data, sharing both successful and unsuccessful experiences. This provided a regional approach to addressing a problem that directly affects farmers in the foliage production area of Cachipay municipality. The advantage of this chat group lies in its multidisciplinary nature, including farmers, technical assistants, export managers, and academia. Subsequently, to ensure the correct use and transmission of information, the data were analyzed using data science tools. This process enabled us to discard inconsistent or unverified information thereby facilitating the identification of patterns in the textual data through natural language processing analysis, which served as a potent instrument for the examination of ingestion of textual data (Choudhury *et al.*, 2018; Khurana *et al.*, 2023). This comprehensive approach improved the management of *Prodiplosis*, optimizing communication and decision-making in the agricultural sector.

The application of digital epidemiology tools in agriculture represents a significant advance in pest management. However, it also brings several challenges. One of the primary challenges is the automation of queries and data downloads, which requires the development of efficient algorithms to process large volumes of information in real-time. In addition, it is essential to have rapid modeling tools capable of generating early warnings of possible outbreaks, enabling a timely and effective response. In this regard, communication of the information obtained is another critical aspect, as the channels must be accessible and understandable to farmers, who often lack an adequate level of familiarity with new technologies. To ensure the effectiveness of these digital tools, it is essential to have validation systems in place that use real field data, guaranteeing that predictions and recommendations are accurate and applicable in practice. Finally, access to the internet and the adoption of new technologies by farmers are crucial factors for the success of digital epidemiology. Without adequate connectivity and willingness to use these innovations, their implementation and effectiveness in the field could be severely limited. In addition, to enhance participation and improve data reliability, capacity-building initiatives are necessary, including training programs and user-friendly digital tools.

Addressing these limitations is crucial for ensuring the long-term success and scalability of digital epidemiology and citizen science in agriculture. Addressing spatial and temporal bias in digital data remains a critical challenge for improving the reliability of pest forecasting models. These biases can limit the accuracy of analyses, particularly when aiming to scale down to finer spatial resolutions necessary for localized decision-making. Spatial inconsistencies driven by uneven internet access and user location combined with time lags in information-seeking behavior reduce the precision of digital epidemiology tools. Overcoming these limitations requires robust frameworks that integrate citizen science strategies with structured data validation protocols. Encouraging participation from producers, technical advisors, and local institutions can enhance data quality and representativeness across key production zones. Citizen science, when properly implemented, offers a valuable avenue to complement digital trace data, providing contextual insight and ground-truthing capacity. Future efforts should prioritize the development of participatory systems that merge digital tools with on-the-ground validation, enabling more accurate and scalable models for agricultural risk management under dynamic environmental and socio-economic conditions.

Conclusions

Integration of digital epidemiological and citizen science tools within agricultural systems offers significant advances in understanding phytosanitary challenges. By enabling indirect monitoring and identifying searching trends at a regional scale, these tools provide valuable insights into the biology, ecology, and management strategies of pests. In the case of emerging pests such as *Prodiplosis*, the application of digital epidemiology permitted a more accurate and comprehensive approximation of real-time phytosanitary status, helping to delineate the pest's distribution, impact and emerging threats across diverse cropping systems.

In addition, we have successfully identified the extent of damage this emerging pest inflicts on foliage crops and its consequent impact on production systems, particularly in terms of economic losses. While this represents an initial approximation, it underscores the critical need for continued research into the life cycle and ecology of this pest as well as the influence of environmental factors on its population dynamics. Such efforts are crucial for development more effective management strategies to minimize both crop losses and production costs.

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Conflict of interest statement

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

Author's contributions

LAVG: conceptualization, research, writing - original draft, visualization, writing, and editing. HJVM: conceptualization, writing - original draft, and editing. LMPR: conceptualization, writing - original draft, and editing JGRG: conceptualization, writing - original draft, and editing. All authors have read and approved the final version of the manuscript.

Supplementary information

Supplementary Information 1 can be consulted in the publicly available repository: https://github.com/agrocompuepidemlab/Citizen-science-and-digital-data-to-trend-analysis-and-impact-assessment-of-Prodiplosis

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UV-C radiation and gaseous ozone improve the physicochemical characteristics of refrigerated strawberries

La radiación UV-C y el ozono gaseoso mejoran las características fisicoquímicas de fresas refrigeradas

Juver Andrey Jiménez Moreno[1*], Edna Lizeth Higuera Castro[1], Raysa Maduro Alves[2], Carolina María Sánchez-Sáenz[1], and Franciane Colares Souza Usberti[2]

ABSTRACT

The use of new preservation techniques, such as the application of gaseous ozone (OZ) and ultraviolet (UV-C) radiation, enables agricultural products to be maintained in optimal conditions for consumers. Additionally, these methods reduce risks to human health by decreasing the presence of pathogens in treated foods. The aim of this study was to evaluate the effect of gaseous ozone and UV-C radiation exposure on the physicochemical characteristics of strawberries. Strawberries were exposed to radiation doses of 0 kJ m^{-2} (control), 2.02 kJ m^{-2}, 3.04 kJ m^{-2}, and 4.05 kJ m^{-2} and to gaseous ozone concentrations of 0.267 and 0.586 mg L^{-1} for 35 and 70 s at each concentration. The experimental units were then packaged in PET packaging and stored at 5±1°C and 85±5% relative humidity (RH) for 5 d. Physicochemical tests included weight loss, color, pH, titratable acidity, total soluble solids, and respiration rate. The UV-C and ozone treatments demonstrated better performance compared to the control maintained at 5±1°C. Notably, the treatments that showed the most favorable outcomes for the physicochemical properties of strawberries were OZ3 (exposure to 0.586 mg L^{-1} of O_3 for 35 s) and UVC3 (exposure to radiation dose of 4.05 kJ m^{-2}), indicating these methods are suitable for the preservation of strawberries.

Key words: ozonation, postharvest quality, ultraviolet radiation, ripening, *Fragaria x ananassa*.

RESUMEN

El uso de nuevas técnicas de conservación, como la aplicación de ozono (OZ) gaseoso y radiación ultravioleta (UV-C), permite mantener los productos agrícolas en condiciones óptimas para los consumidores. Además, estos métodos reducen los riesgos para la salud humana al disminuir la presencia de patógenos en los alimentos tratados. El objetivo de este estudio fue evaluar el efecto de la exposición al ozono gaseoso y a la radiación UV-C sobre las características fisicoquímicas de las fresas. Las fresas fueron expuestas a dosis de radiación de 0 kJ m^{-2} (control), 2,02 kJ m^{-2}, 3,04 kJ m^{-2} y 4,05 kJ m^{-2}, y a concentraciones de ozono gaseoso de 0,267 y 0,586 mg L^{-1} durante 35 y 70 s para cada concentración. Las unidades experimentales se envasaron en empaques de PET y se almacenaron a 5±1°C y 85±5% HR durante 5 d. Las pruebas fisicoquímicas realizadas incluyeron pérdida de peso, color, pH, acidez titulable, sólidos solubles totales y tasa de respiración. Los tratamientos con UV-C y ozono demostraron un mejor rendimiento en comparación con el control mantenido a 5±1°C. Notablemente, los tratamientos que mostraron los resultados más favorables para las propiedades fisicoquímicas de las fresas fueron OZ3 (tratamiento con exposición a 0,586 mg L^{-1} de O_3 durante 35 s) y UVC3 (tratamiento con exposición a dosis de radiación de 4,05 kJ m^{-2}), indicando que estos métodos son adecuados para la conservación de las fresas.

Palabras clave: ozonización, calidad poscosecha, radiación ultravioleta, maduración, *Fragaria x ananassa*.

Introduction

Preserving the quality of fruits and vegetables after harvest is an important matter involving several attributes such as visual appearance, texture, flavor, aroma, nutritional value, and food safety (Bajaj *et al*., 2023). From the perspective of microbiological quality and food safety, it is necessary to guarantee their safety and nutritional value to promote consumer health (Fung *et al*., 2018). In recent years, an aspect that has gained importance is the preservation of agricultural products under optimal conditions to prolong shelf life and reduce microbial load (Gil *et al*., 2015; Snyder & Worobo, 2018). Preservation techniques include irradiation with short-wave ultraviolet light (UV-C), ozone exposure, hydrogen peroxide, and peracetic acid, among others (Shehata *et al*., 2021; Templalexis *et al*., 2023).

Ozone exposure is an ecological and economical alternative for preserving quality in fruits and vegetables, as ozone does not leave any residue in the food. It is also a

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[1] Universidad Nacional de Colombia, Departamento de Ingeniería Civil y Agrícola, Bogotá (Colombia).

[2] School of Agricultural Engineering, Universdade Estadual de Campinas, Campinas, SP (Brazil).

[*] Corresponding author: juajimenezmo@unal.edu.co

germicidal agent that reduces the microbial load of foods (Sarron *et al*., 2021). Ozone is an oxidizing agent that can be applied in gaseous form or dissolved in water (Sarron *et al*., 2021; Vettraino *et al*., 2020). Its action is based on its high oxidizing power, which provides strong disinfection and sterilization capacity, allowing the sanitizing action to occur with a lower concentration and shorter contact time (Pandiselvam *et al*., 2019). However, it is necessary to use the appropriate concentration, as high concentrations of compounds can affect the nutritional and sensory value of vegetables, altering their color and flavor (Coelho *et al*., 2015).

The use of ultraviolet irradiation to preserve fruits and vegetables has notable effects, such as delaying the ripening of some climacteric fruits, such as tomato (Lu *et al*., 2016), and delaying physiological changes in non-climacteric fruits, such as pepper fruits (Ma *et al*., 2021). In addition, ultraviolet radiation does not produce toxic organic compounds compared to chlorine-based disinfectants (Souza, 2014). Ultraviolet radiation is classified as non-ionizing radiation with a wavelength between 100 and 400 nm and is used in post-harvest preservation of fruits and vegetables due to its ability to reduce microbial load and maintain firmness of the fruits (Peng *et al*., 2022; Singh *et al*., 2021). The maximum inactivation peak is at 254 nm, and it has been shown to be most effective at this wavelength (Bhullar *et al*., 2018).

Ozone exposure doses or UV-C irradiation energies vary depending on the type of agricultural product (Delorme *et al*., 2020). Exposure of tissues to low doses of UV radiation can produce fungicidal compounds such as phytoalexins and delay ripening and senescence processes (Darré *et al*., 2022; Sethi *et al*., 2018). One of the benefits of this treatment is the reduction of postharvest losses caused by physiological disorders such as chilling injury, susceptibility to phytopathogen attack, mechanical damage, and loss of firmness, among others (Lemessa *et al*., 2022). For instance, one of the benefits of UV-C irradiation of strawberries (*Fragaria x ananassa*) is the reduction of firmness loss, a critical factor in postharvest life (Xu *et al*., 2017).

The application of UV-C radiation and ozone treatments to strawberries has been shown to significantly influence their physicochemical properties. Several studies have demonstrated that controlled exposure to UV-C can reduce pathogen incidence, slow respiration rates, and extend the postharvest shelf life of strawberries while preserving key quality attributes (Cote Daza, 2011). Similarly, ozone treatment at optimal concentrations has been reported to effectively reduce microbial load and maintain the sensory quality of strawberries, enhancing their postharvest preservation (Macías-Gallardo *et al*., 2023). These findings support the adoption of UV-C and ozone treatments as viable technologies for maintaining fruit quality and prolonging the postharvest longevity of strawberries (Contigiani, 2019).

Strawberries are among the most accepted and commercialized fruits in the import and export of fresh products, in the frozen food industry, as a flavoring in the preparation of medicines, and others (Gonçalves *et al*., 2018). Although the strawberry is a non-climacteric fruit, it is highly perishable and susceptible to mechanical damage, water loss, and physiological and microbiological deterioration (Gol *et al*., 2013; Qureshi Quarshi *et al*., 2023). The quality characteristics and chemical composition of strawberries are influenced by a combination of several genetic (cultivar) and geographical (climate, soil, among others) factors (Ornelas-Paz *et al*., 2013; Pinheiro *et al*., 2021). The aim of this study is to evaluate the effect of exposure to gaseous ozone (OZ) and UV-C radiation, as product preservation techniques, on the physicochemical characteristics of strawberries.

Materials and methods

Strawberries of the Campinas variety (IAC-2712) were purchased at an intermediate stage of ripening (3/4 red on the surface) at the Brazilian State Supply Centre (CEASA) in Campinas, Brazil, and then taken to the postharvest laboratory at the Faculty of Agricultural Engineering (FEAGRI) of the University of Campinas (UNICAMP). The strawberries had an average length and diameter of 32.4 mm and 25.5 mm, respectively. They were stored at 5±1°C until they were exposed to ozone gas and UV-C radiation.

The effect of two non-thermal preservation techniques, ozone gas exposure and UV-C radiation, on the maintenance of the physicochemical characteristics of strawberries was evaluated. For this purpose, strawberries were exposed to different concentrations of ozone gas at different exposure times and doses of short-wave ultraviolet radiation (UV-C), together with control strawberries packaged in PET packaging and stored at a temperature of 5±1°C. A summary of the treatments is given in Table 1.

TABLE 1. Summary of the treatments applied to the strawberry fruits.

Ozone			UV-C	
Treatment	Concentration (mg L^{-1})	Exposure time (s)	Treatment	Energy (kJ m^{-2})
OZ1	0.586	70	UVC1	2.02
OZ2	0.267	35	UVC2	3.04
OZ3	0.586	35	UVC3	4.05
OZ4	0.267	70		

The effect of exposure to gaseous ozone on strawberries was evaluated using an ozonation system. This consisted of an application chamber with a volume of 15 L and an oxygen gas cylinder with 99% purity, which was connected to an ozone generator, model DCGM-2007/Ecozon® operating on the corona discharge principle according to the specifications of Cavasini (2017).

Before carrying out the tests, the ozonation system was calibrated. This calibration consisted of preparing a solution of potassium iodide at 6.64 g in 2 L of distilled water. In the ozonation system, the ozone flow into the chamber was adjusted to 1 L min^{-1} (LPM) and left on for 2 min. At the same time, the potentiometer was adjusted to 1.6 mg m^{-3}, 4.0 mg m^{-3} and 7.0 mg m^{-3}, and the ozone concentrations obtained were 0.165 mg L^{-1}, 0.267 mg L^{-1} and 0.586 mg L^{-1}. From these results, four treatments were defined for the combination of different ozone concentrations (0.586 mg L^{-1} and 0.267 mg L^{-1}) and exposure times (35 s and 70 s) (Tab. 1). After ozonation, the product was packaged in PET containers and stored at 5±1°C for 5 d, as the fruits showed no signs of damage or visible contamination.

The product was irradiated in a chamber with a wooden structure, lined with aluminum foil and equipped with 12 UV lamps with a wavelength of 254 nm, distributed in the upper and lower parts. The irradiated product was placed at an average distance of 22 cm, with a UV-C light fluence rate of 0.044 W m^2, determined by Souza (2012). The time used for each treatment made it possible to obtain different irradiation energies (Tab. 1). After irradiation, the product was packaged in PET packaging and stored at 5±1°C for 5 d.

For each treatment evaluated, tests for weight loss, color, titratable acidity, soluble solids, pH, and respiration rate were carried out, with three replicates per treatment on days 0, 3, and 5 of storage.

The fruits were weighed on a GEHAKA BG2001 balance, with a minimum load of 2.5 g, a maximum load of 2020 g, and a division of 0.1 g. Weight loss (%) was determined by the difference in mass between the day of the experiment and each day of analysis in the laboratory. The result was expressed as a percentage.

Color was evaluated using the CIELab space, where L* is lightness (0 for black to 100 for white), a* = (-60.0 for green to +60.0 for red), and b* = (-60.0 for blue to +60.0 for yellow). Additionally, the system parameters C* and h° were determined, where C* is the chroma and h° is the hue (Eqs. 1 and 2). Data were collected using a Spectro Photometer CM-700d Konica Minolta colorimeter with a 10° angle and a D65 illuminant in the central area of the fruits. Five replicates were made for each treatment with two readings each on days 0, 3, and 5 of storage.

$$C^* = \sqrt{(a^*)^2 + (b^*)^2} \tag{1}$$

$$h^\circ = \arctan\left(\frac{b^*}{a^*}\right) \tag{2}$$

The pH was determined using an ANALION PM 608 digital benchtop pH meter, with the electrode immersed in the product juice until the reading stabilized.

The total soluble solids (TSS) of the strawberries were estimated using an Abbe Mark III automatic Reichert refractometer. The temperature correction value was obtained by placing two drops of distilled water on the instrument lens, followed by the soluble solids value reading, which was taken by placing two drops of sample juice on the lens. The results were expressed in °Brix.

Total titratable acidity (TTA) was calculated according to the methodology developed by Gabriel *et al.* (2015). The results were expressed in mg of citric acid per 100 g. The maturity index was determined as the ratio between TSS and TTA.

The respiration rate was determined using a static system (closed container lid) with an average weight of 100 g in each airtight container. Each of the samples was kept in the refrigerator at a temperature of 5±1°C for 30 min after closing the containers. Gas analysis was carried out using the Mocon Portable Pac Check 325. The results were expressed in mg CO_2 kg^{-1} h^{-1}.

Statistical analysis

The data obtained in the laboratory were subjected to analysis of variance. In addition, the means of each treatment were compared using Tukey's test at 5% probability using the SISVAR 5.6 software.

Results and discussion

During storage, the control treatment showed greater weight loss (7.95%) compared to the gaseous ozone treatments, which showed losses between 4.87% and 6.99%, and the UV-C irradiation treatments, which showed losses between 4.24% and 5.88%. Of the treatments evaluated, the one with the best response was OZ3, with a loss of 4.07%, as shown in Table 2. Strawberry weight loss is an important index that reflects the respiration rate and moisture evaporation between the fruit tissue and the ambient air (Tahir *et al.*, 2018). In addition, strawberries are highly susceptible to rapid water loss due to their extremely thin epidermis (Jiang *et al.*, 2020). The reduction in mass loss during storage is related to the reduction in water loss caused by transpiration and respiration and may also help prevent fruit weakening and shrinkage (Muley & Singhal, 2020).

TABLE 2. Weight loss in strawberries exposed to UV-C radiation and ozone and stored at 5±1°C for 5 d.

Treatment	Day	
	3	5
Control	4.67 ± 0.45 b, B	7.95 ± 1.68 a, C
OZ1	3.11 ± 0.22 ab, B	4.87 ± 0.18 a, C
OZ2	3.41 ± 0.71 ab, B	5.42 ± 1.01 a, C
OZ3	2.37 ± 0.83 a, B	4.07 ± 1.21 a, B
OZ4	3.66 ± 1.34 ab, AB	6.99 ± 3.70 a, B
UVC1	2.83 ± 0.35 ab, B	4.74 ± 0.73 a, C
UVC2	3.14 ± 0.40 ab, B	5.88 ± 0.93 a, C
UVC3	2.36 ± 0.46 a, B	4.24 ± 0.15 a, C

Lowercase letters indicate differences between treatments and uppercase letters indicate differences between analysis days. Same letters do not differ significantly according to the Tukey test ($P<0.05$). ± indicates standard error (n=5). The treatments are described in Table 1.

Table 3 shows the color parameters of the strawberries on days 3 and 5 of the analysis. On the first day, the color parameters of the strawberries were: L*=50.80 ± 2.46, C*=19.27 ± 3.05, and h°=31.51 ± 5.08 a, A. The treatments that preserved the color of the strawberries, evaluating the parameters of luminosity, chroma, and hue, were OZ1, OZ2, UVC2, and UVC3, as they did not show any significant variation ($P>0.05$) over the storage period. On day 3, a significant difference ($P>0.05$) was observed in ozonated strawberries compared to non-ozonated strawberries, indicating that UVC preserved the color of the fruit. This response shows that the treatments with UV-C radiation, UVC2 and UVC3, and the treatments with ozone, OZ1 and OZ2, did not cause significant changes in the color of the strawberries, indicating that the specific concentrations and exposure times did not alter the original characteristics of the fruits.

TABLE 3. Mean values of color characteristics (luminosity L*, chroma C* and hue h°) of strawberries exposed to UV-C radiation and ozone and stored at 5±1°C for 5 d.

Variables	Treatment	Day	
		3	5
L*	Control	48.13 ± 0.94 a, A	48.47 ± 1.09 ab, A
	OZ1	49.01 ± 1.18 ab, A	49.32 ± 1.65 ab, A
	OZ2	48.82 ± 1.57 a, A	48.79 ± 1.25 ab, A
	OZ3	48.96 ± 0.85 ab, AB	48.29 ± 1.42 ab, A
	OZ4	50.31 ± 0.96 b, B	47.70 ± 1.20 a, A
	UVC1	49.01 ± 1.13 ab, AB	48.64 ± 0.79 ab, A
	UVC2	49.25 ± 0.55 ab, A	49.23 ± 1.27 ab, A
	UVC3	49.53 ± 0.93 ab, A	49.68 ± 1.77 b, A
C*	Control	15.97 ± 2.05 a, A	15.22 ± 3.24 a, A
	OZ1	19.52 ± 2.12 b, A	20.55 ± 3.00 b, A
	OZ2	17.84 ± 2.91 ab, A	15.80 ± 4.22 a, A
	OZ3	18.53 ± 2.69 ab, A	17.14 ± 3.71 ab, A
	OZ4	21.04 ± 2.17 b, B	14.90 ± 2.20 a, A
	UVC1	18.76 ± 2.46 ab, AB	15.95 ± 2.62 a, A
	UVC2	18.79 ± 3.06 ab, A	17.74 ± ab, A
	UVC3	18.48 ± 2.28 ab, A	17.05 ± 3.86 ab, A
h°	Control	31.07 ± 3.51 a, A	31.62 ± 3.74 ab, A
	OZ1	28.81 ± 4.61 a, A	29.56 ± 1.92 a, A
	OZ2	30.59 ± 4.39 a, A	30.32 ± 2.24 ab, A
	OZ3	29.55 ± 1.85 a, A	29.88 ± 3.29 a, A
	OZ4	31.75 ± 2.13 a, A	28.77 ± 3.38 a, A
	UVC1	28.42 ± 3.66 a, A	31.17 ± 2.45 ab, A
	UVC2	29.61 ± 3.11 a, A	30.76 ± 4.09 ab, A
	UVC3	31.12 ± 2.77 a, A	34.08 ± 1.66 b, A

Lowercase letters indicate differences between treatments and uppercase letters indicate differences between analysis days. Same letters do not differ significantly according to the Tukey test ($P<0.05$). ± indicates standard error (n=5). The treatments are described in Table 1.

Luminosity of the fruits was maintained during storage in treatments OZ1, OZ2, UVC2, and UVC3. However, treatments OZ4, OZ3, and the control showed a significant variation ($P<0.05$) during storage, with the lowest values (47.70, 48.27, and 48.47, respectively) at the end of the treatment. This decrease in luminosity is undesirable during storage, as fruits with intense and bright colors are preferred by consumers (Schifferstein, 2019), and color retention in strawberries during storage is a desired quality attribute (Azam *et al.*, 2019). In general, the luminosity values during storage ranged from 50.8 at the beginning to 47.7 at the end of storage, with a difference of 6.10% for treatments that did not show significant variation. This is a better outcome than that observed by Octavia and Choo (2017), who obtained a variation of 7.14% during storage. However, São José and Vanetti (2015) obtained L* values of 28.45 at the beginning of storage and 19.66 at the end of storage for ultrasound treatments in strawberries, resulting in a 30.9% decrease in fruit brightness. This is unfavorable, as maintaining brightness in strawberries is essential for preserving the quality and appearance of the strawberries. Therefore, the choice of concentrations, exposure times and irradiation energies can influence the preservation of strawberries during storage. UV-C radiation (UVC2 and UVC3) and ozone (OZ1 and OZ2) treatments showed preservation of brightness in strawberries.

During storage, the chroma or color intensity was maintained in treatments OZ1, OZ2, OZ3, UVC2, and UVC3. However, treatments OZ4 and the control showed significant differences ($P<0.05$) in color saturation of strawberries during storage. Hue did not show significant variation ($P>0.05$) in all treatments during the storage period. The average hue values remained between 28.77° and 31.51°, with hue values closer to zero for redder fruits and closer to 90° for fruits with a predominance of yellow color. Navas Cajamarca (2015) found that the hue in ozonized strawberries was between 27.51° and 39.03°, which is consistent with the results obtained in this experiment. Treatments that showed significant differences resulted in a decrease in color variables. This is attributed to high exposure doses, which caused changes to the strawberry epidermis, as noted by Macías-Gallardo *et al.* (2023) and Panou *et al.* (2021). Likewise, changes in color could be related to the change in pH and turgor of the fruits, as suggested by Ortiz-Araque (2021).

The pH of the fruits on the first day of analysis was 3.28 ± 0.03. The pH of the stored strawberries showed a decrease of 2.44% in control, a decrease of between 4.78 and 7.83% in the gaseous ozone treatments, and a decrease of between 8.14 and 8.44% in the UV-C radiation treatments. According to the data presented in Table 4, neither the control nor the treatments affected the pH. However, on day 5, the control showed a significant difference ($P>0.05$) compared to the treatments studied. These significant pH changes during storage may contribute to the accelerated deterioration of strawberries (Cherono *et al.*, 2018; São José & Vanetti, 2015). However, during the analysis period (5 d), no visible changes were observed in the fruits treated with ozone and UV-C radiation that would indicate any surface damage, keeping the strawberries in good storage conditions and demonstrating adequate preservation techniques.

TSS are an indicator of quality and flavor, as they represent the acids, sugars, salts, pectin, and soluble vitamins present in the fruit (Ladika *et al.*, 2024; Yan *et al.*, 2020). On the first day of storage, the total soluble solids had a value of 7.47 °Brix, within the range of 6.8 to 8.7 described by Navas Cajamarca (2015). On days 3 and 5, a decrease in sugar production in strawberries was observed, with values between 9.18 and 14.01 °Brix (Tab.5), within the range found for strawberries by Xie *et al.* (2016). This variable was not affected by treatment exposure, as there were no significant differences ($P>0.05$). However, significant differences were observed between days 3 and 5 for most treatments (except for treatments OZ3 and OZ4). This significant decrease in TSS during the storage period is possibly related to an improved respiration rate and a less active metabolism in strawberries under refrigerated storage. Similar results were obtained by Mishra and Kar (2014) and Ali *et al.* (2022). As there was no significant variation between treatments, but

TABLE 4. Mean pH values of strawberries exposed to UV-C radiation and ozone and stored at 5±1°C for 5 d.

Treatment	Day	
	3	5
Control	3.24 ± 0.03 a, A	3.20 ± 0.04 b, A
OZ1	3.22 ± 0.05 a, AB	3.12 ± 0.08 ab, A
OZ2	3.21 ± 0.03 a, B	3.05 ± 0.05 a, A
OZ3	3.24 ± 0.07 a, B	3.02 ± 0.02 a, A
OZ4	3.21 ± 0.02 a, B	3.04 ± 0.04 a, A
UVC1	3.19 ± 0.03 a, B	3.01 ± 0.02 a, A
UVC2	3.23 ± 0.04 a, B	3.00 ± 0.06 a, A
UVC3	3.23 ± 0.05 a, B	3.01 ± 0.04 a, A

Lowercase letters indicate differences between treatments and uppercase letters indicate differences between analysis days. Same letters do not differ significantly according to the Tukey test ($P<0.05$). ± indicates standard error (n=5). The treatments are described in Table 1.

TABLE 5. Mean values of soluble solids content in strawberries exposed to UV-C radiation and ozone and stored at 5 ± 1°C for 5 d.

Treatment	Soluble solids (°Brix)	
	Day	
	3	5
Control	14.01 ± 1.18 a, C	10.98 ± 0.32 a, B
OZ1	13.40 ± 0.69 a, B	9.58 ± 1.79 a, A
OZ2	13.40 ± 1.01 a, B	9.84 ± 2.56 a, AB
OZ3	10.91 ± 3.26 a, A	10.78 ± 0.78 a, A
OZ4	10.83 ± 0.37 a, A	9.83 ± 2.41 a, A
UVC1	11.05 ± 0.69 a, AB	9.18 ± 1.17 a, B
UVC2	10.75 ± 0.50 a, B	11.25 ± 1.10 a, B
UVC3	11.51 ± 0.39 a, B	9.45 ± 1.99 a, AB

Lowercase letters indicate differences between treatments and uppercase letters indicate differences between analysis days according to the Tukey test ($P<0.05$). ± indicates standard error (n=5). The treatments are described in Table 1.

a significant decrease between storage days, this suggests that storage temperature may have influenced and slowed down the metabolic changes in the fruit. This behavior was also observed by Ladika *et al.* (2024), who attributed the decrease in TSS to the low and controlled temperature in a refrigerated environment, which helped extend the shelf life of the strawberries.

As shown in Table 6, the TTA of the strawberry treatments ranged from 0.99 to 1.04 mg citric acid 100 g^{-1}. The TTA obtained on the first day of the analysis was 1.01 ± 0.08. These obtained values are consistent with those reported by Liu *et al.* (2018). The total titratable acidity did not show significant differences ($P>0.05$) in the treatments evaluated during the storage period. This behavior can be attributed to the fact that strawberries are non-climacteric fruits and do not undergo drastic biochemical changes after harvesting (Alvarado-Cepeda *et al.*, 2020).

Likewise, the applied treatments suggest that none significantly affected titratable acidity, in accordance with Ali *et al.* (2022) and Mussin *et al.* (2014). When compared with the results obtained by Chen *et al.* (2020) on cantaloupe melons exposed to different doses of ozone, they found that the delayed loss of TTA values is associated with the metabolic activity and respiration rates of the fruits and can be attributed to the action resulting from ozone exposure. Another contributing factor could be the suitable storage conditions, as discussed by Ali *et al.* (2022), Ladika *et al.* (2024), and Yan *et al.* (2020).

TABLE 6. Mean values of titratable acidity and maturity index of strawberries exposed to UV-C radiation and ozone and stored at 5±1°C for 5 d.

Treatment	Titratable acidity (mg citric acid 100 g^{-1})	Maturity index
	Day	Day
	5	5
Control	1.03 ± 0.03 a, A	10.70 ± 0.59 a, B
OZ1	1.02 ± 0.05 a, A	9.41 ± 1.68 a, A
OZ2	1.02 ± 0.02 a, A	9.72 ± 2.69 a, A
OZ3	1.03 ± 0.01 a, A	10.51 ± 0.80 a, B
OZ4	1.04 ± 0.06 a, A	9.50 ± 2.73 a, A
UVC1	0.99 ± 0.05 a, A	9.34 ± 1.59 a, A
UVC2	1.03 ± 0.09 a, A	11.05 ± 2.09 a, B
UVC3	1.04 ± 0.04 a, A	9.11 ± 1.76 a, A

Lowercase letters indicate differences between treatments and uppercase letters indicate differences between analysis days according to the Tukey test ($P<0.05$). ± indicates standard error (n=5). The treatments are described in Table 1.

The maturity index of the strawberries on the first day of analysis was 7.44±0.45. Table 6 shows the 5-d maturity index, and this parameter was higher on the fifth day of storage for both the control and the evaluated treatments. However, the control and the UVC2 treatment presented a higher TSS/TTA ratio, indicating that the product underwent a more accelerated maturation process than the products subjected to the other treatments. The treatment with the lowest maturity index was UVC3. It is possible to conclude that the treatments had no effect on this variable, since they did not show a significant difference. However, the duration of storage affects the response of the control and the OZ3 and UV-C treatments. The values obtained for the TSS/TTA ratio are similar to those reported by Alves *et al.* (2019).

Due to their high respiration rate, strawberries are susceptible to water loss, mechanical damage, and fungal spoilage, which may result in a short postharvest shelf life (Liu *et al.*, 2018; Pott *et al.*, 2020). In general, the respiration rate of strawberries ranges between 18.37 and 22.69 mg CO_2 kg^{-1} h^{-1}, which agrees with Cunha Junior *et al.* (2011) and Zhang *et al.* (2020), who obtained similar respiration rates. The respiration rate on the first day of analysis was 21.68±0.96 mg CO_2 kg^{-1} h^{-1}. The control treatment showed a significant variation ($P<0.05$) with respect to both factors (storage time and treatments). Table 7 shows that the OZ3 and UVC3 treatments presented a significant decrease ($P<0.05$) during the first three days of storage due to two factors: the cold environment and the effect of exposure to the OZ3 and UVC3 treatments, which reduced the respiration rate of the product until day 3. The significant increase observed in the control treatment indicates that the UV-C and ozone treatments were effective in slowing down the respiration rate of the treated fruits, which positively contributes to preserving their quality and extending their shelf life, consistent with the findings of Ali *et al.* (2022).

This behavior was also observed by Cunha Junior *et al.* (2011), who reported a reduction from 21.8 to 10.83 mg CO_2 kg^{-1} h^{-1} in strawberries exposed to a controlled atmosphere with different O_2 concentrations during the first 3 d, after which the concentrations remained stable until the end of the experiment. By not allowing the respiration rate to increase significantly, relevant changes in the fruit components, which could be undesirable from a quality standpoint, are avoided. For this reason, the treatments used proved to be effective in preventing a significant increase in the respiration rate of the strawberries.

TABLE 7. Mean values of respiration rate in strawberries exposed to UV-C radiation and ozone and stored at 5±1°C for 5 d.

Treatment	Respiration rate [mg CO_2 kg^{-1} h^{-1}]	
	Day	
	3	5
Control	20.93 ± 1.10 ab, A	22.69 ± 1.03 b, A
OZ1	20.26 ± 0.36 ab, A	20.82 ± 0.91 ab, A
OZ2	21.82 ± 1.05 b, A	22.03 ± 0.94 ab, A
OZ3	18.37 ± 0.53 a, A	20.44 ± 0.67 a, B
OZ4	20.41 ± 0.48 ab, A	21.85 ± 0.65 ab, A
UVC1	19.36 ± 1.40 ab, A	21.97 ± 0.10 ab, B
UVC2	19.41 ± 1.36 ab, A	22.08 ± 0.57 ab, B
UVC3	19.84 ± 0.19 ab, A	21.07 ± 0.39 ab, AB

Lowercase letters indicate differences between treatments and uppercase letters indicate differences between analysis days according to the Tukey test ($P<0.05$). ± indicates standard error (n=5). The treatments are described in Table 1.

Conclusions

Strawberries exposed to UV-C radiation and ozone performed better than the control treatment kept at 5±1°C during storage, demonstrating the benefits of these preservation techniques on the physicochemical characteristics of the product. However, the best results were obtained with the OZ3 (higher concentration and shorter exposure time) and UVC3 treatments. These doses were sufficient for the conservation of strawberries under natural conditions. Conversely, low ozone concentrations were not favorable for the natural preservation of strawberries, regardless of the exposure time.

Experimental tests to analyze the physicochemical characteristics of strawberries, such as weight loss, color, pH, TSS, TTA, maturity index and respiration rate, proved to be adequate indicators for assessing their quality. However, it is recommended that tests be conducted over extended periods and with varying doses to establish the optimal concentration range for the proper preservation of strawberries.

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Conflict of interest statement

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

Author's contributions

JAJM: methodology, data curation, formal analysis, research, writing – original draft. ELHC: methodology, data curation, formal analysis, research, writing – original draft. RMA: conceptualization, methodology, data curation, writing – review & editing. CMSS: conceptualization, methodology, writing – review & editing, supervision. FCSU: conceptualization, methodology, writing – review & editing, resources, supervision. All authors approved the final version of the manuscript.

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Fallow improves the growth and yield of green beans and changes the rhizosphere microbial communities

Suelos en barbecho mejoran el crecimiento y rendimiento de habichuela y modifican las comunidades microbianas de la rizosfera

Nicolás Rodríguez-Romero[1], Vanessa Otero-Jiménez[1,2], and Daniel Uribe-Vélez[1]*

ABSTRACT

Soil microorganisms support key ecosystem services for agriculture, and some agricultural practices can increase soil microbial activity and improve crop productivity. Fallow periods have been considered a strategy for restoring biological activity. However, our understanding of the link between fallow periods and the biological activity restored remains limited. The present study evaluated soil microbial communities under two different management strategies: continuous agriculture and fallow. Soil physicochemical parameters and microbial communities were determined through microbiological, biochemical, and molecular techniques. The results showed that fallow soil had significantly higher values ($P<0.05$) of organic matter, pH, counts of soil microorganisms, and soil enzymatic activities than agricultural soil. Finally, the evaluation of plant growth showed that plants in fallow soil grew significantly better ($P<0.05$) than those in agricultural soil. However, after sterilization, the differences between the two soils disappeared. Leaving the soil in fallow periods allows the accumulation of organic matter, the growth of key microbial functional groups, the enhancement of soil enzymatic activities, and a significant improvement in plant growth and yield.

Key words: enzymatic activity, microbiome, *Phaseolus vulgaris*, soil health, soil fertility.

RESUMEN

Los microorganismos del suelo soportan servicios ecosistémicos clave para la agricultura, y algunas prácticas agrícolas pueden aumentar la actividad microbiana del suelo y mejorar la productividad de los cultivos. Los períodos de barbecho se han considerado una estrategia para la restauración de la actividad biológica. Sin embargo, nuestra comprensión de la relación entre los períodos de barbecho y la actividad biológica restaurada sigue siendo limitada. El presente estudio evaluó las comunidades microbianas del suelo bajo dos estrategias de manejo diferentes: agricultura continua y barbecho. Se determinaron parámetros fisicoquímicos del suelo y comunidades microbianas mediante técnicas microbiológicas, bioquímicas y moleculares. Los resultados mostraron que el suelo en barbecho presentó valores significativamente más altos ($P<0.05$) de materia orgánica, pH, conteo de microorganismos del suelo y actividades enzimáticas en comparación con el suelo agrícola. Además, la evaluación del crecimiento vegetal reveló que las plantas en suelo en barbecho crecieron significativamente mejor ($P<0.05$) que en suelo agrícola. Sin embargo, tras someter los suelos a un proceso de esterilización, las diferencias entre ellos desaparecieron. Dejar el suelo en barbecho permite la acumulación de materia orgánica, el crecimiento de grupos funcionales microbianos clave, la mejora de las actividades enzimáticas del suelo y un aumento significativo en el crecimiento y rendimiento de las plantas.

Palabras clave: actividad enzimática, microbioma, *Phaseolus vulgaris*, salud del suelo, fertilidad del suelo.

Introduction

Green bean (*Phaseolus vulgaris* L.) is one of the world's most important food legumes for direct use, particularly in least-developed countries, due to its contribution as a rich source of protein, vitamins, minerals, and fiber (Chaurasia, 2020). The demand for this crop is expected to increase based on current trends in population growth and green bean consumption, with a worldwide production of 28.3 million t by 2022, with an estimated value of US$ 31 billion (Chaurasia, 2020; FAOSTAT, 2024). Currently, this legume is widely distributed in almost all areas of the tropics and subtropics (Tridge, 2021).

Green bean production is the primary source of income for most farmers in the eastern province of Cundinamarca, Colombia. However, crop yields have considerably decreased in recent years due to inadequate practices like overfertilization, high input of herbicides, insecticides, and fungicides, and over-mechanization. These practices are

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[1] Universidad Nacional de Colombia, Instituto de Biotecnología, Grupo de Investigación Microbiología Agrícola, Bogotá (Colombia).
[2] Department of Soil and Water Systems, University of Idaho, Moscow, ID (USA).
* Corresponding author: duribev@unal.edu.co

common in the tropics, where the lack of seasons encourages farmers to plant year-round. As a result, they lead to severe soil deterioration due to accelerated erosion, depletion of soil organic carbon (SOC), and loss of biodiversity (Romdhane *et al*., 2022).

Soil fertility depends on physicochemical properties and microbial composition (Kandasamy *et al*., 2019). Microorganisms play a key role in soil because they conduct many biological processes crucial for agroecosystems, such as biological nitrogen fixation and mineralization of nutrients by organic matter decomposition (Banerjee & van der Heijden, 2022). Moreover, they respond to changes in abundance, diversity, and activity with differentiated reactions under various agronomic management strategies, thereby influencing crop productivity (Mann *et al*., 2019). Consequently, a continuous cropping strategy decreases soil quality and has detrimental effects on soil microbial communities (Romdhane *et al*., 2022).

Fallow is the practice of resting land for short periods, especially in subtropical or temperate countries during the non-growing season (Garba *et al*., 2022). In some cases, the land is left fallow for several years to allow soil quality regeneration through the restoration of its natural structure and the improvement of its agrophysical properties (Burdukovskii *et al*., 2020). There are different indicators to measure this biological response. The first alternative is the evaluation of microbial communities using culture-dependent and culture-independent techniques such as Denaturing Gradient Gel Electrophoresis (DGGE) or target sequencing. An alternative approach to assessing soil quality restoration is measuring functional groups through enzymatic activities, which allows characterization of microbial processes in the soil (Klein *et al*., 1985).

Soil enzymes catalyze the essential biochemical reactions for the cycling of nutrients in the soil through the degradation and transformation of organic matter (Reardon *et al*., 2019); in this way, their activity can be used to make inferences about the quality of the soil (Sinsabaugh *et al*., 2008). Particularly, phosphatases and the phosphorus they release have been used as indicators of the effects of management practices like amendment application or reduced tillage (Bandinck & Dick, 1999; Klein *et al*., 1985). On the other hand, nitrogenases are sensitive to nitrogen availability, since the application of nitrogen fertilizers inhibits their activity (Roper *et al*., 1994). Finally, cellulases are part of a larger group of enzymes known as glycosidases (Eivazi & Tabatabai, 1990). Cellulose degradation is a critical process in soil ecosystems, playing a vital role in nutrient cycling and organic matter decomposition (Datta, 2024). These characteristics allow for the assessment of soil quality in different land uses (Dotaniya *et al*., 2019).

Traditionally, research on soil microbial communities has focused on economically important crops like wheat, rice, soybean, or maize (Brisson *et al*., 2019; Chen *et al*., 2019; Edwards *et al*., 2018; Otero-Jiménez *et al*., 2021). However, there are few studies on the microbial communities of other crops like common beans, which have primarily examined the effect of the crop domestication on rhizosphere microbial communities (Pérez-Jaramillo *et al*., 2017; 2019). In this study, we tested the effect of continuous agriculture and a five-year fallow period on green bean yield and the structure and activity of the rhizosphere microbiome. We hypothesized that fallow may improve crop productivity, enhance plant vigor, and increase soil microbial activity.

Materials and methods

Field location and soil conditions

The present study was carried out on La Colorada farm (4°29′42.96′′ N, 73°51′38.6′′ W) located in Fómeque, Cundinamarca, Colombia. The average temperature and annual precipitation were 18.4°C and 1256 mm, respectively. Soils were classified as clay loam (Pachic Melanudands) (Soil Survey Staff, 2022). We evaluated soils under two contrasting conditions: Soil A (eight years of continuous agriculture; crop rotations included passion fruit, gladiolus, tree tomato, and cape gooseberry; this field was planted with green beans in 2019 for this study) and Soil F (five years uncultivated and planted again with green bean crops in 2019 for this study).

Agronomic conditions

One green bean crop cycle was carried out in 2019 on La Colorada farm in a soil field with continuous agricultural practices (field A, 5709 m^2), and a soil field left fallow for five years (field F, 6012 m^2). Fertilization was carried out by applying NPK 10-30-10 at a dose of 250 kg ha^{-1}; the sowing density was 17 plants m^{-2}. Each field, A and F, was divided into three plots of equal area: soil A, 1903 m^2, and soil F, 2004 m^2 per plot. Each plot was considered one replicate.

Soil physicochemical parameters

Bulk soil samples were taken (depth 25 cm) in triplicate from soil A and F before the crop cycle, air-dried, and sieved (mesh size 2 mm in diameter). The physicochemical parameters were measured following the protocols of Pansu and Gautheyrou (2006). Organic matter (OM) was measured using the Walkley-Black method; the texture was measured by the hydrometer method; apparent density

(AD) was measured by the paraffin clod method; and pH was measured using the water method (1:1 v/v).

Soil sampling for microbial analysis

Six plants in each plot were pulled out and the roots were shaken vigorously to remove bulk soil. The six plant roots were combined and stored in Ziplock® bags to obtain a composite sample for each experimental plot. The bags were transported in cooler boxes (4°C) to the Agricultural Microbiology laboratory at the Biotechnology Institute of the Universidad Nacional de Colombia (IBUN), and rhizosphere soils were collected according to Barillot *et al.* (2013) to evaluate the microbial community function and structure as described below.

Study of culturable microorganisms

For the rhizosphere soil collected, heterotrophic bacteria and phosphate solubilizers were evaluated using the plate count technique on a Plate Count Agar (OxoidTM) and NBRIP culture medium (tricalcium phosphate as the sole source of phosphate), respectively (Nautiyal, 1999). Free-living nitrogen fixers were evaluated using the most probable number (MPN) technique (Chandrapati & Williams, 2014), with NFB culture medium (Alef & Nannipieri, 1995).

Soil enzymatic activities

Acid and alkaline phosphatase activities were evaluated according to the procedure proposed by Eivazi and Tabatabai (1977) in the rhizosphere soil collected. This evaluation is based on the determination of *p*-nitrophenol released after incubation of soil samples in a bis-*p*-nitrophenyl phosphate solution for 1 h at 37°C. Cellulase enzymatic activity was determined by the procedure proposed by Schinner and von Mersi (1990), which is based on the determination of reducing sugars released after incubation of soil samples using carboxymethylcellulose (CMC) as a substrate for 24 h at 50°C. Finally, nitrogenase activity was measured by the Acetylene Reduction Assay (ARA), based on the quantification of ethylene produced from a hermetically sealed bottle containing soil, where the gas phase was replaced with acetylene (Zuberer & Silver, 1978).

Bacterial community analysis by denaturing gradient gel electrophoresis (DGGE)

A DGGE analysis was carried out on the collected rhizosphere soil to determine the effect of soil management on the structure of the soil bacterial community. This technique was implemented following the protocol of Vanegas *et al.* (2013). Total DNA from rhizosphere soil was extracted using the DNeasy PowerSoil Pro Kit (Qiagen Hilden, Germany). DNA was quantified using a NanoDrop spectrophotometer (Thermo Scientific NanoDrop One) and visualized by agarose gel electrophoresis (1%). The concentration of all samples was above 30 ng μl^{-1}. The total DNA isolate was used as a template to amplify the V4 region of the 16S rRNA gene using the universal primers 338F and 518R (Muyzer & Smalla, 1998). The PCR mix contained 1X buffer, 2 mM $MgCl_2$, 0.1 mM dNTPs, 0.5 μM of each primer, 1U Taq polymerase, and 4 ng μl^{-1} of DNA per reaction tube (50 μl). PCR amplification was performed in a Thermal Cycler S1000 (Bio-Rad Laboratories, Hercules, CA, USA). The conditions were: 92°C for 2 min for initial denaturation, followed by 30 cycles (1 min of denaturation at 92°C, annealing at 55°C for 30 s, extension at 72°C for 1 min), with a final extension at 72°C for 6 min.

The DGGE was carried out using a Dcode Universal Mutation Detection System (Bio-Rad Laboratories, Hercules, CA, USA). The PCR products were run in duplicate on a 6% polyacrylamide gel in a 1X TAE buffer; the denaturant gradient was 30-80% (urea:formamide). Electrophoresis was run for 16 h at 60°C and 75 volts; the gel was stained with SYBR™ Gold and photographed under UV light using a Gel Doc™ XR+ (Bio-Rad Laboratories, Hercules, CA, USA).

Effect of soil management on crop productivity

For each replicate, which consisted of a plot of 1903 m^2 for soil A and 2004 m^2 for soil F, yield data were measured and the number of leaves and root nodules per plant were counted at the flowering crop stage in six plants per replicate. For yield, weekly harvests of the fruits were conducted, and the fresh weight of each harvest was recorded. The weekly records were summed at the end of the crop cycle to calculate total production and then divided by the area to calculate the yield.

Effect of soil microbial biomass on initial plant growth

A pot assay was implemented to assess the effect of a five-year fallow period on the initial growth of green bean plants in comparison to conventional agricultural management. A factorial design was adopted to evaluate the effect of the soil-living microbial biomass. The first factor, soil sterility, had two levels: sterile and non-sterile soil. The second factor, soil management strategy, had two levels, agricultural soil (soil A) and fallow soil (soil F). Soil sterilization was done by two sterilization cycles at 121°C and 15 psi for 40 min each. A total of four treatments with 14 independent plants were applied, where each plant was considered an experimental unit. The number of replications was calculated using the Harris-Hurvitz-Mood method. Green bean seeds were planted outdoors in plastic pots filled with 400 g of collected bulk soil collected from the top 25 cm of the soil profile, since this is the effective depth of green bean roots (Vallejo Cabrera *et al.*, 2004). The experiment was performed in the same location as the field experiment. The plant response variables were plant height, fresh weight, dry weight, and number of root nodules measured 30 d after planting.

Statistical analysis

The results were subjected to an analysis of variance ($P<0.05$) and a subsequent post hoc Tukey test ($P<0.05$) to evaluate differences between soil management strategies. For each case, the assumptions of normality were tested using the Shapiro-Wilk test ($P<0.05$), and the homogeneity of variances was tested using the Bartlett test ($P<0.05$). Multivariate analysis, principal component analysis, and Pearson correlation were calculated. All analyses were performed in R v4.2 (R Core Team, 2021) using agricolae (Mendiburu, 2023) and ggplot2 (Wickham, 2016) packages.

Results

Fallow period improves soil physicochemical parameters, microbial culturable counts, and soil enzyme activities

The soil organic matter (OM) and pH showed significantly higher values in fallow soil (F) compared to with agricultural soil (A) (Tab. 1). Apparent density was significantly higher in soil A compared to soil F (Tab. 1). Total soil microbial counts showed statistically significant higher values in soil F compared with soil A for total heterotrophic bacteria, phosphate solubilizers and nitrogen-fixing bacteria (Tab. 1). There were significant differences between the two soils, with soil F showing the highest activity for all enzymes (Fig. 1).

TABLE 1. Physicochemical properties and microbial counts across study areas on La Colorada farm.

	OM (%)	**AD**	**pH**	**TB**	**PS**	**NF**
	%*	mg m^{-3}*	*	LogCFU gds^{-1}*	LogCFU gds^{-1}*	LogMPN gds^{-1}*
Soil F	7.48 ±0.71 a	1.46±0.09 b	6.43±0.13 a	8.35±0.23 a	5.95±0.22 a	6.51±0.22 a
Soil A	5.91±0.6 b	1.71±0.04 a	4.99±0.03 b	8.14±0.36 a	5.60±0.34 b	5.69±0.30 b

*Significant at the 0.05 probability level. Values are presented as means ± standard deviation. Means with the same letter do not differ significantly at $P\leq0.05$, n = 3. Statistical test ANOVA-Tukey. OM: soil organic matter, AD: apparent density, TB: total heterotrophic bacteria, PS: phosphate solubilizers, and NF: nitrogen fixers, CFU: colony-forming units, MPN: most probable number, gds: g of dry soil.

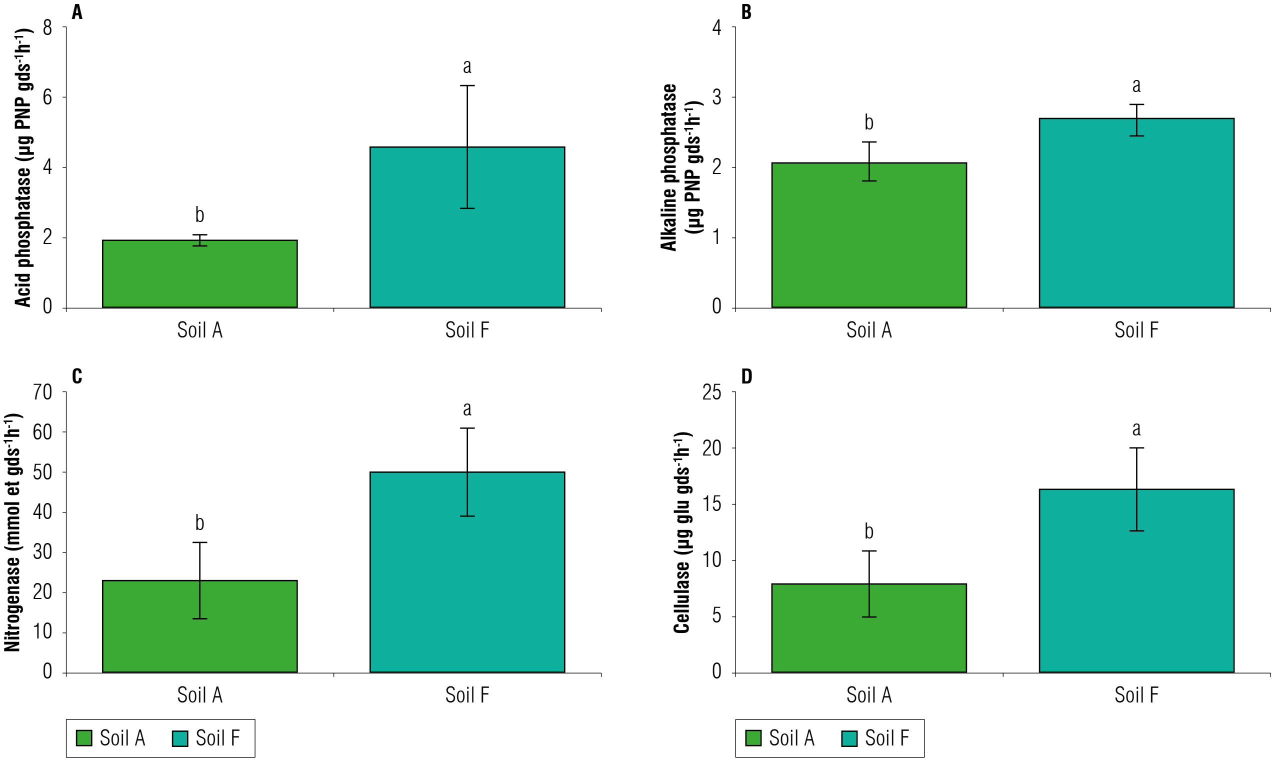

FIGURE 1. Enzymatic activities of: A) acid phosphatase, B) alkaline phosphatase, C) nitrogenase, and D) cellulase. PNP: *p*-nitrophenol; et: ethylene; glu: glucose; A: agricultural soil, F: fallow soil, gds: g of dry soil. Different letters indicate significant differences (ANOVA-Tukey, $P\leq0.05$), n=3. The bars represent the standard deviation.

Crop yield is improved in fallow soil under field conditions

The crop measurements under field conditions showed that soil F had significantly higher values for the number of nodules and leaves per plant and an increase in yield compared to soil A (Tab. 2).

TABLE 2. Effect of soil management on crop performance under field conditions at La Colorada farm.

Soil	Number of leaves per plant*	Number of nodules per plant*	Yield (t ha^{-1}) *
Soil F	38.9 ±2.8 a	33.2 ±5.06 a	14.4 ±0.14 a
Soil A	31.0 ±1.93 b	19.4 ±5.25 b	8.47±0.27 b

*Significant at the 0.05 probability level. The values are presented as means ± standard deviation. Means followed by the same letter do not differ significantly at $P\leq0.05$, n = 3. Statistical test: ANOVA followed by Tukey's test.

Bacterial communities change under different soil management strategies

Figure 2A shows a UPGMA (Unweighted Pair Group Method using Arithmetic Averages) clustering, formed from the banding patterns obtained by a DGGE approach. The analysis shows two large clades, where soil A is separated from soil F, which indicates that changes in soil management generated differences in the composition of the rhizosphere bacterial community (Fig. 2A). Figure 2B shows a correlation matrix that relates enzymatic activities, and functional group counts with physicochemical parameters and plant growth variables. Principal component analysis (Fig. 2C) shows a different clustering for samples from soil A and soil F. The response variables measured explain more than 70% of the total variance in the experiment.

Soil management and soil sterilization affect plant growth

We hypothesized that there is a differentiated effect in terms of plant growth between both soil management strategies: continuous agriculture and the fallow period. To demonstrate this effect, we took bulk soil from fields A and F and evaluated green beans growth in a pot assay. The results showed that soil management during the initial phases of green bean growth significantly influences the four variables measured, where soil F showed higher values (Fig. 3). It is important to point out that, leaving soil A and soil F with similar conditions after sterilization, these four variables did not present significant differences (Fig. 3), thus demonstrating the importance of soil microorganisms for the development and growth of the plants.

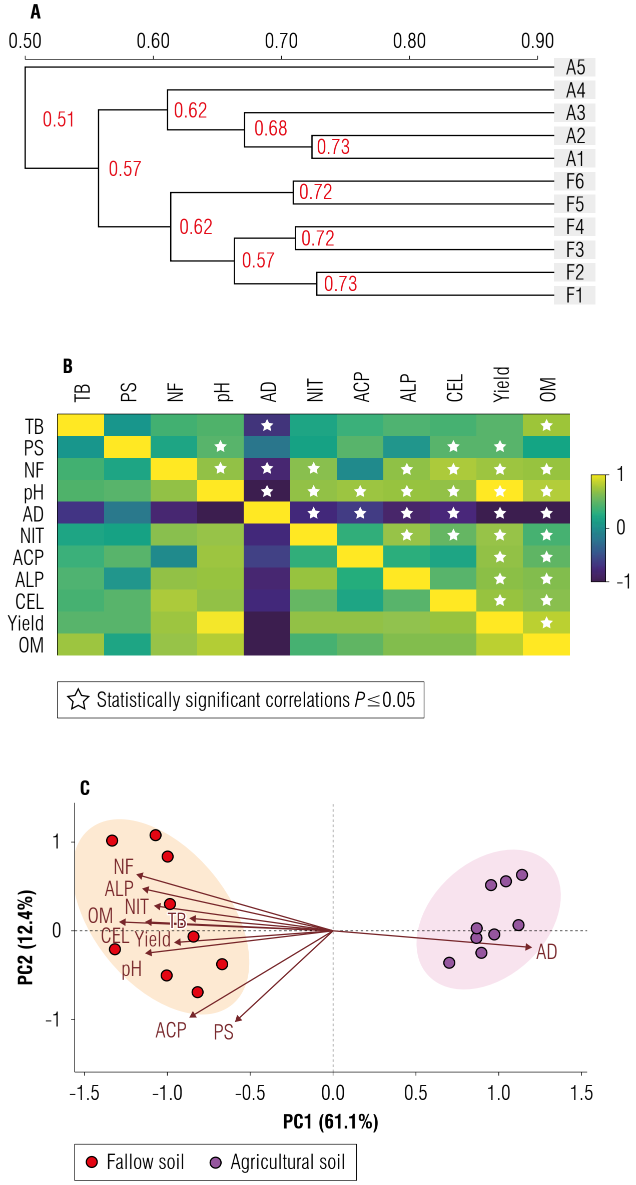

FIGURE 2. A) Analysis of relationships between non-culturable microbial communities obtained by DGGE using the UPGMA hierarchical grouping method. (B) correlation matrix between the variables measured. The result of Pearson's correlation is shown in R^2. White stars show statistically significant correlations $P<0.05$. ACP – acid phosphatase, ALP – alkaline phosphatase, CEL – cellulase, NIT – nitrogenase, OM – soil organic matter, AD – apparent density, TB – total heterotrophic bacteria, PS – phosphate solubilizers, NF – nitrogen fixers. The figure was made in R using the ggplot package. (C) Principal component analysis establishes relationships between the two soil management dynamics and the variables measured in the experiment.

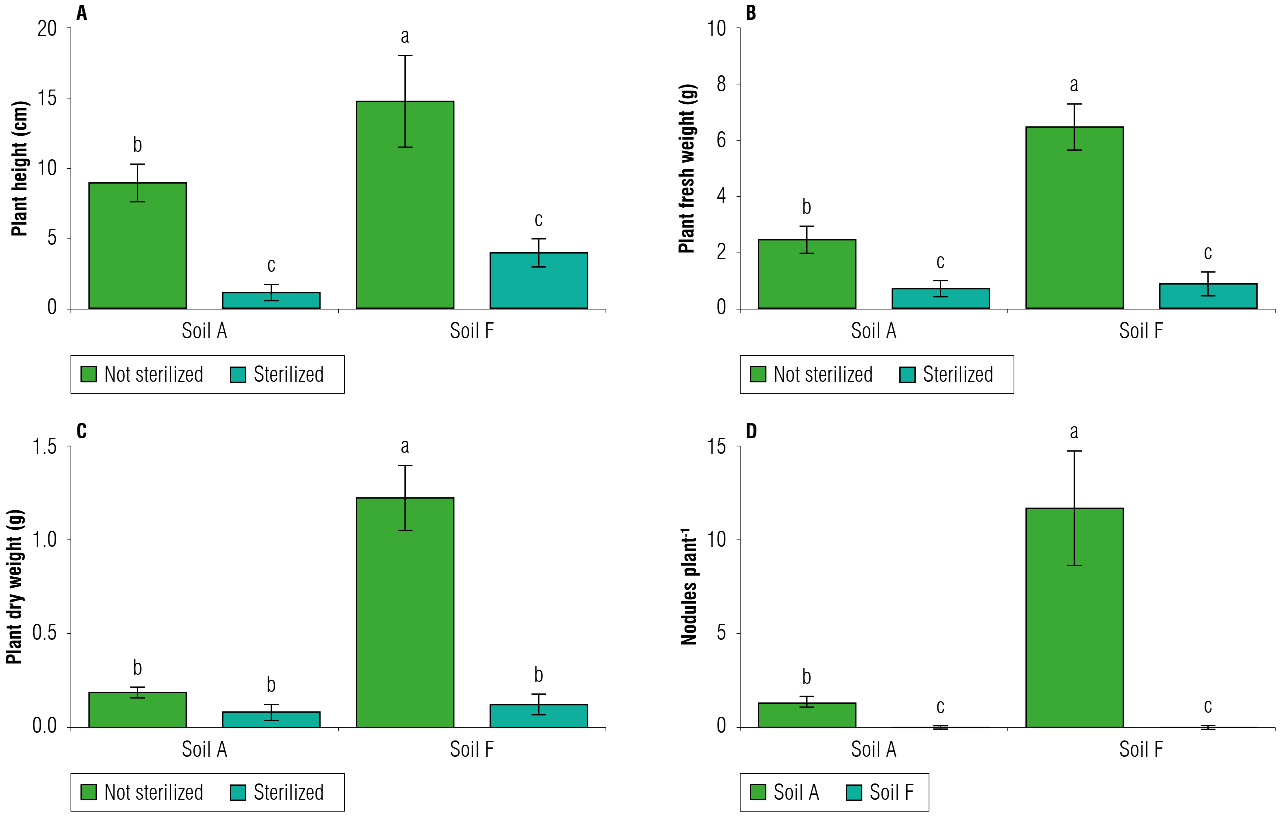

FIGURE 3. Agronomic variables measured in the green bean growth test: A) Plant height, B) Plant fresh weight, C) Plant dry weight, and D) Mean number of nodules produced per plant. Different letters indicate significant differences (ANOVA-Tukey, $P \leq 0.05$). The bars correspond to the standard deviation. Soil A: agricultural soil, Soil F: fallow soil, n=14. The figure was made in R using the ggplot package.

Discussion

This study is one of the few published reports that assesses the effect of fallow on soil microbiological activity from a functional perspective and relates it to the yield of the subsequent crop. Our results provide a reference framework, suggesting that leaving soil fallow for five years can contribute to the recovery of biological activity and, consequently, productivity. However, further research is needed to determine whether shorter fallow periods could achieve similar effects. Some meta-analyses have attempted to explore whether there is a relationship between fallow duration and crop yield (Mertz, 2002; Mertz *et al.*, 2008). Although there is empirical evidence indicating that longer fallow periods are correlated with higher yields, other factors influence yield, making it difficult to establish causal relationships between fallow duration and yield (Mertz, 2002; Mertz *et al.*, 2008).

Brennan and Acosta-Martinez (2017) showed that six years are sufficient to positively shift the microbial community in California soils under intensive agriculture when transitioning to organic cropping. Other interesting approaches demonstrated that soil amendments under different fallow periods (1, 3, and 16 years) in crops grown in continuously cultivated soils led to improved growth and resistance to pests (Howard *et al.*, 2020). However, beyond fallow periods, the long-term sustainability of organic systems also requires practices such as reduced tillage, the application of organic amendments, and the use of cover crops. Among these, cover crops are particularly effective as they provide a readily available source of labile carbon for microorganisms (Brennan & Acosta-Martinez, 2017).

We recognize that five years is a long period for a farmer to leave a crop field fallow. However, this timeframe allows us to assess the effect of fallow on the recovery of biological activity in agricultural soils. It also serves as a starting point to evaluate shorter, more economically sustainable fallow periods and management strategies. Additionally, it helps identify biological activities that indicate soil health in *P. vulgaris* crops under tropical conditions.

The soil OM content, pH, and apparent density of the soil have been reported as major determinants of the distribution of edaphic microorganisms (Fierer, 2017). These three variables in soil F showed the most favorable values for the development of microorganisms. For instance, higher OM content will provide a greater supply of carbon to soil microorganisms (Martínez-García *et al*., 2018), suggesting better microbial activity. In our study, this was indicated by the correlation between OM and total heterotrophic bacteria, nitrogen fixers, nitrogenase activity, phosphatase activity, and cellulase activity (Fig. 2B). A lower apparent density results in greater pore space, which is necessary for gas exchange and water retention; these conditions facilitate the formation of niches, which are where microorganisms live and where most biological processes of agricultural interest occur (Totsche *et al*., 2018). Our results suggest that these phenomena can be observed in the negative correlation between total heterotrophic bacteria and nitrogen fixers with apparent density (Fig. 2B).

One of the physicochemical variables that showed the greatest differences was pH, which decreased from 6.43 in the fallow soil to 4.99 in the agricultural soil (Tab.1). This pH reduction is commonly reported in soils subjected to intensive agricultural activity (Zhou *et al*., 2021). For instance, the frequent application of nitrogen fertilizers such as urea has been associated with an increase in the nitrification process (Ayiti & Babalola, 2022), contributing to soil acidification. On the other hand, a slight increase in pH has been reported in fallow soils (Fachin *et al*., 2021). Although no concrete data are available regarding the amount of fertilizers and pesticides applied to the agricultural soil, it is known that this soil had been under continuous cultivation for the past 12 years before this study, with at least three fertilizer applications per year.

A higher pH improves the survival conditions of many bacterial populations (Xiao *et al*., 2018); in this study, we identified a correlation between pH and phosphate solubilizers (Fig. 2B). From an agronomic perspective, a pH of 6.43 is optimal for the availability of most nutrients, whereas at a pH of 4.99, macronutrient availability is significantly reduced (Hartemink & Barrow, 2023). This suggests that not only are soil microorganisms affected, but plant growth is also directly limited by the low pH in agricultural soils. However, Figure 3 shows that after soil sterilization, where the only variable altered is the presence of living microorganisms, there are no differences in plant growth between fallow and agricultural soils. This suggests that the main effect of pH is exerted on soil microorganisms.

A high phosphatase activity indicates better soil quality, especially in tropical soils (Ferreira *et al*., 2016; Stone & Plante, 2014). The results obtained in our study showed a positive response to leaving agricultural soils for a five-year fallow period, since both types of phosphatases (acid and alkaline) were higher in soil F than in soil A. This suggests a more abundant fraction of organic P in this soil, as expected given the significantly higher content of OM (Tab. 1). On the other hand, it is worth mentioning that phosphatases, both acid and alkaline, are more active in the rhizosphere (Tarafdar & Jungk, 1987), especially in leguminous plants such as the green bean (Yadav & Tarafdar, 2001). We did not find a significant correlation between phosphate solubilizers and alkaline phosphatase or acid phosphatases, suggesting that the enzymatic activity observed corresponds to another type of phosphate-mineralizing microorganisms that are functionally active, or even extracellular enzymes attached to mineral and organic particles, as discussed below.

The significant correlation found between OM and alkaline phosphatase, nitrogenase, and cellulase activity (Fig. 2B) indicates that a greater amount of OM facilitates the stabilization of these enzymes in the soil matrix. These enzymes are usually excreted by microorganisms and adhere to organic particles or clays (Nannipieri *et al*., 2018). Once stabilized, enzymes remain catalytic for long periods (Dotaniya *et al*., 2019), being a potential source of enzymatic activity associated with the soil (Skujiņš & Burns, 1976). The correlation found between cellulase and nitrogenase enzymes (Fig. 2B) can be explained by considering that the energy necessary for nitrogenase activity comes mostly from carbon sources, resulting from cellulose and hemicellulose hydrolysis (Deng & Tabatabai, 1994).

Other studies analyzing the diversity and microbial structure by DGGE found differences in bacterial diversity between soils subjected to different soil management strategies. In this context, Wallis *et al*. (2010) observed lower richness in soils of intensive crop production compared to soils of natural pastures and forests. Other researchers have also shown that alternatives such as reduced tillage, cover crops, and organic amendments improve microbial diversity and abundance in soil (Kuntz *et al*., 2013; Martínez-García *et al*., 2018). Here, we demonstrated that a fallow period of five years and continuous agriculture can generate a differential configuration of the soil bacterial community, indicating that soil microbes respond to soil management practices. Principal component analysis (Fig. 2C) showed two clearly separated groups based on the type of soil management evaluated, supporting the previous discussion and the hypothesis.

Legume crops such as green beans have the inherent capacity for biological nitrogen fixation mediated by the interaction with diazotrophic bacteria (Zhong *et al*., 2024), which catalyze the conversion of nitrogen to ammonia via the nitrogenase enzyme (Chaulagain & Frugoli, 2021). Nitrogen is one of the principal macronutrients associated with plant growth and productivity. This research showed that soils under fallow for five years increased the number of leaves; in this way, more carbon can be fixed through photosynthesis. This, in turn, can be used in exchange for nitrogen produced in the nodules by nitrogen-fixing microorganisms (Chaulagain & Frugoli, 2021). Finally, this is reflected in a higher yield in fallow treatment, suggesting that plant growth on fallow plots offers a niche that allows the establishment of different microbial communities that promote greater nitrogen fixation and, consequently, increase plant growth and yield.

Our results highlight the importance of soil management, particularly its biological component, and the consequent effects on plant vigor and plant productivity, since soil fertility depends largely on biological activity, especially in the rhizosphere (Mendes *et al*., 2013).

Conclusions

The intensive use of soil leads to the continuous impoverishment of microbial communities and their functions. On the other hand, leaving fallow for five years increases organic matter and improves certain physicochemical properties, which leads to an improvement of microbial activity as well as plant vigor and productivity of the crop. All these conditions are responsible for the differences that occur in terms of plant height and weight, the number of nodules per plant, and the improvement of crop yield. Our results indicate that proper management of the biological component of the soil stimulates greater microbial activity, which leads to healthier and more productive soils. Acknowledging that leaving crop fields fallow for five years generates a greater green bean yield, the challenge is to generate similar effects using shorter periods or soil management strategies that lead to similar results.

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Conflict of interest statement

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

Author's contributions

All authors contributed to the study conception and design. NRR: writing the original draft, data curation, formal analysis, visualization, review, and editing. VOJ: writing, reviewing, and editing. DUV: funding acquisition, supervision, writing, reviewing, and editing. All authors have read and approved the final version of the manuscript.

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Classification of maize hybrids using UAV-based multispectral remote sensing and machine learning algorithms

Clasificación de híbridos de maíz utilizando detección remota multiespectral basada en UAV y algoritmos de aprendizaje automático

João Lucas Gouveia de Oliveira[1], Dthenifer Cordeiro Santana[1], Izabela Cristina de Oliveira[1], Ricardo Gava[1], Fábio Henrique Rojo Baio[1], Carlos Antônio da Silva Junior[2], Larissa Pereira Ribeiro Teodoro[1], Paulo Eduardo Teodoro[1], and Job Teixeira de Oliveira[1]*

ABSTRACT

Novel methodologies for phenotypic evaluation in maize have been developed through the integration of advanced sensing technologies and machine learning algorithms. The aim of this study was to identify the most accurate machine learning algorithm for the classification of maize hybrids and to determine the optimal input data to enhance model performance. Seven maize hybrids were used in the experiment. After 60 d of crop emergence, the remotely piloted aircraft SenseFly® eBee RTK was used to obtain reflectance values at the following spectral bands (SB): blue (475 nm, B_475), green (550 nm, G_550), red (660 nm, R_660), red edge (735 nm, RE_735) and near-infrared (790 nm, NIR_790). Following the acquisition of spectral band (SB) data, vegetation indices (VIs) were calculated. The resulting dataset was subsequently analyzed using machine learning techniques, evaluating six algorithms: artificial neural networks (ANN), J48 decision trees (J48), REPTree (DT), random forest (RF), support vector machine (SVM) and logistic regression (LR) as the baseline model. Three accuracy metrics were used to evaluate the performance of the algorithms in classifying maize hybrids: correct classifications (CC), Kappa coefficient, and F-score. Among the algorithms tested, ANN showed the highest performance in all three metrics, proving its superiority and potential for real-world applications. Although all three input configurations enhanced classification accuracy for ANN algorithm, the optimal approach is to use only SB as input due to reduced data processing time and increased simplicity.

Key words: artificial neural networks, spectral bands, spectral curve, vegetation indices, machine learning classification, UAV-based image analysis.

RESUMEN

Se han desarrollado nuevas metodologías para la evaluación fenotípica en maíz mediante la integración de tecnologías de detección avanzadas y algoritmos de aprendizaje automatizado. El objetivo de este trabajo fue identificar el algoritmo de aprendizaje automático más preciso para la clasificación de híbridos de maíz y determinar los datos de entrada que mejoran el rendimiento del modelo. Se utilizaron siete híbridos de maíz. A los 60 d de emergencia del cultivo se utilizó la aeronave pilotada remotamente SenseFly® eBee RTK para obtener la reflectancia en las siguientes bandas espectrales (BE): azul (475 nm, B_475), verde (550 nm, G_550), rojo (660 nm, R_660), borde rojo (735 nm, RE_735) y NIR (790 nm, NIR_790). Luego de la adquisición de datos de la banda espectral (BE), se calcularon los índices de vegetación (Vis). El conjunto de datos resultante se analizó posteriormente utilizando técnicas de aprendizaje automático, evaluando seis algoritmos: redes neuronales artificiales (RNA), árboles de decisión J48 (J48), REPTree (DT), bosque aleatorio (BA), máquina de vectores de soporte (MVS) y regresión logística (RL) como enfoque de referencia. Se utilizaron tres métricas de precisión para evaluar el desempeño de los algoritmos en la clasificación de híbridos de maíz: clasificaciones correctas (CC), coeficiente Kappa y F-score. Entre los algoritmos probados, el algoritmo de ANN se destacó con el mayor desempeño en las tres métricas, demostrando su superioridad y potencial para aplicaciones reales en clasificación. Aunque las tres configuraciones de entrada mejoraron la precisión de clasificación para el algoritmo RNA, el enfoque óptimo es utilizar solo las BE como entrada debido al menor tiempo de procesamiento de datos y la mayor simplicidad.

Palabras clave: redes neuronales artificiales, bandas espectrales, curva espectral, índices de vegetación, clasificación mediante aprendizaje automático, análisis de imágenes basado en UAV.

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[1] Universidade Federal de Mato Grosso do Sul, Chapadão do Sul, MS (Brazil).
[2] Universidade Estadual do Mato Grosso (Brazil).
* Corresponding author: job.oliveira@hotmail.com

Introduction

Maize (*Zea mays* L.) is a highly versatile crop with numerous applications, representing a substantial portion of cultivation in major grain-producing countries (Liu *et al.*, 2023). This versatility results from the extensive selection and improvement processes that the plant has undergone. These processes have enabled maize to overcome global challenges, improve productivity and nutritional quality, and meet diverse human needs (Swarup *et al.*, 2021).

Over the years, plant breeding techniques have advanced, especially at the molecular level, such as genetic sequencing of several species and gene interventions, enabling the development of populations resistant to biotic and abiotic stresses (Iqbal *et al.*, 2021; Rivero *et al.*, 2022; Zafar *et al.*, 2022). However, phenotypic analysis has not progressed at the same rate, facing challenges such as a shortage of qualified personnel capable of efficiently and accurately selecting desirable characteristics. Therefore, it is essential to develop and implement advanced techniques to enhance the precision and performance of phenotypic evaluations (Santos *et al.*, 2014).

The advancement in technologies such as remote sensing, combined with efforts to accelerate maize genetic improvement, has led to the development of new high-precision phenotyping (FAP) techniques. These techniques address the extended duration required by traditional methods for the evaluation and selection of materials (Herzig *et al.*, 2021). FAP provides significant advantages in reducing fieldwork time, labor and costs by using sensors to characterize phenotypes with high accuracy and efficiency in a non-destructive manner (Andrade *et al.*, 2021; Dobbels *et al.*, 2019; Santana *et al.*, 2023). FAP also enables the correlation of several plant traits, such as the selection of soybean genotypes based on precocity and grain productivity (Santana *et al.*, 2022).

The emergence of remotely piloted aircraft (unmanned aerial vehicle - UAV) was significantly benefited agriculture by integrating various sensors to capture data across different wavelengths. This technology has become an important tool for non-destructive field data collection throughout the crop cycle (Das Choudhury *et al.*, 2019). It is capable of collecting data with high speed and spatial resolution (Kar *et al.*, 2021).

The amount of data generated through remote sensing is substantial, posing challenges for traditional statistical methods in correlating sensor data with plant characteristics, due to the non-linearity between spectral variables and physiological or morphological characteristics of plants (Van Eeuwijk *et al.*, 2019). Thus, there is a need for enhanced data processing capacity, which can be achieved through the application of machine learning (ML) algorithms.

Machine learning (ML) algorithms enable the prediction of leaf nitrogen concentration and height in maize (Osco *et al.*, 2020). Additionally, ML algorithms can be used for classifying different soybean cultivars (Gava *et al.*, 2022), evaluating leaf nutritional traits (Santana, Teodoro *et al.*, 2023), and accurately classifying genotypes based on industrial soybean characteristics (Santana, Teixeira *et al.*, 2023). Corn plants subjected to different irrigation management practices exhibit distinct spectral behaviors, allowing their classification through machine learning modeling (Oliveira *et al.*, 2025).

This study hypothesizes that maize hybrids can be effectively distinguished based on spectral data using machine learning models. The aim was to identify the most accurate machine learning algorithm for classifying maize hybrids and to determine the optimal input data to enhance the performance of these models.

Materials and methods

Experiment conditions

The experiment was conducted in the experimental area of the Federal University of Mato Grosso do Sul (18°41'33" S, 52°40'45" W, 810 m a.s.l.), located in Chapadão do Sul, Brazil. The conventional soil preparation method, consisting of plowing and leveling harrowing, was employed. The climate of the region is classified as Tropical Savanna (Aw) according to the Köppen classification. The soil, classified as clayey Dystrophic Red Oxisol (Santos *et al.*, 2018), exhibits the following characteristics in the 0-0.20 m layer: $pH_{(H_2O)}$ = 6.2; exchangeable Al (cmol kg^{-1}) = 0.0; Ca+Mg (cmol kg^{-1}) = 3.59; P (mg kg^{-1}) = 41.3; K (cmol kg^{-1}) = 0.16; organic matter (%) = 1.97; base saturation (BS, %) = 45; aluminum saturation (%) = 0.0; sum of bases (cmol kg^{-1}) = 1.92; cation exchange capacity (CEC) (cmol kg^{-1}) = 4.25.

Seed sowing was carried out using a seeder, with seeds placed 0.45 m apart between rows. Additionally, 200 kg ha^{-1} of monoammonium phosphate (MAP) with a formulation of 11-52-00 was applied. Top dressing was performed using 150 kg ha^{-1} of urea (45% N) when the plants reached the V4 growth stage. The experiment was conducted using a strip-plot design, with plot dimensions of 3.8 m x 4.9 m (18.62 m^2). The maize hybrids used in the experiment were: H1 (AS 1868), H2 (DKB 360), H3 (FS 615 PWU), H4 (K 7510 VIP3), H5 (NK 520 VIP3), H6 (P 3858 PWU), and H7 (SS 182E VIP3). Each hybrid's seed amount per meter was 2.67, 2.68, 2.74, 2.65, 2.64, 2.63, and 2.55, respectively.

Acquisition and processing of multispectral images

A flight was conducted 60 d after crop emergence, at the stage of full bloom for the maize varieties, using the SenseFly® eBee RTK, a fixed-wing remotely piloted aircraft with autonomous takeoff and landing capabilities. The eBee was equipped with a Parrot® Sequoia multispectral sensor (Parrot Drones SAS). The images were obtained at 09:00 a.m. under clear skies, at an altitude of 100 m and a spatial resolution of 0.10 m. The aerial survey employed Real-Time Kinematic (RTK) technology, which enables image acquisition with a positional accuracy of 2.5 cm. Image mosaicking and orthorectification were subsequently performed using Pix4Dmapper software.

Radiometric calibration of the entire scene was conducted using a calibrated reflective surface provided by the manufacturer. The Parrot Sequoia multispectral sensor is equipped with a sunshine sensor for the calibration of acquired reflectance values. The sensor captured data across the following spectral bands (SB): blue (475 nm, B_475), green (550 nm, G_550), red (660 nm, R_660), red edge (735 nm, RE_735) and near-infrared (790 nm, NIR_790). Following the acquisition of spectral band (SB) data, 41 vegetation indices (VIs) (Suppl. Tab. 1 (S1)) were calculated, ranging from simple ratios to indices incorporating atmosphere and soil correction (Oliveira *et al.*, 2025; Ramos *et al.*, 2020), using ESRI ArcGIS 10.5 geographic information systems software.

Machine learning models and statistical analysis

The data were subjected to machine learning analyses, evaluating six algorithms: artificial neural networks (ANN) (Egmont-Petersen *et al.*, 2002), J48 decision tree (J48) (Quinlan, 1993) and REPTree (Al Snousy *et al.*, 2011), random forest (RF) (Belgiu *et al.*, 2016), support vector machine (SVM) (Nalepa *et al.*, 2019), and logistic regression (LR) (Štepanovský *et al.*, 2017). Logistic regression (LR) was chosen as the baseline model due to its widespread adoption in binary and multiclass classification problems. It is frequently used as a reference in the literature because of its interpretability, computational efficiency, and consistent performance on linearly separable data. Its comparison with traditional methods (such as discriminant analysis or Naive Bayes) was implicitly addressed through stratified k-fold cross-validation (k=10 with 10 replicates), ensuring a robust and generalizable evaluation. Although LR does not always outperform more complex approaches, its use as a baseline allows for quantifying the actual improvement of the proposed hybrid models, distinguishing significant gains from marginal ones. Additionally, its simplicity facilitates the identification of biases or underfitting, making it a valid substitute for traditional methods in scenarios where variable relationships are predominantly linear. All algorithm parameters were established according to the default configuration of Weka 3.8.5, which proved efficient for the problem under study, with its impact on model performance validated through comparative tests.

Three accuracy metrics were used to evaluate the performance of the algorithms in classifying maize hybrids: correct classification (CC), Kappa coefficient and F-score. An analysis of variance (ANOVA) was conducted to assess the significance of ML algorithms, input variables and their interactions. To illustrate model performance and statistical significance, boxplots were generated using the mean values of CC, Kappa and F-score, grouped according to the Scott-Knott test (Scott & Knott, 1974) at a 5% significance level. All analyses and graphical representations were performed using the ggplot2 and ExpDes.pt packages in R (R Core Team, 2013).

Results and discussion

The reflectance of each hybrid at specific wavelengths is shown in Figure 1. Hybrids H4 and H7 exhibited the highest reflectance values across all wavelengths, with statistically significant similarities observed only with hybrid H6 at the B_475 wavelength and with hybrid H1 at the RE_735 and NIR_790 wavelengths. Hybrids H3 and H5 exhibited the lowest reflectance values at the B_475, R_660 and G_550 wavelengths. At the RE_735 wavelength, hybrids H5 and H6 showed reduced reflectance, while at the NIR_790 wavelength, hybrid H6 demonstrated the lowest reflectance.

Performance evaluation of the machine learning algorithms was conducted using three parameters: correct classification (CC), F-score and Kappa coefficient. A significant interaction was observed between the input data and the machine learning algorithms for all accuracy parameters assessed.

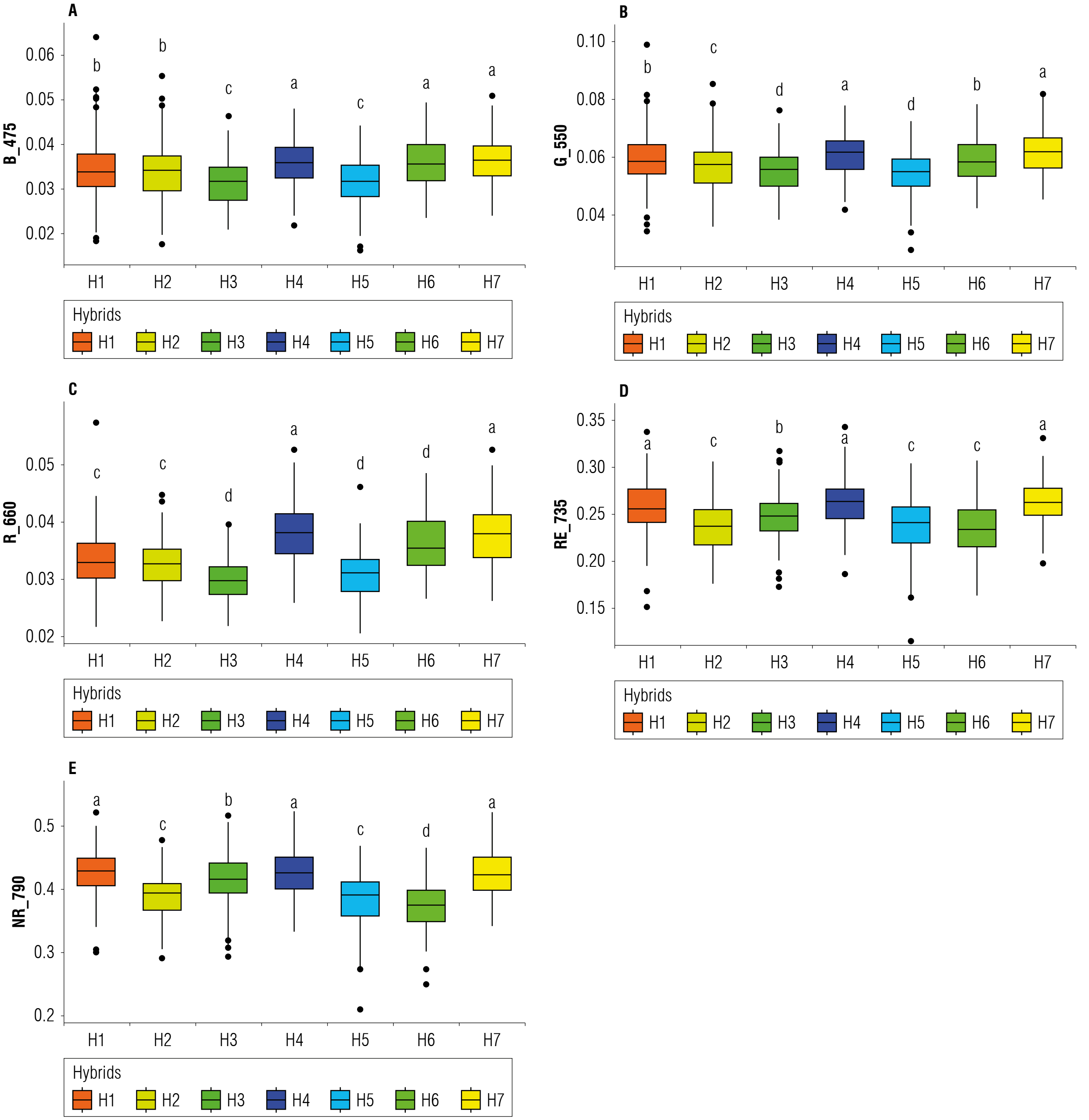

FIGURE 1. Boxplots illustrating the multispectral reflectance of seven maize hybrids across five spectral bands: blue (B_475 nm, A), green (G_550 nm, B), red (R_660 nm, C), red edge (RE_735 nm, D) and near-infrared (NIR_790 nm, E). The data were analyzed using the Scott-Knott test at a 5% significance level. Hybrids followed by the same letters for each wavelength do not significantly differ from each other.

Based on the correct classification metric, the artificial neural network (ANN) was identified as the best-performing algorithm among all input datasets (Fig. 2). When analyzing the ANN algorithm, no statistically significant differences were observed among the input datasets. In contrast, the RF, LR, DT, and SVM algorithms demonstrated superior performance with the IVs and SB+IVs inputs. For the J48 algorithm, the best performance was achieved with the SB and IVs inputs.

Evaluation of the algorithms using F-score metric revealed that ANN was the most effective algorithm for classification among all input datasets (Fig. 3). For the ANN, LR, J48, and DT algorithms, no statistically significant differences

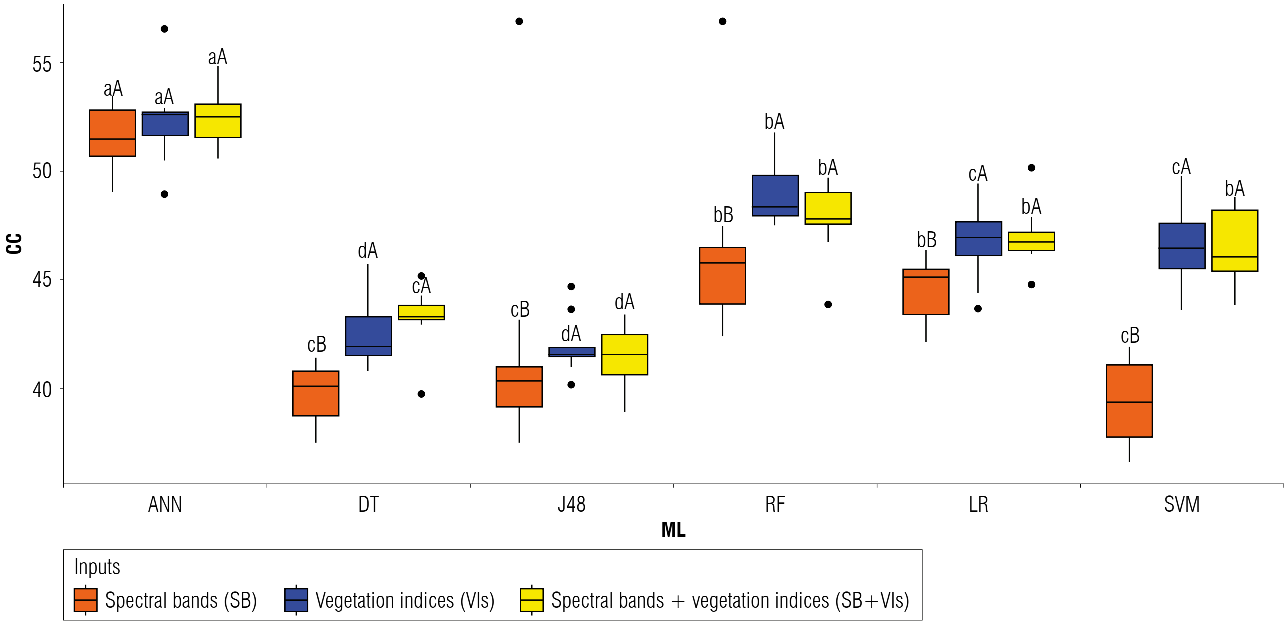

FIGURE 2. Boxplot illustrating the percentage of correct classification (CC) for the significant interaction between the machine learning models and the tested input datasets. Means followed by the same uppercase letters (for the different inputs) and the same lowercase letters (for the different ML algorithms) do not differ significantly according to the Scott-Knott test at a 5% significance level. Algorithms: Artificial neural networks (ANN), J48 decision trees (J48), REPTree (DT), random forest (RF), support vector machine (SVM), and logistic regression (LR).

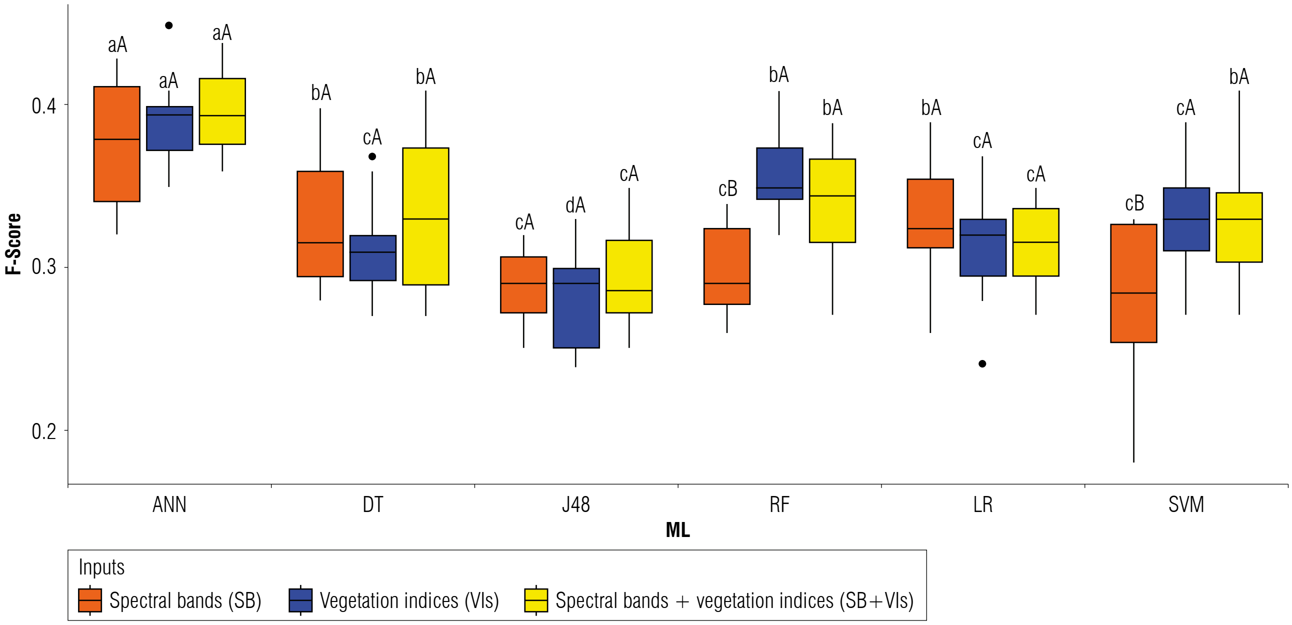

FIGURE 3. Boxplot illustrating the percentage of F-score metric for the significant interaction between the machine learning models and the tested input datasets. Means followed by the same uppercase letters (for the different inputs) and the same lowercase letters (for the different ML algorithms) do not differ significantly according to the Scott-Knott test at a 5% significance level.

were observed among the different input datasets. Conversely, the RF and SVM algorithms exhibited optimal performance with IVs and SB+IVs inputs.

Analysis using the Kappa metric indicated that the artificial neural network (ANN) was the most effective algorithm for classifying all input datasets (Fig. 4). For both the ANN and J48 algorithms, no statistically significant differences

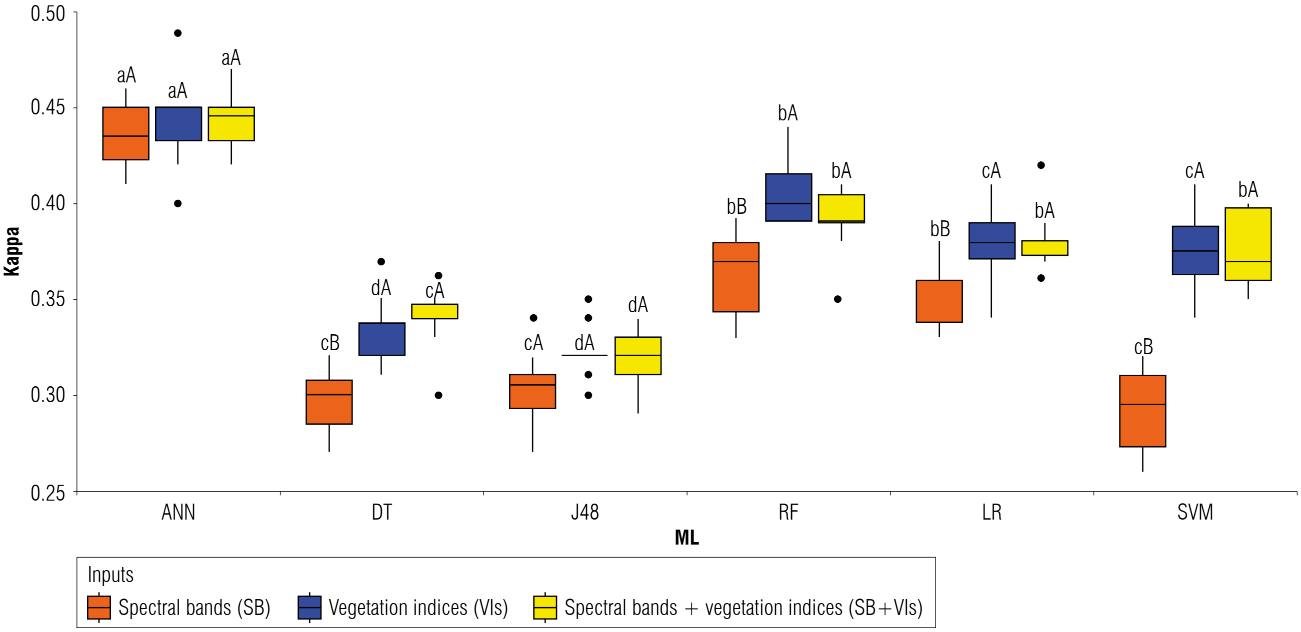

FIGURE 4. Boxplot illustrating the percentage of Kappa metric for the significant interaction between the machine learning models and the tested input datasets. Means followed by the same uppercase letters (for the different inputs) and the same lowercase letters (for the different ML algorithms) do not differ significantly according to the Scott-Knott test at a 5% significance level.

were observed among the input datasets. In contrast, the RF, LR, DT, and SVM algorithms demonstrated optimal performance with VI and SB+VI inputs.

The spectral signature of plants generally exhibits low reflectance in the visible region of the electromagnetic spectrum due to the absorption of these wavelengths by pigments for photosynthesis. In contrast, reflectance increases in the near-infrared and red-edge regions, above 700 nm (Taiz *et al.*, 2017). A study mapping corn crops using Google Earth Engine (GEE), with calculations of vegetation indices, classification by the random forest (RF) algorithm, followed by analysis of the confusion matrix, kappa, and validation statistics, found satisfactory results; kappa values of 81.26% and 84.61% were reported among the image systems considered (Maciel Junior *et al.*, 2024).

The visible spectrum is divided into spectral bands with distinct roles in plant physiology. For instance, blue (475 nm) and red (660 nm) wavelengths are critical for photosynthesis, significantly enhancing plant growth and development (Lu *et al.*, 2021; Shi *et al.*, 2019). The absorption of blue light positively regulates the expression of certain photosynthesis-related genes and increases the rate of electron transport, whereas the absorption of red light influences the expression of other genes involved in photosynthesis (Li *et al.*, 2017; Wu *et al.*, 2014).

The spectral behavior of hybrids varies according to their genetic background. In this context, hybrids H3 and H5 exhibited lower reflectance values across the observed range, suggesting greater use of wavelengths for photosynthesis, due to reduced reflectance and increased absorption. Notably, within the blue region of the spectral signature at 475 nm, minimal differentiation was observed among the hybrids. In contrast, the 550 nm wavelength, which corresponds to the green region of the spectrum, shows a pronounced peak in reflectance due to the strong reflection of green light by chloroplasts (Nishio, 2000). The H4 hybrid showed the highest reflectance in the 550 nm range compared to the other hybrids. From 735-790 nm onwards, a prominent increase in the reflectance within the VIS/NIR region is observed, attributed to the abundance of chlorophyll and organization of the internal leaf structure (Hennessy *et al.*, 2020). This increase can serve as an indicator of stress, senescence, or disease incidence in vegetation (Atta *et al.*, 2023; Dawson *et al.*, 1998; Gholizadeh *et al.*, 2016; Zahir *et al.*, 2022). Thus, hybrids H1 and H3 exhibited high reflectance at 735 nm and lower reflectance at 475 nm and 660 nm. This result suggests that these hybrids have a high concentration of chlorophyll, as indicated by their increased reflectance at 735 nm. Additionally, the low reflectance in blue and red wavelengths implies intense photosynthetic activity in these hybrids. Based on the detailed physiological information provided by the spectral bands, it is possible to investigate relationships between different spectral bands (Oliveira *et al.*, 2023) through mathematical models known as vegetation indices (VIs) (Pantaleão *et al.*, 2022; Silva *et al.*, 2020). The use of these VIs can provide even more accurate information about plant physiological characteristics, including agronomic responses such as productivity (Santana, Santos *et al.*, 2022), leaf nitrogen concentration, and maize plant height (Osco *et al.*, 2020).

Distinguishing maize hybrids based on their spectral characteristics can reduce the costs of the genotype selection process. However, the large volume of data generated can hinder information processing. Consequently, the use of machine learning algorithms to classify plants or leaves (Osco *et al.*, 2020) is a fast and accurate alternative for genotype differentiation. ML involves training algorithms on specific datasets to recognize patterns, which can then be applied to plant genetic improvement studies (Niazian & Niedbała, 2020).

Among the machine learning (ML) algorithms used, artificial neural networks (ANNs) are among the most well-known and widely employed techniques, particularly for classification tasks (Vidyarthi *et al.*, 2020). A key advantage of neural networks over other ML algorithms is their ability to efficiently model complex nonlinear relationships, without requiring prior assumptions (Qi & Zhang, 2001).

Among the three accuracy metrics evaluated, the ANN algorithm achieved the highest performance in classifying maize hybrids, regardless of the input data used. Consequently, the use of SB can simplify and accelerate procedural steps, as it does not require the complex mathematical model calculations needed for IVs (Gava *et al.*, 2022). The use of only specific wavelengths enhances algorithm accuracy in various agricultural analyses, such as predicting days to maturity, plant height and yield in soybeans (Teodoro *et al.*, 2021). Identifying the most effective machine learning algorithms and input variables improves the performance of models for accurate classification (Oliveira *et al.*, 2025). Our findings indicate that maize hybrids can be effectively distinguished using multispectral data, revealing distinct spectral behaviors and unique spectral signatures among different hybrids. Furthermore, the use of machine learning algorithms can be an important tool for classifying these hybrids. It is important to highlight the novelty of this research in maize cultivation. While similar techniques have been applied to other crops, such as soybeans, there is limited literature addressing genotype classification, both commercial and in the improvement process, in maize cultivation. The use of hyperspectral sensors represents a promising approach for obtaining more comprehensive insights into maize hybrids and is suggested as a potential direction for future research.

Conclusions

The Artificial Neural Networks (ANN) algorithm demonstrated the highest performance among the three accuracy metrics evaluated.

Although all three input configurations improved classification accuracy of the ANN algorithm, the most effective approach was using only the spectral bands, as this strategy reduces data processing time and simplifies the overall procedure.

Despite the promising results, this study has some limitations. First, the analysis was restricted to a specific geographic region and a limited number of maize hybrids, which may affect the generalizability of the model to other environments or crop varieties. Second, the use of only multispectral data (without additional features such as thermal or LiDAR data) might not capture all relevant phenotypic variations among hybrids. Third, the performance of the ANN algorithm, while superior in this study, may vary with larger datasets or more complex crop traits.

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Conflict of interest statement

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

Author's contributions

JLGO: conceptualization, methodology, validation, formal analysis, research, writing - original draft; DCS, JTO: experiment design, data analysis, manuscript writing and editing; ICO: validation, writing - review and editing; RG: experiment design, data analysis, manuscript writing and editing; FHRB: validation, resources, writing - review and editing; CASJ, LPRT, PET: validation, writing - review and editing. All authors approved the final version of the manuscript.

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SUPPLEMENTARY TABLE S1. List of 41 vegetation indices.

Acronym	Vegetation index (VI)	Equation
ARVI2	Atmospheric resilient vegetation index 2	$-0.18+1.17*[\frac{(\mathrm{R_{nir}}-\mathrm{R_{red}})}{(\mathrm{R_{nir}}+\mathrm{R_{red}})}]$
ATSAVI	Transformed soil-adjusted vegetation index	$1.22*[\frac{(\mathrm{R_{nir}}-1.22*\mathrm{R_{red}}-0.03)}{(1.22*\mathrm{R_{nir}}+\mathrm{R_{red}}-1.22*0.03+0.0891+1.22^2)}]$
CCCI	Canopy chlorophyll content index	$\frac{(\mathrm{R_{nir}}-\mathrm{R_{rededge}})/(\mathrm{R_{nir}}+\mathrm{R_{rededge}})}{(\mathrm{R_{nir}}-\mathrm{R_{red}})/(\mathrm{R_{nir}}+\mathrm{R_{red}})}$
CIgreen	Green chlorophyll index	$\frac{\mathrm{NIR}}{\mathrm{green}}-1$
CTVI	Correct vegetation index transformed	$\frac{\mathrm{NDVI}+0.5}{\mathrm{NDVI}+0.5}*\sqrt{\mathrm{NDVI}+0.5}$
CVI	Chlorophyll vegetation index	$\mathrm{NIR}\frac{\mathrm{red}}{\mathrm{green}^2}$
DVI	Differentiated vegetation index	$\frac{\mathrm{R_{nir}}}{\mathrm{R_{red}}}$
EVEI2	Enhanced vegetation index 2	$\frac{2.5*(\mathrm{R_{nir}}-\mathrm{R_{red}})}{(\mathrm{R_{nir}}+2.4*\mathrm{R_{red}}+1)}$
EVI	Improved vegetation index	$2.5*\frac{(\mathrm{R_{NIR}}-\mathrm{R_{RED}})}{((\mathrm{R_{NIR}})+(\mathrm{C1}*\mathrm{R_{NIR}})-(\mathrm{C2}*\mathrm{R_{BLUE}})+\mathrm{L})}$
GDVI	NIR difference/vegetation index by green difference	$\mathrm{R_{nir}}-\mathrm{R_{green}}$
GEMI	Global environmental monitoring index	$\frac{2*(1-0.25*2)-((\mathrm{R_{red}}-0.125)}{(1-\mathrm{R_{red}})}$
GLI	Green leaf index	$\frac{(2*\mathrm{R_{green}}-\mathrm{R_{red}}-\mathrm{R_{blue}})}{(2*\mathrm{R_{green}}+\mathrm{R_{red}}+\mathrm{R_{blue}})}$
GNDVI	Green normalized vegetation index	$\frac{(\mathrm{R_{nir}}-\mathrm{R_{green}})}{(\mathrm{R_{nir}}+\mathrm{R_{green}})}$
GSAVI	Green soil adjusted vegetation index	$\frac{(1+\mathrm{L})*(\mathrm{R_{nir}}-\mathrm{R_{green}})}{(\mathrm{R_{nir}}+\mathrm{R_{green}}+\mathrm{L})}$
GTVI	Green triangle vegetation index	$\frac{\mathrm{NDVI}+0.5}{\mathrm{NDVI}+0.5}*[(\sqrt{\mathrm{NDVI}}+0.5)]$
IAF	Leaf area index	$\frac{\ln(\frac{0.69-\mathrm{SAVI}}{0.59})}{0.91}$
IPVI	Infrared vegetation percentage index	$\mathrm{R_{nir}}/\left(\mathrm{R_{nir}}+\frac{\mathrm{R_{red)}}}{2}\right)*(\mathrm{NDVI}+1)$
LnRE	Red rim vegetation index	$(\ln\mathrm{R_{NIR}}-\mathrm{ln_{REDGE}})*100$
LogR	Log r	$\log\frac{\mathrm{R_{nir}}}{\mathrm{R_{red}}}$
MCARI	Modified chlorophyll absorption reflectance index	$\mathrm{R_{700}}-\mathrm{R_{670}}-0.2(\mathrm{R_{700}}-\mathrm{R_{(550)}})\frac{\mathrm{R_{700}}}{\mathrm{R_{670}}}$

To be continue

Acronym	Vegetation index (VI)	Equation
MSAVI	Modified soil-adjusted vegetation index	$\frac{2\mathrm{nir} + 1 - \sqrt{(2\mathrm{nir} + 1)^2 - (8\mathrm{nir} - \mathrm{red})}}{2}$
MSR	Modified soil proportion	$\frac{2 * R_{nir} + 1 - \sqrt{(2 * R_{nir} + 1)^2 - 8 * (R_{NIR} - R_{red})}}{2}$
MSRNir _ Red	Modified simple rate nir/red	$(\frac{R_{nir}}{R_{red}} - 1)/\sqrt{\frac{R_{nir}}{R_{red}} + 1}$
MTVI	Modified triangular vegetation index	$\frac{1.5\ [1.2(\mathrm{nir} - \mathrm{green}) - 2.5(\mathrm{red} - \mathrm{green})]}{\sqrt{(2\mathrm{nir} + 1)^2 - (6\mathrm{nir} - 5\sqrt{\mathrm{red}})} - 0.5}$
NDRE	Rededge normalized difference vegetation index	$\frac{(R_{NIR} - R_{EDGE})}{(R_{NIR} + R_{EDGE})}$
NDVI	Normalized difference vegetation index	$\frac{(R_{NIR} - R_{RED})}{(R_{NIR} + R_{RED})}$
NGRDI	Normalized green-red difference index	$\frac{R_{green} - R_{red}}{R_{green}}/(R_{nir} + R_{red} + R_{green})$
NIR/G	Simple ratio nir and green	$\frac{R_{nir}}{R_{green}}$
NIR/R	Simple ratio nir and red	$\frac{R_{nir}}{R_{red}}$
NIR/RE	Simple ratio nir and rededge	$\frac{R_{nir}}{R_{re}}$
Norm _ R _ 1	R normal	$\frac{R_{red}}{(R_{nir} + R_{red} + R_{green})}$
OSAVI	Optimized soil-adjusted vegetation index	$\frac{(1 + 0.16) * (R_{nir} - R_{red})}{(R_{nir} + R_{red} + 0.16)}$
PNDVI		$\frac{R_{nir} - (R_{green} + R_{red} + R_{blue})}{R_{nir} + (R_{green} + R_{red} + R_{blue})}$
RDVI	Renormalized difference vegetation index	$\frac{(R_{nir} - R_{red})}{\sqrt{R_{nir} + R_{red}}}$
SAVI	Soil-adjusted vegetation index	$(1 + 0.5)\frac{\mathrm{nir} - \mathrm{red}}{\mathrm{nir} + \mathrm{red} + 0.5}$
SQRT _ IR _ R		$\sqrt{\frac{R_{nir}}{R_{red}}}$
TVI	Transformed vegetation index	$\sqrt{\frac{R_{nir} - R_{red}}{R_{nir} + R_{red}}}$
VARI		$\frac{R_{green} - R_{red}}{R_{green} + R_{red} - R_{bllue}}$
WDRVI	Wide dynamic range vegetation index	$\frac{(0.1R_{nir} - R_{red})}{(0.1 * R_{nir} + R_{red})}$

R_{NIR}: reflectance in the near infrared range; R_{GREEN}: reflectance in the green range; R_{RED}: reflectance in the red range; Rre: reflectance in the red transition range (Red-edge); L: ground effect correction factor.

Comparing spectral models to predict manganese content in *Rosa* spp. leaves using VIS-NIR data

Comparación de modelos espectrales para predecir el contenido de manganeso en hojas de *Rosa* spp. usando datos VIS-NIR

Oscar Hernán Franco Montoya[1*] **and Luis Joel Martínez Martínez**[1]

ABSTRACT

This study, conducted on the Freedom rose cultivar grown under greenhouse conditions in the municipality of Tocancipá, Cundinamarca (Colombia), implemented Partial Least Squares Regression (PLSR) and Principal Component Regression (PCR) methods using visible and near-infrared (VIS-NIR) spectroradiometry from 350 to 2500 nm to predict manganese (Mn) content in rose leaves. A randomized complete block design (RCBD) with manganese doses of 0%, 25%, 50%, 75%, and 100% of the reference dose of 2 mg L^{-1} was established in 25 plots with five treatments and five replicates. Samplings were conducted in the five phenological stages of "palmiche", "rice", "chickpea", "scratch color", and "straight sepals", analyzing 10 plants per treatment, and spectral responses were measured on the adaxial leaf surface using the FieldSpec® 4 spectroradiometer. For model generation (PLSR and PCR), 24 predictive models were evaluated, comprising three spectral response ranges: range 1 (350-1000 nm), range 2 (350-1800 nm), and range 3 (350-2500 nm), applying different spectral correction methods: raw data (RD), Savitzky-Golay (SG), range normalization (RN), and Savitzky-Golay followed by range normalization (SG-RN). A total of 100 samples were used: 80 for calibration and 20 for external validation, randomly selected to represent the variability of the treatments. The spectral corrections improved the accuracy and robustness of the predictions, with the RN-PLSR and SG-RN-PLSR models showing the best performance metrics (R^2, RMSE, and RPD). The most relevant wavelengths were 523 nm, 557 nm, and around 720 nm, with correlations greater than 0.6 with the Mn concentration in leaves.

Key words: PLSR, PCR, crop nutrition, spectral reflectance, spectral smoothing, predictive models, multivariate analysis, spectroradiometer.

RESUMEN

Este estudio, realizado en cultivo de rosa variedad Freedom sembrada bajo invernadero en el municipio de Tocancipá, Cundinamarca (Colombia), implementó métodos de Regresión por Mínimos Cuadrados Parciales (RMCP) y Regresión con Componentes Principales (RCP) utilizando espectroradiometría visible e infrarroja cercana (VIS-NIR) de 350 a 2500 nm para predecir el contenido de manganeso (Mn) en hojas de rosa. Se estableció un diseño de bloques completos al azar (BCA) con dosis de manganeso de 0%, 25%, 50%, 75% y 100% (referencia de 2 mg L^{-1}) en 25 parcelas con cinco tratamientos y cinco repeticiones. Se realizaron muestreos en los cinco estados fenológicos "palmiche", "arroz", "garbanzo", "rayando color" y "sépalos rectos", analizando 10 plantas por tratamiento; las respuestas espectrales se midieron en la superficie adaxial de las hojas utilizando el espectroradiómetro FieldSpec® 4. Para la generación de los modelos (RMCP y RCP), se evaluaron 24 modelos predictivos conformados por tres rangos de respuesta espectral: rango 1 (350-1000 nm), rango 2 (350-1800 nm) y rango 3 (350-2500 nm), aplicando diferentes métodos de corrección de espectro: datos crudos (RD), Savitzky-Golay (SG), normalización por rangos (NR) y Savitzky-Golay seguido de normalización por rangos (SG-NR); se usaron 100 muestras en total: 80 para calibración y 20 para validación externa, seleccionadas aleatoriamente para representar la variabilidad de los tratamientos. Las correcciones del espectro mejoraron la precisión y solidez de la predicción, siendo los modelos NR-PLSR y SG-NR-PLSR los que presentaron las mejores valoraciones en las métricas (R^2, RMSE y RDP), mientras que las longitudes de onda más relevantes fueron 523 nm, 557 nm y cerca de 720 nm, con correlaciones superiores a 0,6 con la concentración de Mn en hojas.

Palabras clave: RMCP, RCP, nutrición de cultivos, reflectancia espectral, suavizado espectral, modelos predictivos, análisis multivariado, espectroradiómetro.

Introduction

The rose (*Rosa* spp.) is one of the most economically important cut flowers worldwide and holds a particularly prominent place in Colombia's agricultural exports. Introduced in the country during the 1960s through a partnership between foreign investors and local entrepreneurs, rose cultivation rapidly expanded, finding ideal agroecological

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[1] Universidad Nacional de Colombia, Facultad de Ciencias Agrarias, Bogotá (Colombia).

[*] Corresponding author: ohfrancom@unal.edu.co

conditions in the departments of Cundinamarca and Antioquia (Arbeláez, 1993). Today, Colombia is the leading exporter of flowers in Latin America and the second largest globally, surpassed only by the Netherlands. Roses account for nearly 48% of the total area dedicated to flower production, making them the primary species cultivated. This industry generates over 120,000 direct jobs and approximately 99,000 indirect jobs, with significant participation from women (ICA, 2024). The continued growth of rose exports and increasing international market demands (related to stem uniformity, bud size, color intensity, and harvest stage consistency) highlight the need for precise crop management and the implementation of advanced technological tools for quality optimization (Ruppenthal & Conte, 2005).

Manganese (Mn) is an essential nutrient for the growth and development of rose crops, participating in key physiological processes such as photosynthesis. Mn is a cofactor in at least 35 metabolic processes, playing a crucial role in chlorophyll formation, oxygen evolution in photosystem II, and redox reactions within the electron transport chain during photosynthesis (Rashed *et al.*, 2019). It also acts as an activator of enzymes involved in respiration, amino acid synthesis, and hormonal regulation (Santos *et al.*, 2017).

However, both manganese deficiency and excess can have negative effects on plants. Mn deficiency in plants interferes with photosynthesis, affecting photolysis in photosystem II and damaging chloroplasts, which impedes proper electron production for the process. On the other hand, excess manganese can be toxic, and tolerance to its toxicity varies depending on the species and genotype of the plant (Humphries *et al.*, 2006; Santos *et al.*, 2017). In rose plants, manganese is present in low concentrations at the foliar level and is a relatively immobile nutrient, meaning it does not easily relocate from older leaves, leading to a deficiency in expanding young leaves (Humphries *et al.*, 2006). Therefore, deficiency symptoms are not evident in older leaves or in young leaves that have not yet fully expanded. This may indicate a higher demand in these developing leaves, or it may simply be a matter of source-sink dynamics favoring the faster-growing tissues (Humphries *et al.*, 2006).

The diagnosis of nutritional status in rose cultivation is essential for making informed decisions about the application of exogenous fertilizers, which can be mainly administered through the fertigation system or, to a lesser extent, by foliar application. Fertilizer formulations must be applied at the right time, considering the interactions between soil/substrate, plants, and water, as these factors directly affect the quality and productivity of the crop, which are fundamental for its sustainability. An adequate supply of micronutrients, such as manganese, can significantly increase crop productivity, making it essential to maintain proper nutritional balance through timely diagnostics (Hariyadi *et al.*, 2019). In flower crops, traditional nutritional analysis requires destructive methods, which are slow and resource-intensive, causing delays in diagnosis. For this reason, there has been an increased interest in developing faster, non-destructive alternative methods, such as the use of sensors. In this context, reflectance measurement has emerged as a promising alternative for characterizing the nutritional status of plants, allowing for a quicker and more efficient estimation of key parameters at the foliar level. This approach presents a valuable tool for improving nutritional diagnostics in rose cultivation.

Numerous studies have linked the chemical composition of plants with the absorption of electromagnetic radiation. This relationship has evolved from laboratory near-infrared spectroscopy (NIRS) research to remote sensing, where it has been shown that biochemical concentrations of elements such as nitrogen in foliar tissue are closely related to radiation through spectral absorption features, both in dry and fresh leaves. However, fresh leaves are influenced by water content, which alters their absorption characteristics in the near-infrared region of the spectrum (1000–2500 nm) (Huber *et al.*, 2008). The reflectance of leaves exhibits specific characteristics: in general, pigments present in the leaves have a significant influence on reflectance in the visible region, while cellular structure manifests in near-infrared reflectance. Additionally, proteins and water content primarily respond in the near-infrared to mid-infrared regions (Liang, 2005).

In recent years, significant progress has been made in developing spectral-based models for predicting micronutrient concentrations in plants. For example, Yu *et al.* (2019) demonstrated the high potential of near-infrared spectroscopy (NIRS) combined with chemometric techniques to predict manganese content in cottonseed flour. The study applied spectral corrections such as standard normal variate (SNV) and first derivative (FD), along with advanced variable selection methods like Monte Carlo uninformative variable elimination (MCUVE) and successive projections algorithm (SPA). The resulting models yielded high prediction accuracy, with RMSEP = 1.99, $R^2 = 0.95$, and RPD = 4.37, confirming the effectiveness of optimized spectral preprocessing and variable selection in enhancing model robustness.

Similarly, Boshkovski *et al.* (2020) assessed nutrient dynamics in common bean plants subjected to abiotic stress using spectral reflectance and multivariate regression. Their findings revealed significant correlations between reflectance and several nutrients, including manganese, boron, iron, phosphorus, and zinc, as well as vegetation indices like NDVI. Custom spectral indices developed in the study exhibited strong predictive power for a wide range of macro- and micronutrients. Additionally, Santoso *et al.* (2019) evaluated nutrient content in oil palm leaves using reflectance data and built predictive models through stepwise regression and principal component regression (PCR). The models achieved moderately high R^2 values, ranging from 0.33 to 0.53, particularly for nitrogen and calcium, demonstrating the potential of VIS-NIR data in estimating leaf nutrient status in tropical crops.

Other researchers used hyperspectral spectroscopy to develop spectral indices and multivariate models, combined with machine learning, to predict foliar nutrients in mango. The results showed that conventional models such as partial least squares regression (PLSR) and principal component regression (PCR), were ineffective in predicting the nutrients. However, models combining PLSR with machine learning techniques, such as the Cubist model, support vector regression (SVR), and elastic net, showed good performance, especially in predicting nitrogen, phosphorus, potassium, zinc, magnesium, and sulfur. The results suggest that hyperspectral remote sensing has great potential for non-destructive estimation of foliar nutrients in mango, contributing to more precise nutrient management (Mahajan *et al.*, 2021).

In a related study on citrus, a calibration model based on spectroscopy was developed to predict the foliar concentrations of macro and micronutrients in *Citrus clementina* plantations using fast, non-destructive spectral measurements. The results showed that spectroscopy, using a portable spectrometer to measure spectral absorbance (430–1040 nm), effectively estimated nutrient levels, with determination coefficients ranging from 0.31 to 0.69, with the highest values observed for phosphorus (P), potassium (K), and boron (B). Similarly, the technique showed high potential for rapid and non-destructive prediction of foliar nutrients, reinforcing the idea that hyperspectral remote sensing is a promising tool for nutrient management in crops (Acosta *et al.*, 2023).

Similarly, in oil palm plantations, spectral reflectance was used to predict key nutrients such as nitrogen, phosphorus, potassium, calcium, magnesium, boron, copper, and zinc. In the study, the proposed vegetation indices performed better than conventional indices, and multivariate models based on principal component regression (PCR) were more effective when significant variables selected through stepwise regression were used, with wavelengths in the green region contributing the most to the model (Santoso *et al.*, 2019). Together, these studies demonstrate the great potential of spectral remote sensing to predict foliar nutrients in various crops, facilitating more precise nutrient management in agriculture. In this context, the present research evaluated the potential of reflectance data, obtained in the range of 350 nm to 2500 nm using a spectroradiometer, in rose crops planted under greenhouse conditions. The aim was to develop predictive models based on spectral responses to estimate the foliar manganese content in rose crops, using partial least squares regression (PLSR) and principal component regression (PCR) analysis.

In this context, the nutritional management of rose crops not only directly impacts flower quality but also the country's competitiveness in international markets. However, traditional methods for nutritional diagnosis are destructive, time-consuming, and costly, which delays timely decision-making in the field. In response to this challenge, rapid and non-destructive tools such as visible and near-infrared (VIS-NIR) spectroradiometry have gained relevance, as they allow for the characterization of plant nutritional status through spectral response. Although this technology has shown promising results in other crops, its application in roses remains limited. Therefore, this study aims to contribute to the knowledge of spectral multivariate modeling for predicting foliar manganese content in *Rosa* spp., supporting the development of more efficient monitoring systems adapted to tropical production conditions and with potential for implementation in the Colombian floriculture industry.

Materials and methods

Experimental design

The study was conducted in a greenhouse located in Tocancipá, Cundinamarca (Colombia), at 2,605 m a.s.l. (4°58'40.1" N, 73°59'06.6" W), using *Rosa* spp. (Freedom cultivar). The experimental area comprised 176 m², divided into 25 plots of 1.35 m² (0.3 m × 4.5 m), each containing 60 plants. A randomized complete block design (RCBD) was implemented to minimize environmental variability within the greenhouse. The experimental unit consisted of one plot (60 plants); five manganese (Mn) treatments were applied at concentrations of 0%, 25%, 50%, 75%, or 100%

of the reference dose 2 mg L^{-1} Mn-EDTA, each replicated five times across blocks (hydroponic banks). The response variable was foliar Mn concentration, and the explanatory variables were spectral reflectance values collected at different phenological stages.

Nutrient solutions were supplied through a drip fertigation system, maintaining a pH between 5.3 and 5.8 and electrical conductivity between 1.5 and 1.8 dS m^{-1}. The other macro- and micronutrients were kept constant in the nutrient solution across treatments (Tab. 1).

All treatments received the same concentrations of macronutrients and micronutrients, except for manganese (Mn), which was applied in increasing doses. Nutrient solutions were prepared to ensure consistency across treatments, minimizing confounding effects in the evaluation of Mn-specific responses.

Plant material and sample processing

The samplings were conducted across five key phenological stages: “palmiche”, “rice”, “chickpea”, “scratch color”, and “straight sepals” (Franco Montoya & Martínez Martínez, 2024). For each stage, 10 fully developed leaves were sampled from each plot. The same leaves were used for both spectral measurements and chemical analysis to ensure consistency.

Samples were stored in a cooler and transported to the laboratory within 5 h after collection. Leaves were kept refrigerated (4°C) until processing. Manganese content was determined using microwave-assisted digestion (HNO_3 + H_2O_2), followed by analysis with inductively coupled plasma optical emission spectroscopy (ICP-OES). The standard error of the laboratory method for Mn determination was 0.09 mg kg^{-1} (Ghosh *et al*., 2013).

Spectral measurement

Spectral data were collected using a FieldSpec® 4 spectroradiometer (Malvern Panalytical, UK), which covers a wavelength range from 350 to 2,500 nm. The device has a wavelength accuracy of 0.5 nm, a spectral resolution of <3.0 nm at 700 nm, and a noise-equivalent radiance of 5 × 10^{-10} W/cm^2/nm/sr at 700 nm. It records data across 2,151 spectral channels and weighs approximately 5.44 kg (12 lbs).

Measurements were taken on the adaxial surface of the fourth and fifth fully developed leaves using a leaf clip accessory equipped with an internal halogen light source and an integrated Spectralon® white reference. For each leaf, five reflectance scans were taken and averaged. All measurements were conducted under controlled lighting conditions at the Geomatics Laboratory of the Universidad Nacional de Colombia.

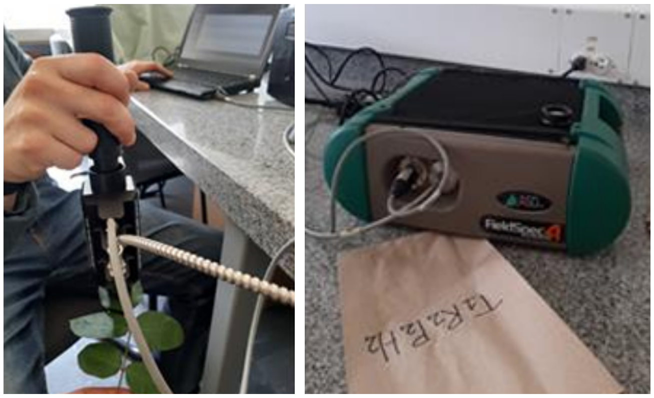

FIGURE 1. Capturing spectral responses: A) Leaf clip accessory, B) FieldSpec 4® spectroradiometer.

Spectral preprocessing and modeling

A total of 100 samples were used to build prediction models: 80 for calibration and 20 for external validation, selected randomly to represent treatment variability. Three spectral ranges were analyzed: 350–1000 nm, 350–1800 nm, and 350–2500 nm. Four preprocessing techniques were applied:

- Raw data (RD);
- Savitzky-Golay smoothing (SG) with a window of 15 points and a second-order polynomial;
- Range normalization (RN);
- SG followed by RN (SG-RN).

TABLE 1. Nutrient composition of the fertigation solution for each manganese (Mn) treatment.

Treatments	(Mn supply, % of the reference dose)	N	P	K	Ca	Mg	S	Mn	Zn	Cu	Fe	B	Mo
		mg L^{-1}											
T1	0%	160	10	180	100	40	14	0	0.7	1.2	2	0.2	0.1
T2	25%	160	10	180	100	40	14	0.5	0.7	1.2	2	0.2	0.1
T3	50%	160	10	180	100	40	14	1	0.7	1.2	2	0.2	0.1
T4	75%	160	10	180	100	40	14	1.5	0.7	1.2	2	0.2	0.1
T5	100%	160	10	180	100	40	14	2	0.7	1.2	2	0.2	0.1

Predictive models were developed using Partial Least Squares Regression (PLSR) and Principal Component Regression (PCR). All analyses were conducted in R software (version 10.0.19045.5487), employing a set of specialized packages for data preprocessing, modeling, and visualization. The following R packages were used: pls, caret, signal and pracma, tidyverse, including dplyr, tidyr, and ggplot2, and lattice.

Model performance was assessed based on the coefficient of determination (R^2), root mean square error of prediction (RMSEP), and residual predictive deviation (RPD).

Results and discussion

Figure 2 shows that the foliar Mn concentration in rose plants varied according to the fertigation applications, indicating that the leaves respond directly to the amount of Mn supplied. In the first sampling, the treatments showed similar Mn concentrations, except for the 0% reference dose treatment T1, which had the lowest value. From the second sampling onward, differences in concentrations were observed, suggesting that Mn accumulation in the foliar tissue depends on its availability in the substrate and the plant response to fertilization applications. In summary, rose leaves accumulate Mn according to the supplied concentration, reflecting a direct response to the fertilization levels.

Table 2 presents the results obtained at different phenological stages, highlighting the best models according to the evaluated prediction metrics. In the palmiche phenological stage, the most accurate model was obtained using the spectral responses from the 350 nm to 1000 nm range, applying Savitzky-Golay smoothing with 15 points and range normalization. This model achieved an RPD of 1.24, an R^2 of 0.36, and an RMSE of 14.37. In the rice phenological stage, the best model was generated using the PLSR technique with raw values in the 350 nm to 2500 nm range, yielding the best results without the need for spectral correction. In the chickpea phenological stage, the standout model was the RN-PLSR in the 350 nm to 1000 nm range, with an RPD of 2.01. This model presented an R^2 of 0.67 and an RMSE of 10.70, with good performance for the evaluated metrics. In the scratch color phenological stage, the best model was obtained using the 350 nm to 1800 nm range, applying the Savitzky-Golay smoothing algorithm and range normalization with the PLSR technique (SG-RN-PLSR). This model showed metric values with an RPD of 1.73, an R^2 of 0.66, and an RMSE of 19.33. Finally, the straight sepals phenological stage showed the best results in terms of RPD, with a model based on the 350 nm to 2500 nm range with the RD-PLSR. This model achieved an RPD of 3.05, an R^2 of 0.88, and an RMSE of 14.66, standing out for its high performance compared to other phenological stages.

In summary, the phenological stages of straight sepals and chickpea stood out for presenting the best values in the evaluated metrics, especially RPD, suggesting that the methodology used at these phenological stages is particularly effective. The straight sepals stage, with an RPD of 3.05, showed the best overall performance, making it recommended for future research and spectral modeling applications, as it provides more accurate and consistent results. The chickpea phenological stage also showed high

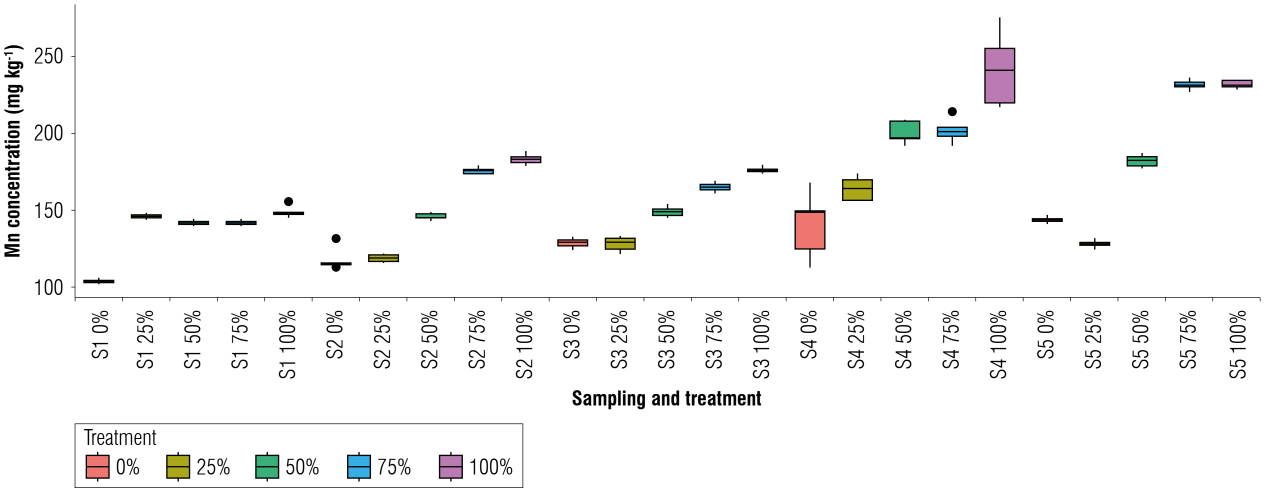

FIGURE 2. Foliar Mn concentration (mg kg^{-1}) in rose plants at five phenological stages: S1, palmiche; S2, rice; S3, chickpea; S4, scratch color; S5, straight sepals. Treatments – Mn supply, % of the reference dose.

TABLE 2. Results of the evaluated metrics for the predictive models.

Palmiche						Rice						Chickpea					
Model	**Range(nm)**	**F**	**R^2**	**RMSE**	**RPD**	**Model**	**Range(nm)**	**F**	**R^2**	**RMSE**	**RPD**	**Model**	**Range(nm)**	**F**	**R^2**	**RMSE**	**RPD**
SG-RN-PLSR	350-1000	7	0.36	14.37	1.24	RD-PLSR	350-2500	4	0.63	16.79	1.66	RN-PLSR	350-1000	6	0.67	10.70	2.01
RD-PLSR	350-1800	4	0.36	14.23	1.24	SG-PLSR	350-2500	3	0.62	16.91	1.64	SG-RN-PLSR	350-1000	6	0.66	10.94	1.98
SG-PLSR	350-1800	4	0.33	14.25	1.23	RD-PCR	350-2500	4	0.59	17.54	1.58	RN-PLSR	350-1800	7	0.73	9.75	1.96
SG-RN-PLSR	350-1800	3	0.29	14.75	1.20	SG-PCR	350-2500	4	0.59	17.54	1.58	SG-PLSR	350-1000	6	0.63	11.33	1.91
SG-RN-PCR	350-1800	4	0.27	14.95	1.20	SG-RN-PLSR	350-1000	7	0.26	23.68	1.32	RD-PLSR	350-1000	6	0.63	11.42	1.90
RN-PLSR	350-1800	3	0.28	14.78	1.20	RN-PCR	350-2500	4	0.42	21.01	1.31	RD-PCR	350-1800	6	0.72	10.09	1.87
RD-PCR	350-1800	7	0.28	15.22	1.16	SG-RN-PCR	350-2500	4	0.42	21.02	1.31	SG-PLSR	350-1800	7	0.75	9.43	1.87
RN-PCR	350-1800	5	0.23	15.28	1.15	RN-PLSR	350-2500	3	0.41	21.06	1.31	SG-PCR	350-1800	6	0.71	10.11	1.87
RN-PCR	350-2500	4	0.10	15.84	1.06	SG-RN-PLSR	350-2500	3	0.41	21.09	1.31	SG-RN-PLSR	350-1800	7	0.73	9.75	1.84
SG-RN-PCR	350-2500	4	0.10	15.84	1.06	RD-PLSR	350-1000	6	0.30	23.08	1.27	SG-RN-PCR	350-1800	6	0.70	10.30	1.84
RN-PLSR	350-1000	4	0.09	16.84	1.05	RN-PLSR	350-2500	4	0.36	22.07	1.25	RN-PCR	350-1800	6	0.70	10.39	1.83
SG-PCR	350-1800	1	0.08	16.78	1.04	SG-RN-PLSR	350-2500	4	0.36	22.07	1.25	RD-PLSR	350-2500	7	0.63	11.92	1.66
RD-PLSR	350-1000	4	0.04	17.67	0.99	RN-PLSR	350-1000	6	0.17	25.08	1.22	RD-PLSR	350-1800	7	0.75	9.47	1.66
SG-PLSR	350-1000	4	0.04	17.68	0.99	RD-PLSR	350-2500	3	0.32	22.69	1.21	RN-PLSR	350-2500	7	0.61	12.38	1.60
RD-PCR	350-2500	1	0.00	16.83	0.99	SG-PLSR	350-2500	4	0.32	22.70	1.21	SG-RN-PLSR	350-2500	5	0.57	12.87	1.53
SG-PCR	350-2500	1	0.00	16.83	0.99	SG-RN-PCR	350-2500	5	0.31	22.78	1.21	SG-PLSR	350-2500	5	0.51	13.80	1.44
RN-PCR	350-1000	5	0.02	17.77	0.99	RD-PCR	350-2500	5	0.23	24.11	1.14	RD-PCR	350-1000	3	0.44	13.95	1.40
SG-RN-PCR	350-1000	5	0.01	17.81	0.99	SG-PCR	350-2500	5	0.37	24.11	1.14	SG-PCR	350-1000	3	0.44	13.95	1.40
SG-PCR	350-1000	4	0.02	18.05	0.97	SG-RN-PCR	350-1000	6	0.11	26.00	1.13	SG-RN-PCR	350-1000	2	0.40	14.45	1.34
RD-PCR	350-1000	4	0.02	18.06	0.97	SG-PLSR	350-1000	2	0.15	25.37	1.13	RN-PCR	350-1000	2	0.40	14.47	1.33
SG-RN-PLSR	350-2500	6	0.14	17.80	0.94	RN-PCR	350-1000	6	0.10	26.11	1.12	RD-PCR	350-2500	5	0.23	17.30	1.14
RN-PLSR	350-2500	6	0.14	17.87	0.94	SG-PCR	350-1000	1	0.11	26.07	1.10	SG-PCR	350-2500	5	0.23	17.30	1.14
SG-PLSR	350-2500	7	0.12	18.31	0.91	RD-PCR	350-1000	1	0.11	26.07	1.10	RN-PCR	350-2500	4	0.13	18.39	1.08
RD-PLSR	350-2500	7	0.12	18.37	0.91	RN-PCR	350-2500	1	0.16	25.16	1.09	SG-RN-PCR	350-2500	4	0.11	18.58	1.07

Scratch color						Straight sepals					
Model	**Range(nm)**	**F**	**R^2**	**RMSE**	**RPD**	**Model**	**Range(nm)**	**F**	**R^2**	**RMSE**	**RPD**
SG-RN-PLSR	350-1800	7	0.66	19.33	1.73	RD-PLSR	350-2500	6	0.88	14.661	3.05
RN-PLSR	350-1800	7	0.65	19.54	1.69	RN-PLSR	350-2500	5	0.84	17.213	2.54
RN-PLSR	350-1000	6	0.48	27.60	1.41	SG-PLSR	350-2500	5	0.84	17.349	2.53
SG-RN-PLSR	350-1000	6	0.46	27.93	1.39	SG-RN-PLSR	350-2500	5	0.83	17.806	2.47
SG-RN-PLSR	350-2500	7	0.37	29.03	1.27	RN-PCR	350-2500	2	0.65	25.499	1.69
RN-PLSR	350-2500	7	0.36	29.18	1.26	SG-RN-PCR	350-2500	2	0.65	25.584	1.68
RD-PLSR	350-1800	2	0.30	27.47	1.21	RD-PCR	350-2500	3	0.62	26.48	1.66
SG-PLSR	350-1800	2	0.30	27.47	1.21	SG-PCR	350-2500	3	0.62	26.481	1.66
RD-PCR	350-1800	2	0.29	27.74	1.20	RN-PCR	350-1800	2	0.53	30.493	1.46
SG-PCR	350-1800	2	0.29	27.74	1.20	SG-PLSR	350-1800	3	0.35	36.058	1.32
RD-PLSR	350-1000	5	0.30	31.97	1.19	RD-PLSR	350-1800	3	0.35	36.058	1.32
SG-PLSR	350-1000	5	0.30	31.97	1.19	RD-PCR	350-1800	3	0.34	36.303	1.31
RD-PCR	350-1000	6	0.24	33.18	1.15	SG-PCR	350-1800	3	0.34	36.304	1.31
SG-PCR	350-1000	6	0.24	33.20	1.15	RN-PLSR	350-1800	2	0.29	37.618	1.25
RN-PCR	350-2500	2	0.22	32.31	1.13	SG-RN-PLSR	350-1800	2	0.28	37.673	1.24
SG-RN-PCR	350-2500	2	0.21	32.33	1.13	SG-RN-PCR	350-1800	2	0.28	37.796	1.24
RN-PCR	350-1000	1	0.18	34.49	1.12	RD-PCR	350-1000	4	0.31	36.815	1.21
SG-RN-PCR	350-1000	1	0.18	34.53	1.11	SG-PCR	350-1000	4	0.31	36.822	1.21
RN-PCR	350-1800	2	0.16	30.15	1.09	RD-PLSR	350-1000	3	0.29	37.434	1.19
SG-RN-PCR	350-1800	2	0.15	30.40	1.08	SG-PLSR	350-1000	3	0.29	37.435	1.19
RD-PLSR	350-2500	2	0.04	35.79	1.02	RN-PCR	350-1000	4	0.22	39.253	1.16
SG-PLSR	350-2500	2	0.04	35.80	1.02	SG-RN-PCR	350-1000	4	0.22	39.261	1.15
RD-PCR	350-2500	2	0.19	36.65	1.00	RN-PLSR	350-1000	2	0.18	40.171	1.15
SG-PCR	350-2500	2	0.19	36.65	1.00	SG-RN-PLSR	350-1000	2	0.18	40.175	1.14

Factors: R^2, coefficient of determination; RMSE, root mean square error; RPD, residual predictive deviation.

performance, especially in models with R^2 values above 0.7 and RPD values close to 2.0, indicating that the methodology is suitable for predicting manganese content in rose plants.

Figure 3 presents the prediction results according to the reference data for the best-performing models in each of the evaluated phenological stages, using 20 validation data points per stage. In the case of the palmiche and rice phenological stages, since the values of their metrics do not show good performance, this methodology is not recommended. In contrast, starting from the chickpea phenological stage, the models show better performance according to their validation metrics. In the chickpea stage, an R^2V of 0.44, RMSEV of 14.68, and RPD of 2.0 were obtained; for the scratch color phenological stage, an R^2V of 0.85, RMSEV of 28.82, and RPD of 1.73 were obtained; and for the straight sepals stage, an R^2V of 0.69, RMSEV of 23.61, and RPD of 3.05 were obtained.

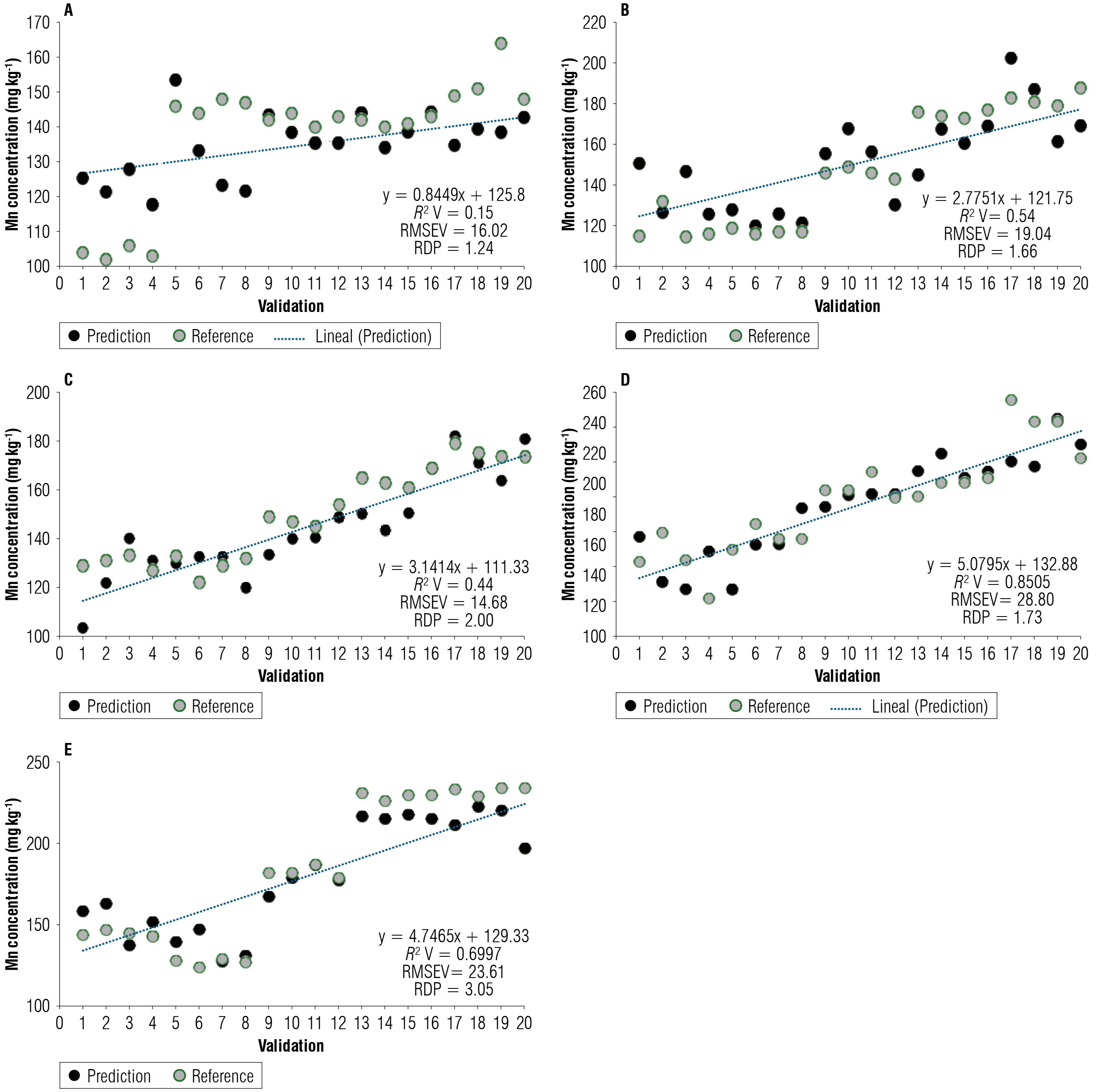

FIGURE 3. Prediction vs reference data in the phenological stages of rose plants: A) palmiche, B) rice, C) chickpea, D) scratch color, and E) straight sepals.

From the results presented, in the palmiche and rice phenological stages, there were no good correlations with the evaluated reflectances, nor good performance of the predictive models. Starting from the chickpea phenological stage, the models showed better performance, with the RN-PLSR model being the best for the chickpea stage, SG-RN-PLSR for the scratch color stage, and Row-PLSR for straight sepals stage.

Figure 4 indicates the spectral regions that had the greatest impact on the predictive models with the best performance for each evaluated phenological stage. For the chickpea phenological stage, where the best-performing model was RN-PLSR (350 nm - 1000 nm), the most relevant regions were 523 nm, 557 nm, 703 nm, and 714 nm. In the scratch color phenological stage, where the best model was SG-RN-PLSR (350 nm - 1800 nm), the key regions were 711 nm, 1341 nm, and 1394 nm. In the straight sepals phenological stage, where the best-performing model was RD-PLSR (350 nm - 2500 nm), the most influential regions were 720 nm, 1420 nm, and 1840 nm. From the chickpea to straight sepals stages, three spectral regions that had the greatest influence on the model performance were around 523 nm, 557 nm and 720 nm.

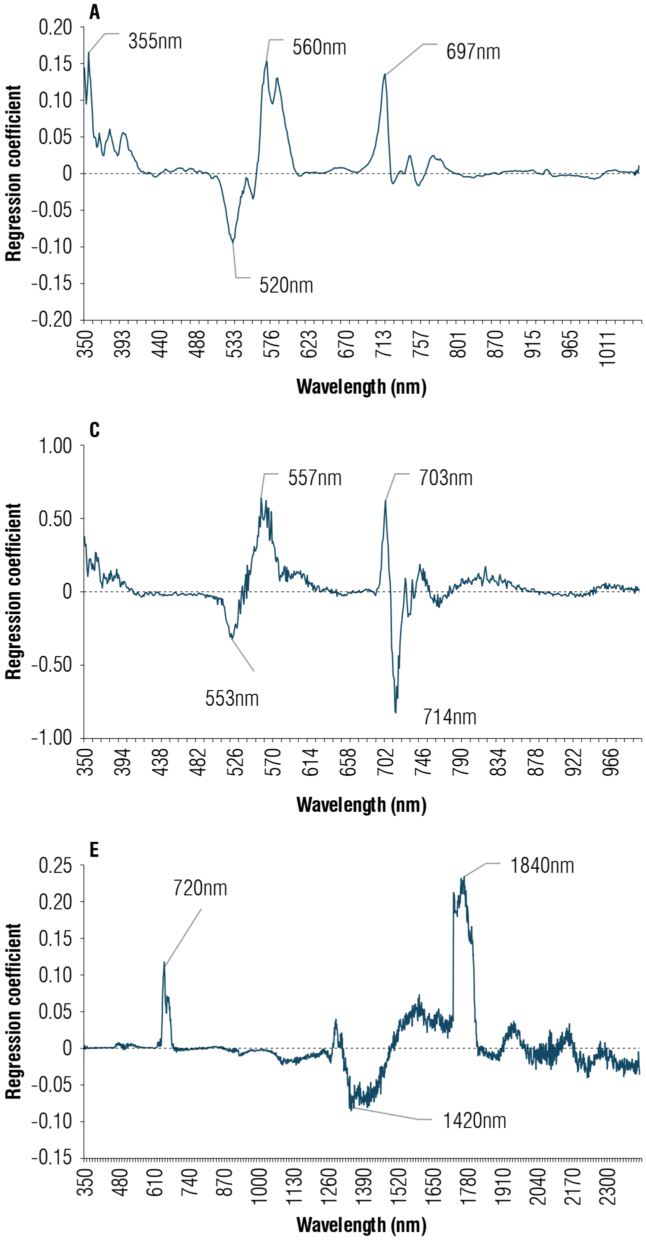

FIGURE 4. Wavelengths with the greatest influence in the predictive models: A) palmiche, B) rice, C) chickpea, D) scratch color, and E) straight sepals.

To determine the confidence of the regressions, an F-test was performed for the selected PLSR models in the phenological stages "chickpea," "scratch color," and "straight sepals". The results are summarized in Table 3. The F-test was conducted with a 95% confidence interval, meaning the significance level is $\alpha = 0.05$. The p-value obtained is less than the significance level α, indicating that the three regression models are statistically significant.

TABLE 3. F-test results.

Phenological stage	Model	DF	F value	*P* value
Chickpea	RN-PLSR	18	67.22	1.73E-04
Scratch color	SG-RN-PLSR	18	39.21	6.62E-03
Straight sepals	RD-PLSR	18	258.00	4.07E-09

From the results presented above, it can be inferred that in the phenological stages of palmiche and rice, no good correlations with the evaluated reflectances were observed, nor did the predictive models perform well according to the metric values. From the phenological stage of chickpea onwards, the models showed better performance, with the RN-PLSR, SG-RN-PLSR, and RD-PLSR models being the most effective. From the phenological stage of rice to straight sepals, three spectral zones were identified, whose reflectances have a greater contribution to the performance of the models: 523 nm, 557 nm, and near 720 nm. These zones are more influential in each sampled period and are key to predicting manganese concentrations in the rose crop.

Figure 5 presents the dual-axis correlations between the predicted values and the actual Mn contents for the selected models in each phenological stage, comparing the regressions using the PLSR and the PCR regression method. The results show that the models that performed better were those using the PLSR method. In the palmiche phenological stage (Fig. 5A), for the same spectral range with the SG-RN techniques, the PLSR model obtained an R^2 of 0.36, while the PCR model reached an R^2 of 0.14. In the rice phenological stage (Fig. 5B), the RD-PLSR model presented an R^2 of 0.63, and the PCR model an R^2 of 0.60. This trend in favor of PLSR was maintained in the subsequent phenological stages.

In chickpea (Fig. 5C), the PLSR model achieved an R^2 of 0.78, while the PCR model obtained an R^2 of 0.46. For the scratch color phenological stage (Fig. 5D), the R^2 value was 0.68 for PLSR, while for PCR it was 0.16. In the straight sepals phenological stage, PLSR presented an R^2 of 0.93, while PCR achieved an R^2 of 0.64. Although in some phenological stages both methods achieved good R^2 values, the PLSR model consistently performed better across all the evaluated phenological stages.

The findings of this study, where PLSR models consistently outperformed PCR in predicting foliar manganese (Mn) concentration in *Rosa* spp., are consistent with previous research across various crops. As reported by Mahajan *et al.* (2021), PLSR yielded better predictions of foliar nutrients in mango compared to PCR and traditional regression approaches. Similar results were observed in oil palm (Santoso *et al.*, 2019) and common bean (Boshkovski *et al.*, 2020), where PLSR demonstrated robustness, especially when applied alongside spectral preprocessing techniques such as Savitzky-Golay (SG) and range normalization (RN). Unlike PCR, which maximizes variance in the predictor variables without considering the response variable, PLSR optimizes the covariance between both sets, making it ideal for collinear spectral data, such as that obtained through VIS-NIR spectroscopy (Wold *et al.*, 2001).

The improved modeling of Mn is particularly relevant, as this nutrient has proven difficult to predict in citrus crops, as noted by Gálvez-Sola *et al.* (2015). In contrast, the present study achieved superior performance metrics during key phenological stages ("straight sepals" and "chickpea"), which may be attributed to the specific optical properties of the rose crop, as well as the experimental design and preprocessing techniques employed. Spectral regions around 523, 557, and 720 nm were consistently important, aligning with findings from other studies (Hu *et al.*, 2011; Mahajan *et al.*, 2021), further reinforcing their relevance as indicators of nutrient content.

These results are also aligned with the study by Mahajan *et al.* (2024), who combined hyperspectral remote sensing with machine learning algorithms (PLSR-Cubist, SVM, elastic net) to estimate foliar nutrients in cashew. In that study, Mn prediction using PLSR-Cubist was particularly strong, with a coefficient of variation of 21% in the calibration set and a slightly positively skewed distribution, indicating its suitability as a model variable.

Similarly, Acosta *et al.* (2023) reported the effectiveness of PLSR in citrus crops, achieving reliable predictions of macro and micronutrients, with the best results for P, K, and B. In this case, Mn showed optimal and consistent foliar values throughout the crop cycle, which—along with the identification of visible-range wavelengths associated with foliar pigments—supports its diagnostic utility through spectroscopy.

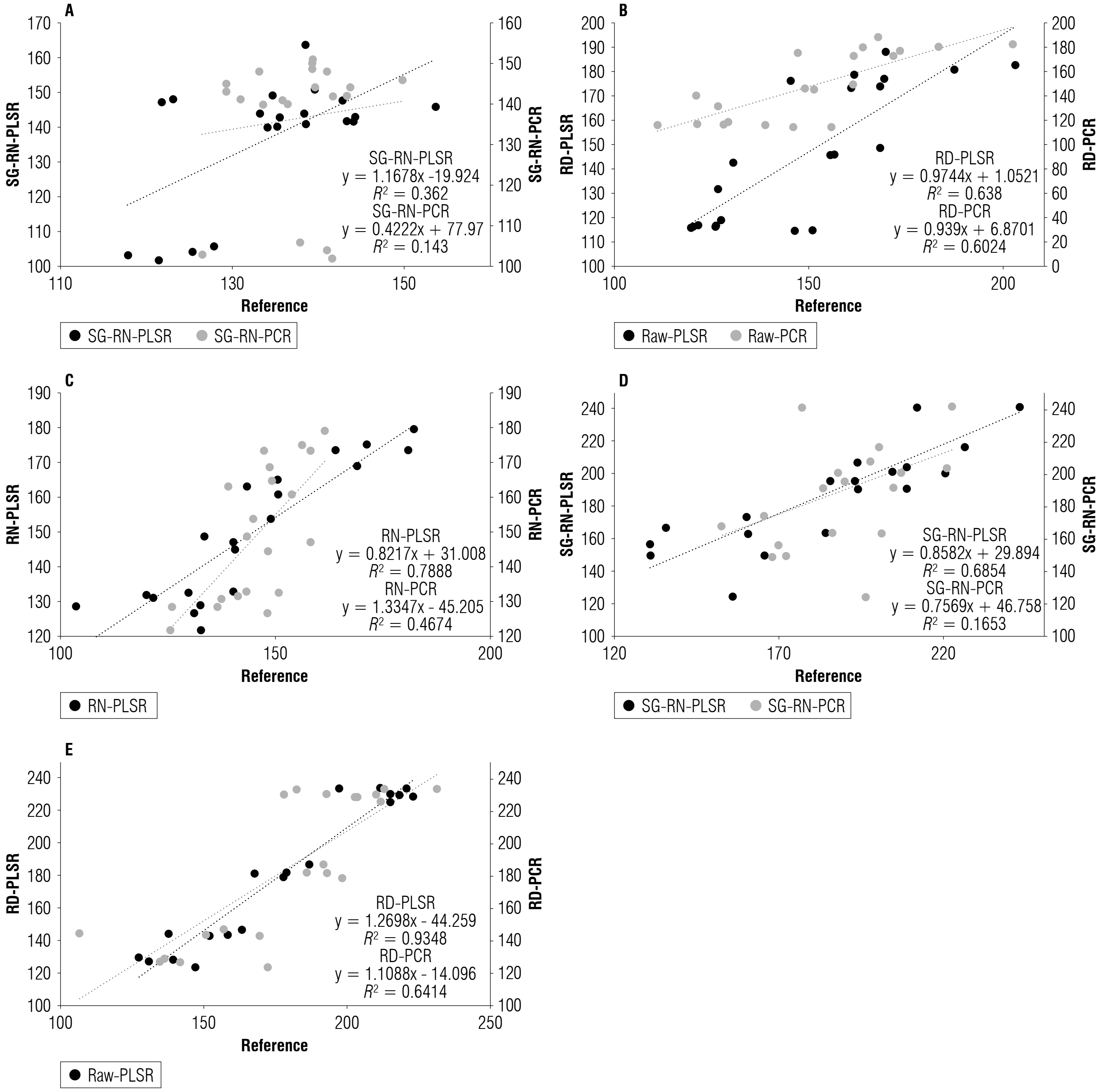

FIGURE 5. Dual-axis correlation comparing the regression techniques PLSR and PCR: A) palmiche, B) rice, C) chickpea, D) scratch color, and E) straight sepals.

In a different but complementary approach, Kusumiyati *et al.* (2022) validated the use of VIS-NIR spectroscopy for estimating nutritional compounds in green cayenne pepper fruits ('Ratuni UNPAD'). Their models achieved high accuracy for carotenoids, water, and capsaicin (RPDs ranging from 1.90 to 2.29), positioning this technique as an efficient, non-destructive alternative for quality control. Finally, studies in Chinese cabbage (*Brassica campestris* L. ssp. Pekinensis 'Norangbom') have shown that VIS-NIR spectroscopy combined with SMLR significantly outperforms conventional sap tests for estimating foliar nitrogen. SMLR produced an R^2 of 0.846 and PLS yielded an R^2 of 0.840 with significantly lower errors (Min *et al.*, 2006). These findings support the applicability of similar models in other species such as *Rosa* spp., where non-destructive Mn detection through PLSR emerges as a promising tool for nutrient monitoring under controlled greenhouse conditions.

Conclusions

This study demonstrated that VIS-NIR spectroscopy, combined with multivariate models such as Partial Least Squares Regression (PLSR), allows for accurate prediction of foliar manganese content in *Rosa* spp. under greenhouse conditions. The models developed—particularly those using Savitzky-Golay smoothing and range normalization—showed robust performance metrics, validating their potential as non-destructive tools for nutritional diagnosis.

The application of this methodology represents an efficient and rapid alternative to conventional foliar analysis, which is especially relevant in highly technical and quality-sensitive crops like roses. The possibility of implementing real-time nutritional monitoring can lead to improved crop management decisions, greater uniformity in floral quality, and reduced laboratory costs.

As a future projection, it is recommended to expand the sample size and validate the models under different environmental conditions and in other rose cultivars. Additionally, hybrid approaches that combine spectral regression with machine learning algorithms could be explored to further enhance prediction accuracy and model adaptability. The results contribute to the advancement of precision agriculture tools in floriculture, with direct application to sustainable rose crop management.

Conflict of interest statement

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

Author's contributions

LJMM was responsible for the conceptual design and objectives of the study; OFM supervised the experiments, managed data collection and maintenance, performed statistical analyses and wrote computer codes, and coordinated the project funding; OFM conducted field and laboratory experiments, as well as created effective visual representations of the data and findings; LJMM and OFM designed and developed the research methodology, including methods and equipment for data collection, and ensured the accuracy and reliability of the results through a rigorous validation process; OFM wrote the initial draft. All authors contributed to the critical review and approved the final version of the manuscript.

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Challenges and strategies to promote the industrialization of cassava crop in Colombia

Desafíos y estrategias para promover la industrialización del cultivo de yuca en Colombia

Eduardo Rodríguez-Sandoval[1]*, Alejandro Mejia-Villota[2], and Víctor Hernández[2]

ABSTRACT

Cassava (yuca) is a major crop in Colombia, with a production of more than 2.5 million t in an estimated area of more than 260,000 ha. However, the average yield in the country is 11.34 t ha^{-1}, a low level compared to other world-class producing countries. About 94.4% of the total production of cassava nationwide is used for direct human consumption, and only 5.6% is destined for industrialization. The aim of this research was to verify the problems faced by the industrialization of cassava crop in Colombia and to establish possible strategies to help deal with them. The strategies proposed were identified through field observations, iterative testing, stakeholder feedback, and comparative analysis. Among the most noticeable challenges in the low industrialization of cassava crop are low availability of land and high-quality planting material, low technological development, and seasonality of production. Some outstanding strategies to overcome this problem are sowing by pre-sale, access to suitable technology, change of crop varieties, and the producer's business culture. Large industrialized crops are adopting pre-sale planting as a business strategy for agribusiness; moreover, a crop with agricultural mechanization and irrigation systems on large tracts of land is economically efficient. However, the shift from subsistence agriculture to agribusiness is not enough. Government support with public policies and subsidies is needed to safeguard the cassava production chain in times of national or global crisis.

Key words: *Manihot esculenta,* mechanization, pre-selling, agribusiness, business culture, public policies.

RESUMEN

La yuca es un cultivo muy importante en Colombia, con una producción mayor a 2.5 millones t en un área estimada de más de 260 mil ha; sin embargo, el rendimiento promedio en el país es de 11,34 t ha^{-1}, un nivel bajo si se compara con otros países productores de talla mundial. Alrededor del 94,4% del total de la producción de yuca a nivel nacional es para consumo humano directo y sólo 5,6% se destina a la industrialización. El objetivo de esta investigación fue verificar los problemas que enfrenta la industrialización del cultivo de yuca en Colombia y establecer posibles estrategias para ayudar a superar este desafío. Las estrategias propuestas se identificaron mediante observaciones sobre el terreno, pruebas iterativas, comentarios de las partes interesadas y análisis comparativos. Entre los problemas que destacan en la poca industrialización del cultivo de yuca se encuentran la baja disponibilidad de tierras, de material de siembra de alta calidad, escasa tecnificación y estacionalidad de la producción. Algunas de las estrategias planteadas para superar esta problemática son la siembra por venta anticipada, acceso a tecnología adecuada, el cambio de las variedades del cultivo y la cultura empresarial de los productores. Los grandes cultivos industrializados están adoptando la siembra por venta anticipada como una estrategia de negocio para la agroindustria; además, un cultivo con mecanización agrícola y sistemas de riego en grandes extensiones de tierra es económicamente eficiente. Sin embargo, el cambio de agricultura de subsistencia a agronegocios no es suficiente; se necesita el apoyo del gobierno con políticas públicas y subsidios para salvaguardar la cadena productiva de la yuca en épocas de crisis nacional o mundial.

Palabras clave: *Manihot esculenta*, mecanización, venta anticipada, agronegocio, cultura empresarial, políticas públicas.

Introduction

Cassava or yuca (*Manihot esculenta*) is a tropical crop native to South America. It was domesticated for food about 5,000 years ago by hunter-gatherer groups from the Caribbean and Amazon regions (Aguilera Díaz, 2012; Otálora *et al.*, 2024). Cassava is grown in more than 102 countries for its edible starchy tubers and is consumed as an energy source in the diet of thousands of people (Jisha *et al.*, 2008). It is rich in carbohydrates, calcium, vitamins B and C, and essential minerals, but the nutrient composition differs according to the variety, age of the harvested crop, the soil conditions, the climate, and other environmental factors during cultivation. Cassava is the basis of several

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[1] Universidad Nacional de Colombia sede Medellín, Facultad de Ciencias Agrarias, Departamento de Ingeniería Agrícola y Alimentos, Grupo de Investigación de Alimentos Funcionales, Medellín, Antioquia (Colombia).

[2] Alimentos y Productos en Polvo Poltec S.A.S., La Estrella, Antioquia (Colombia).

[*] Corresponding author: edrodriguezs@unal.edu.co

products, including flour, animal feed, alcohol, starches for the food industry, paper and textiles, sweeteners, prepared foods, and biodegradable products (Aguilera Díaz, 2012; Chavarriaga-Aguirre *et al.*, 2016).

World cassava production in 2023 was 334 million t (FAO, 2024). Africa has the highest cassava production in the world (62% of the global volume), with more than 207 million t in 2023; the remaining 29% is produced in Asia and 9% in Latin America (Ritchie *et al.*, 2023).

In Africa, the largest producer is Nigeria, with an average yield of 9 t ha^{-1} and production of about 60 million t (Ikuemonisan *et al.*, 2020). The second largest producer in the region is the Democratic Republic of Congo, with an average yield of 8.1 t ha^{-1} and production of about 40 million t. The third largest producer is Ghana, with an average yield of 14 t ha^{-1} and production of about 23 million t, with a growing industry led by smallholders (Adebayo, 2023; Poku *et al.*, 2018).

In Asia, Thailand is one of the leaders in industrial cassava production. This has placed it as one of the priority crops for its bioeconomy development (Arthey *et al.*, 2018). These authors propose to implement biorefineries to develop value-added products such as lactic acid, starches, and bioplastics from cassava and sugar cane to maintain its competitive advantage as a leading exporter of cassava worldwide (Lane, 2017; Saardchom, 2017). Thailand is a leader in the global market, exporting 26% of fresh, frozen, or dried cassava and 72% of cassava starch.

The leading producer in Latin America is Brazil, followed by Paraguay (FAO, 2020). Brazil has one of the most modern industrial parks since the beginning of 2000 and serves the starch industries in the center-south and flour in the northeast, mainly for domestic consumption (Felipe, 2020). The predominant producers of flour are small-scale farmers. The production of starch occurs on a larger scale. The main buyers of these inputs are pasta, biscuit and bakery, tapioca, and meat products companies. In Paraguay, 70% of production is for consumption (human and animal food), while 30% is marketed (Canales & Trujillo, 2021). The main production areas are in the eastern region (69%), where small producers grow from 1 to 10 ha (Enciso Rodríguez *et al.*, 2015). Paraguay is the fourth largest exporter of cassava starch in the world and the first in the American continent; 60% of Paraguay's cassava starch exports are produced by Codipsa, the only processing plant with European technology in South America. In 2018, this company produced 60 thousand tons of starch and exported 22 thousand tons (Diaz, 2019). Science, technology, and innovation focused on cassava cultivation began about 30 years ago worldwide. In 1976, cassava was considered an orphan crop without the support of any country (Hershey, 1994).

Colombia is the third largest producer of cassava in Latin America (Canales & Trujillo, 2021). Cassava is an ancestral crop used for direct but not industrial consumption. It has traditionally been a relevant crop for agriculture, constituting an essential element in the diet of many people in various regions of the country. The products that generate added value to the crop are native cassava starch, sour or fermented cassava starch, and dry and fresh cassava (Parra Olarte, 2019). It is the fifth most produced agricultural good in volume in the country, after sugarcane, banana, potato, and rice. In 2022, Colombia produced 2.1 million t in a harvested area of 187,268 ha (Agronet, 2023). The average yield in Colombia is low (11.22 t ha^{-1}) compared to world leaders such as Thailand (22 t ha^{-1}) and, in the region, Brazil (14 t ha^{-1}) and Paraguay (18 t ha^{-1}) (FAO, 2020). Cassava is grown in all 32 departments of the country. The most prominent production centers are the Caribbean, Orinoquia, and Cauca regions. The leading producers, in general, are the departments of Bolívar (17% of national production), followed by Córdoba (11%), Sucre (8%), and Magdalena (7.5%) (Parra Olarte, 2019). From 2015 to 2018, the areas planted with the cassava crop increased by 7%, while harvested areas increased by 21%. In the same period, total cassava production varied by 34%, reaching 2.37 million t of tubers harvested by 2018 (MADR, 2021). However, production and area have decreased to values close to 2 million t and less than 190,000 ha from 2018 to 2022 (Agronet, 2023).

Different varieties of cassava are produced in the country. However, commercial production is divided into two large segments: sweet cassava, mainly aimed at human consumption, and industrial (bitter) cassava, aimed at industrial segments for processing into starch and other products (MADR, 2021). The main characteristics of industrial cassava are the high yield of fresh roots and its high dry matter content, resulting in a high dry yield per area. Likewise, industrial cassava doesn't need high contents of cyanogenic glycosides. The terms bitter cassava and industrial cassava are used interchangeably. The industry often processes non-bitter cassava (Taborda Andrade *et al.*, 2023).

Of the total production of cassava nationwide, 94.4% is for direct human consumption, and only 5.6% is focused on industrialization (Canales & Trujillo, 2021). The departments that have the highest industrial cassava production in the country are Sucre (40%), Córdoba (27%), and Cauca (30%); nevertheless, industrial cassava is produced in 11 out of the 32 departments of the country (Rivera *et al.*, 2021). Almost all cassava transformation is carried out by small and medium-sized companies in the communities, with intensive labor and traditional techniques (Canales & Trujillo, 2021). The agricultural production system is mainly small-scale (85%), grown in areas less than 10 ha, with traditional practices and low technology. The crop is grown primarily on leased land (70%). It generates an average of 53 working days per hectare of industrial cassava and 40 working days per hectare of fresh cassava (MADR, 2017). Fresh cassava cultivation for direct human consumption is conducted in rotation or associated with corn, yam, or cotton. In contrast, industrial cultivation is undertaken in monoculture and is more technological (MADR, 2017).

Colombia's main product of cassava cultivation is the fresh root for direct human consumption. One of the challenges of fresh cassava is its perishability since it must be consumed or transformed within the first 48 h after harvest. The roots can be waxed with paraffin or processed (peeled, chopped, used to prepare flour or dried pieces) to extend their shelf-life and sell it in frozen and pre-cooked presentations (Canales & Trujillo, 2021). Products from washed and peeled roots generate root wash effluents and solid by-products (shells and root tips). Large industries reuse washing water. The shells are often given to producers for use in compost or to farmers for consumption as animal feed without processing (Canales & Trujillo, 2021).

Cassava products can be fermented or unfermented and contribute to the growth of its industrial applications. Primary unfermented cassava products include flour and starch. Fresh cassava roots can be processed into "chips," and dried cassava chips can be transformed into flour or starch after milling (Chisenga *et al.*, 2019). On the processed product side, cassava flour is used as an additive in preparing bakery products, and starch as a stabilizing agent for soups, frozen foods, and snacks, among other uses (Aguilera Díaz, 2012). Cassava flour and starch, which do not contain gluten, are alternatives that could compete in this market. Another market opportunity for cassava is an increased demand for bioplastics, as it can be used to obtain thermoplastic biopolymers, films, and biofuels. Thus, there is potential for the development of industrial-scale multi-product biorefineries, complex systems where agricultural residues are transformed to obtain a portfolio of high-value-added products and energy sources for integration into cassava starch processing, in a scheme that promotes socio-economic growth, as these facilities provide the opportunity to create new jobs, increase the flow of profits, and improve people's purchasing power (García-Vallejo & Cardona-Alzate, 2024; Padi & Chimphango, 2021; Ravichandran *et al.*, 2024). Likewise, cassava could be an essential source of energy for animal feed and compete with the corn imports currently made in the country (Canales & Trujillo, 2021; Contexto Ganadero, 2022). Therefore, our research was to verify the problems faced by the industrialization of cassava crops in Colombia and establish strategies that can serve in the future for a clear development in strengthening this productive chain, which is very important in social and economic terms for the country.

Current problems in the industrialization of cassava crop

The cassava chain vision in Colombia 2010-2024 acknowledges that the low transfer of innovative technologies to the cassava transformation process does not allow consolidating a competitive and economically, socially, and environmentally sustainable agri-food chain, active in national and international markets and with technological innovation, business development, and support for food security (MADR, 2014). The precariousness and inefficiency of technology transfer in primary production has been one of the main cassava production chain weaknesses in Colombia. In addition, the planting area of industrial cassava varieties should be tripled. Therefore, the country must overcome barriers like low access to or availability of land, low availability of high-quality cassava planting material (seed stalks), low profitability of current industrial cassava production systems, low commercial confidence between producers and industry, low use of technology, use of improved varieties, lack of mechanization, lack of irrigation systems, production seasonality (i.e., all producers plant and harvest at the same times, thus causing market shortages or saturation at the same time of year), cassava substitution for illicit coca cultivation, and low generational relay in the rural population to work in the crop (Taborda Andrade *et al.*, 2023). Moreover, other challenges in the cassava industry must be considered: high production costs, high consumption of resources, loss of roots and starch, increasing environmental impacts, and difficulties in adapting to climate variation derived from the effects of climate change (Pingmuanglek *et al.*, 2017; Van Giau *et al.*, 2023; Van Giau *et al.*, 2024).

The historical prices of different types of commercial cassava are volatile (2013-2021), and prices can vary by 100% from year to year for some cultivars. This is possibly related to periods of scarcity, low production of cassava in some parts of the country, and the oversupply of periods of cassava or other substitute products (Taborda Andrade *et al.*, 2023). Harvest times are generally between November and February, and one of the current difficulties is the availability of fresh cassava throughout the year, since there are seasonal periods where production levels are very low, and prices increase considerably.

Immersion in a globalized economy model can stimulate rural-to-urban migration and generate an absence of labor, minimal generational change, loss of local practices and knowledge, and low remuneration for selling farmer products (Ocampo, 2014). Rural areas face a peasant deactivation scenario, mainly due to a generational absence with no renewal. This can be a vicious circle since there is no work or the remuneration is very low; therefore, the worker leaves. Since there are no people to work, there is no labor to cultivate.

Strategies for industrializing the cassava crop

Cassava is a versatile crop that can serve as an important source of income and economic development for agribusinesses by expanding raw material supply, enabling value-added products, and stabilizing supply chains. Transforming cassava root into products with added value and with greater demand in the market initially requires the crop to be industrialized. Thus, the problems mentioned above can be improved through strategies suitable to each crop's conditions and situations. The proposed strategies were identified through a mixed-methods approach that combined: 1) Field observations and iterative testing through longitudinal engagement with cassava-producing regions to document systemic bottlenecks and direct participation in pilot projects in order to assess the feasibility of mechanization, irrigation systems, and contract farming models; 2) Stakeholder feedback through interviews with smallholder farmers to identify pain points in production, pricing, and supply chain coordination where operations face difficulties or inefficiencies, and support for workshops with agricultural cooperatives and research institutions (*e.g.*, Agrosavia, CIAT) to validate technological and policy barriers; and 3) Comparative analysis through benchmarking with cassava industrialization models from Thailand, Brazil, and Paraguay, adapting lessons learned from the Colombian agricultural context. The strategies to fill this long-term gap are presented in Figure 1, highlighting sowing by pre-sale or forward contracts, access to adequate technology, switching to extensive crops, and an adequate producer business culture.

Sowing by pre-sale or forward contract

The forward sale contract is a way of marketing a crop that enables pre-selling the harvested product that acts as a form of risk management for its future price. Under this modality, the farmer has the possibility of fixing the sale price of their product previous to the physical delivery, but the producer assumes the risk. The main difference between pre-selling and futures market is that in the latter more financial instruments enable product price management, without the physical delivery commitment required by the forward contract (Gutiérrez, 2009; Molina & Victorero, 2016). An important feature is that companies generally constantly give technical training and advice to farmers so that the harvest is obtained in the best conditions.

However, there are several risks associated with forward contracts, and one of the most incurred is trust between stakeholders. During the 2021-2022 period, competition for cassava roots in the Sucre and Córdoba departments increased. Furthermore, it is estimated that more than 60% of the cassava grown in Cauca was replaced by coca leaf crops. Therefore, the demand shifted to the Caribbean, causing increases in root prices. In 2022, one ton of fresh cassava roots of industrial varieties reached a historical maximum of $1,400,000 Colombian peso (COP) (US$ 283 at the Representative Market Rate - RMR of that year). The situation resulted in breaches of up to 70% of the forward contracts signed between industries and producers (Taborda Andrade *et al.*, 2023). This situation generated uncertainty in the industry and the economic groups that carry out these contracts, changing conventional policies and procedures in this business.

If there is no confidence in a forward contract in agricultural production, several problems and negative consequences arise: for instance, credit risk, price uncertainty, negative impact on the supply chain, and damaged reputation, among other possibilities. In the end, if there is no agreement between the parties, companies can resort to legal actions to assert their rights, seeking a solution in court. However, these actions can take too much time and economic resources, and they do not guarantee a return on the investment. Additionally, the business group can take another path, such as leasing land and contracting all the services for agricultural activity development throughout a considerable area thus benefiting from the scale economies that this enables (Gutiérrez, 2009).

Access to adequate technology

Production includes three major activities: sowing, cultivation, and harvesting. Small producers practice traditional or manual planting in the Colombian Caribbean (Fig. 2), starting with soil preparation in the dry season from the first days of April to June, with the purpose of harvesting between May and November. The seeds (cassava stem cuttings) are buried in soil 5 to 10 cm of their average 20 cm length, and they are usually planted in an upright position, ensuring that many buds are below ground. This task requires between 6 to 8 d ha^{-1} (Martínez García & Tordecilla-Acevedo, 2019).

The harvesting technique involves cutting and selecting foliage and seeds (cuttings). Only a stem (20-40 cm long) is left attached to the roots to extract them more easily from the soil by pulling them with the hands. The roots are collected and packed in sacks (*costales*) or plastic bags that are then collected and taken to the market (Fig. 3). The production of cassava roots is intended for self-consumption, artisanal chopping, industry, and fresh produce traders (Martínez García & Tordecilla-Acevedo, 2019).

At the time of starting cassava industrial production, farmers are recommended to use planting material from quality vegetative seed (genetic, sanitary, physical, and physiological) to reduce and avoid the spread of sanitary problems (Floro *et al.*, 2018; Leon *et al.*, 2021; Nassar & Ortiz, 2007). Therefore, uniformity of the crop stand, vigor in the plant establishment, and a high yield of commercial roots during the harvest can be achieved (Rodríguez Henao *et al.*, 2021). It is estimated that at least 60% of a good crop lies in the use of cuttings suitable for the different places where it is grown. In Colombia, many commercial cassava cultivars have been developed by the Alliance Bioversity International and the International Center for Tropical Agriculture (CIAT, by its Spanish acronym), partnered with the Colombian Corporation for Agricultural Research (Agrosavia, by its Spanish acronym) (Rodríguez Henao *et al.*, 2021). The most widely used industrial cassava varieties in Colombia are Corpoica Tai, Corpoica Belloti, Corpoica Sinuana, and Corpoica Ropain in the Caribbean region; Corpoica Cumbre and Corpoica La Francesa in the department of Cauca; and Agrosavia Melúa-31 in the Orinoco region (Rosero Alpala *et al.*, 2023). It is necessary to make agreements with these research centers to plant certified cassava that is pest-resistant and that can last more than 14 months without loss of dry matter (starch). Currently, 9-month harvest crops are sown, and dry matter loss has been noticed after that time.

Experience and data have shown that the mechanization of cassava cultivation is relatively difficult to execute and adopt, although there are solutions for cassava cultivation automation that are newly developed every year. Land preparation, irrigation, transport, and pest and disease management systems are readily available and adaptable to cassava cultivation. Moreover, specialized machinery, such as stem planting machines, cassava root harvesters, and cassava harvesters are being developed to improve the mechanization process (Adekunle *et al.*,

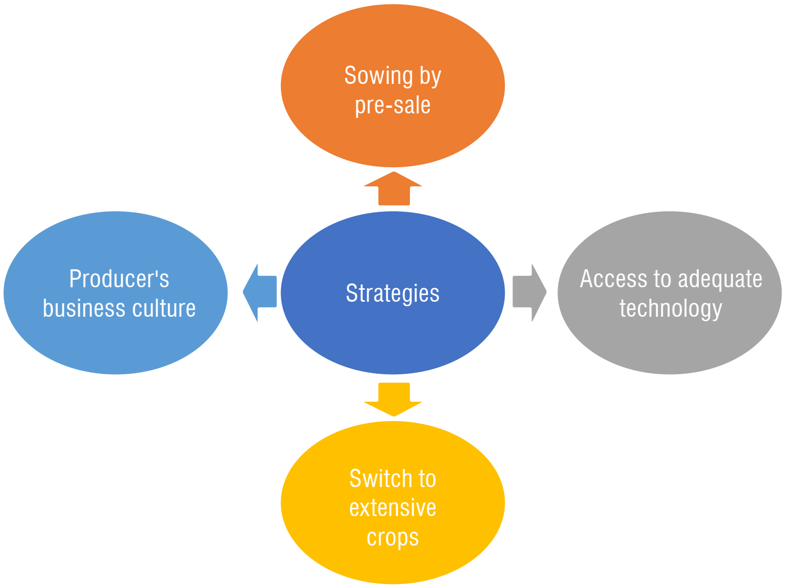

FIGURE 1. Strategies for industrializing cassava crop in Colombia.

2016). Unfortunately, technological advances often do not translate into real applications in the field, mainly due to the cost of these technologies. However, in most cases, the social aspect is linked to economic limitations with regard to the users of these technologies.

Cassava cultivation mechanization is one of the main agriculture needs in Colombia considering the projection of that crop in national and international markets and its application in the industry. Agricultural development not only depends on the opening of new land or more intensive use of already used land but also on the mechanization of crops and the use of different technologies for agro-industrial processing, *e.g.*, mechatronics, the internet of things, the use of drones and sensors, precision agriculture, autonomous vehicles, and rural and renewable energies. Smart production can greatly contribute to the productive efficiency of these processes and the reduction of their environmental impact (Alonso-Gomez *et al.*, 2024; ONU & CEPAL, 2018; Tran *et al.*, 2015). However, mechanization for crop extraction from the field is conditioned on the dimensions reached by the variety sown, its developmental level, and the soil type (García-Pereira *et al.*, 2017).

Mechanization in Colombian cassava production is currently limited to land clearing (preparation for planting), seedbed preparation, and transportation-practices adopted primarily by large-scale agro-industrial farms. Small-scale producers (85% of farmers) remain dependent on manual labor due to land fragmentation, financial constraints, and limited access to machinery (Rodríguez Henao *et al.*, 2021). Since implementing mechanization in agriculture drastically reduces the total person-hours needed to perform a specific task, a typical effect of technological improvements in agriculture is a temporary increase in the number of unemployed people in society (Adekunle *et al.*, 2016). While it seems logical to assume that such an increase in unemployment would be restricted to the sector directly affected by innovation, the overall impact on unemployment in the country could be large, as the adoption of technologies is known to reduce labor needs by up to 84%. This problem can only be avoided if the government is receptive enough to create proactive programs that stimulate the economy, including initiatives such as programs for acquiring skills that target workers who will be affected by structural unemployment (Adekunle *et al.*, 2016).

Cassava is considered a relatively drought-tolerant crop, since it reduces water use by reducing leaf area and stomatal closure (Polthanee, 2018). However, water stress causes a reduction in yield, especially during early growth stages. All supplemental irrigation water regimens increase the leaf area index and yield of cassava roots in contrast to a lack of irrigation during the dry season. With supplementary crop irrigation during the dry season, a higher tuber yield and higher starch content can be achieved, translating into greater productivity (Polthanee, 2018). Dry matter and starch content are commonly considered quality factors and can vary widely among cassava varieties. The factors described are closely related to the potassium content of the soil, the age of the crop, the climate, especially rainfall and soil moisture content (Santos *et al.*, 2019). In general, cassava is considered to be more resistant than other crops to harsher climatic conditions (Guerrero Hernandez & Ramos de Arruda, 2021). However, the effect of climate chang on this crop would be less if an adequate irrigation system is implemented.

As mentioned above, the average yield in Colombia is 11.22 t ha^{-1}, which is low when compared to cassava root production in Thailand with an average yield of 22 t ha^{-1}. This can vary greatly depending on the area and agricultural

FIGURE 2. Traditional sowing of cassava in the Colombian Caribbean: A) Soil preparation, B) cuttings for sowing, C) manual planting of cassava cuttings (Photos with permission of Poltec SAS).

FIGURE 3. Cassava harvest in the Colombian Caribbean: A) Manual harvest, B) transport of harvested cassava (Photos with permission of Poltec SAS).

management practices. However, it is estimated that using a certified and suitable seed for each type of soil, applying mechanization technology and irrigation systems with good agricultural management practices (*e.g.*, the application of manure or compost, mulching, fertilizer optimization, cassava rotation with grain legumes to fix atmospheric nitrogen, and conservation tillage) could increase yield up to 50 t ha^{-1} (Pingmuanglek *et al.*, 2017; Rubiano-Rodriguez & Cordero-Cordero, 2019).

Switch to extensive crops

In line with global trends, the large land tracts in Colombia destined for a specific crop seem to be related to agro-industrial transformation, especially biofuels from sugarcane, oil palm, and soy, which are highly encouraged by the State. These species are interesting because they serve multiple agro-industrial purposes (Pardo & Ocampo-Peñuela, 2019; Torres-Mora, 2020). A relevant example is the use of large land tracts in the Valle del Cauca department by the sugar cane agro-industry. This industry does not necessarily acquire the land where they grow the cane but they lease the land and buy the harvested product through forward contracts, as mentioned above. In this new model used today, more than 70% of the land is owned by suppliers while only 25% is owned by sugar mills (Torres-Mora, 2020).

Considering the case of Almidones de Sucre – a company that transforms cassava into native starch and generates 60 direct jobs in the plant and 600 indirect jobs in the field in the Sucre, Córdoba, and Bolívar departments – processing requires maintaining 3,000 ha of cassava for plant operation at a maximum of 80 km around the company, so that the freight does not increase the price of the product, which must be brought to the factory no later than 48 h after harvest (Martínez García & Tordecilla-Acevedo, 2019). However, producers are scattered throughout the department and are not always part of associations, thus they sell their product to the highest bidder. Incentives are lacking that generate loyalty to the customer companies, so, there is little clarity about their product's importance in the links of the production chain, and the derivatives and diverse uses of the product (Martínez García & Tordecilla-Acevedo, 2019; Pabon-Pereira *et al.*, 2019). Traditionally, cassava has been grown by smallholders with less than 5 ha of cassava per farm that were mostly intercropped with maize and yams. More recently, larger plantations of more than 10 ha of cassava per farm have arisen in response to a sharp increase in demand from cassava processors (Pabon-Pereire *et al.*, 2019).

For the market, around 11,000 t of *fécula de mandioca* (cassava starch) (the batch code name registered in international trade and revised in Legiscomex for 2022) were imported. In addition, the current scenario in the post-pandemic context (global container crisis, increase in the USD to COP exchange rate, conflicts in Ukraine and Israel, among others) has dynamized national demand for native, fermented, and modified starches (Taborda Andrade *et al.*, 2023). Thus, an industrialized cassava crop to meet this demand, (*i.e.*, large extensions of crops using modern technology) need a continuous supply for agro-industrial transformation.

One of the alternatives that could be implemented to industrialize the crop and link it with the agro-industry is to convert large land tracts dedicated to low-density livestock into cassava crops with technological applications and locate them near the processing centers. This is the case in some areas of Sucre and Córdoba. Nevertheless, associations or cooperatives of small producers could be created to generate scale economies that respond to the industry demands with forward contracts respecting the agreed contractual clauses. However, this strategy requires farmers, who produce cassava roots, to change these practices.

Producer business culture

Smallholder farmers have low financial and human capital, as well as limited physical and social capital. Low financial and human capital reflects poor access to financial resources such as savings, credit, or assets, and lower educational attainment, skills, or health status to perform well in agricultural activities. This, in turn, makes it difficult for them to face and adapt to the numerous crises and challenges they face (Hendrawan *et al.*, 2024). This means that the majority of cassava farmers have educational attainment below fifth grade, do not maintain accounting of their production, live in extreme poverty conditions, are geographically dispersed, have no knowledge of the value chain, have access to little market information, and have poor assimilation of the value concepts and intermediation margins throughout the chain. Furthermore, the vast majority of farmers are an older population with little actual generational relay (Ariza García *et al.*, 2021; Martínez García & Tordecilla-Acevedo, 2019).

This situation can be improved if policy interventions are implemented with a comprehensive approach that should focus primarily on covering and strengthening the financial and human capacity of these smallholder farmers (Taborda *et al.*, 2018). This could be achieved through direct financing, such as the provision of direct financial aid or subsidies, and capacity-building interventions, such as extension support to agricultural management practices (Hendrawan *et al.*, 2024). Along with the above, a strengthening of producer organizations and companies must be structured; that should enable the producers to collectively negotiate conditions of financial services packages, zoning according to the sizes of areas dedicated to cultivation to establish stocks that reduce transport costs, and to coordinate the product placing on the market and the technology needed to cultivate (Ariza García *et al.*, 2021; Martínez García & Tordecilla-Acevedo, 2019).

Farmers should have information on the sector's behavior and the suitable technology in the crop that is translated for their educational attainment. The implementation of any technology can have positive or negative effects on society, depending on the pace at which it is introduced and adopted by the population. The adoption of agricultural technology by a rural population will normally depend to a large extent on the socio-cultural and economic ideologies, gender, religion, and the individual's economic position, as well as on the application of these technologies to local production systems. The economic and social structure of society, more than any benefit or improvement arising from new technologies, plays a crucial role in the way in which the population accepts innovation (Adekunle *et al.*, 2016).

Another challenge agricultural production faces is rural population aging, a phenomenon that is observed worldwide and that must be taken with great caution by government entities and society in general. Making agriculture attractive to young people requires changes in the economic, social, and political structures of the food system by adopting new agricultural approaches. Changes include civic agriculture that considers the development of activities from agriculture to food, giving life to a social movement to resist isolation and inequality (Farkas *et al.*, 2023; Salgado Sánchez, 2015).

It is important to highlight that the technology applied to cultivation can only benefit farmers willing to specialize, mechanize, and expand their agricultural operations. The research and extension programs were designed to help farmers turn their farms into agribusinesses. Large specialized agricultural operations can be economically efficient, but they are also risky and vulnerable to economic collapse, as shown during the agricultural financial crisis of the 1980s and the COVID-19 crisis that started in 2020. The agricultural policies of the 1980s were an experiment to see if large, specialized farms could survive without government assistance. They could not. Government price support, deficiency payments, subsidized crop insurance and crop income, guaranteed loans, and disaster payments are means by which governments have absorbed the risks of industrial agriculture (Ikerd, 2024). Large specialized agricultural tracts can be the product of cooperatives or associations of cassava farmers. However, there is another alternative: transform the land dedicated to low-density livestock into cassava crops using technology and taking advantage of the fact that people in the livestock sector have a credit culture and access to the financial sector.

Conclusions

Cassava has traditionally been a relevant crop for agriculture in Colombia. The country ranks third as a producer in

Latin America. However, the average yield in the country is only 11.34 t ha^{-1}, a low level when compared to Paraguay (18 t ha^{-1}) or Thailand (22 t ha^{-1}). About 94.4% of total production of cassava nationwide is dedicated to direct human consumption, and only 5.6% is destined for industrialization. Among the problems that stand out from the low industrialization of cassava cultivation in Colombia are low access to or availability of land, low availability of high-quality planting material, low commercial trust between producers and industry, lack of technology, production seasonality, and aging of the rural population. Among the strategies proposed in this research to overcome these challenges are as follow: sowing by pre-selling, access to appropriate technology, switching to extensive crops, and the producer business culture. These stand out. It is estimated that at least 60% of a good crop lies in the use of suitable and certified seeds, suitable for the different soils and growing conditions in the country. In addition, cassava cultivation mechanization is one of the main agricultural needs in Colombia. The application of supplementary irrigation systems can lead to a higher tuber yield and higher starch content that translates into greater productivity. Large extensions of crops with technology are necessary, favoring a continuous supply in the agro-industrial transformation to meet the demand for industrial cassava. However, it should be noted that not only the approach of specialized farmers willing to mechanize and expand their agricultural operations is sufficient, rather, a shift from subsistence agriculture to agribusiness with government support is also essential to face different crises. Some ways of absorbing the industrial agriculture risks by the government could be the offer of subsidized crop insurance, guaranteed loans, and disaster payments.

Conflict of interest statement

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

Author's contributions

AMV and VH formulated the overarching research goals and aims, ERS wrote the initial draft, AMV and VH obtained the financial support for the project leading to this publication. All authors approved the final version of the manuscript.

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Characterization of yeast communities vectored by *Drosophila melanogaster* during post-harvest fermentation of coffee

Caracterización de comunidades de levaduras transmitidas por *Drosophila melanogaster* durante la fermentación postcosecha del café

José Julián Apraez Muñoz[1] *, Álvaro Javier Ceballos Freire[1], and Jesús Geovanny Solarte Guerrero[1]

ABSTRACT

Drosophila melanogaster, present in coffee processing stages, feeds on mucilage and coffee "cherries", inoculating yeasts that influence coffee bean fermentation. This pioneering study in Colombia evaluated the microorganisms associated with the larvae of this fly, determining their kinetic behavior, observing their morphophysiological structures, and characterizing the types of yeasts present. Understanding the interaction between these microorganisms and coffee bean quality is crucial in the coffee industry, given that in other industries, such as winemaking, they have been shown to directly affect the organoleptic characteristics of the final product. This study was carried out in three coffee-growing municipalities in northern Nariño. The yeasts present in the oral apparatus of *D. melanogaster* larvae were analyzed. These were cultivated in YGC media differentiated by color and growth type. Quantification was performed using colony forming units (CFUs) and lactophenol blue staining. Additionally, the number of CFUs was correlated with spectrophotometric measurements. The results revealed the presence of yeast species such *as Geotrichum, Galactomyces, Trichosporon*, and *Blastoschizomyces*. This research provides an approximation to the microbiome of coffee-growing environments and its biotechnological potential. Moreover, it lays the foundation to formulate methodologies for the morphophysiological classification of yeast strains associated with insects, their role in coffee fermentation processes, and their potential to determine flavor and quality of the final product.

Key words: microbiology, associated yeasts, fermentative microbiome, *Coffea arabica*.

RESUMEN

Drosophila melanogaster, presente en los procesos de beneficio del café, se alimenta del mucílago y la cereza, inoculando levaduras que influyen en la fermentación del grano. Esta investigación, pionera en Colombia, evaluó los microorganismos asociados con las larvas de esta mosca, determinando su comportamiento cinético, observando sus estructuras morfofisiológicas y caracterizando el tipo de levaduras presentes. En la industria cafetera, comprender la interacción entre estos microorganismos y el grano es crucial dado que, en otras industrias, como la vinícola, se ha demostrado que influyen directamente en las características organolépticas del producto final. El estudio se realizó en tres municipios cafeteros del norte de Nariño, donde se analizaron las levaduras presentes en el aparato bucal de las larvas *de D. melanogaster*. Estas se cultivaron en medios YGC, diferenciándose por color y tipo de crecimiento, y se cuantificaron mediante unidades de formación de colonias (UFC) y tinción con azul de lactofenol. Además, se correlacionó el número de UFC con mediciones espectrofotométricas. Los resultados mostraron la presencia de especies como *Geotrichum, Galactomyces, Trichosporon* y *Blastoschizomyces*. Esta investigación ofrece una aproximación al microbioma de los entornos cafetaleros y a su potencial biotecnológico. Además, sienta las bases para formular metodologías de clasificación morfo-fisiológica de cepas de levaduras asociadas a insectos, su papel en los procesos de fermentación del café y su potencial para determinar el sabor y la calidad del producto final.

Palabras clave: microbiología, levaduras asociadas, microbioma fermentador, *Coffea arabica*.

Introduction

Yeasts are vectored by *Drosophila melanogaster* and concurrently constitute a source of nutrients for this species (Ganter, 2006). Indeed, the availability of different yeast species as nourishment influences the developmental process of *D. melanogaster* larvae. In the same vein, the composition of microbial communities within the larval substrate is instrumental in determining the vulnerability of the larvae to parasitic attacks (Anagnostou *et al*., 2010). It has been observed that more complex yeast communities are favored as dietary options (Rohlfs & Kurschner, 2010).

The larvae of *Drosophila* spp. exhibit opportunistic behavior and are usually found in coffee during post-harvest processes and in the decaying fruits. The adult stage of this

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[1] Universidad de Nariño, Pasto (Colombia).

* Corresponding author: apraez.julian@gmail.com

insect is commonly referred to as the 'fruit fly' due to its presence in the aforementioned contexts. While it does not pose a direct threat to coffee crops, the invasion of its larvae during the stages of fermentation, harvesting, and "cherry" pulping is commonplace in the Andean region of the Department of Nariño.

Yeasts play a role in *Drosophila* reproduction. The composition of yeast in the fly's diet affects egg production, particularly in terms of egg size. This relationship indicates the interplay between nutritional availability and reproductive success. In certain *Drosophila* species, yeasts are even presented as nuptial gifts during courtship. Moreover, yeast-colonized substrates emerge as preferred oviposition sites for most fruit-breeding *Drosophila*, surpassing sites dominated by bacteria or molds. In particular, *Drosophila buzzatii* displays a strong affinity for feeding and ovipositing on specific yeast species. Likewise, *Drosophila,* both as larvae and in the adult stage, prefer to feed on particular yeast species. Common synthetic volatiles used to attract *Drosophila* spp. include chemicals released by yeast fermentation of fruits, such as ethanol, acetic acid, methyl acetate, ethyl acetate, acetaldehyde, and *n*-propanol. When offered a choice of pure yeast cultures, the interaction between larvae and yeasts provides essential nutrients such as sterols and, at the same time, produces chemicals that attract scattered insects and favor their migration toward favorable environments (Blackwell, 2017).

The importance of the interaction between insects and yeasts is not clear-cut. However, it has been shown that the presence of *K. ohmeri* increases the invasive and reproductive capacities of insects in environments with ripe fruits (Arbogast *et al*., 2012). This yeast is responsible for producing volatile components in food, which serve as strong attractants for other dipterans. For both larvae and adults, the most frequently identified yeast species is *H. uvarum*, suggesting a potential association between *D. suzukii* and *H. uvarum*. Brix grades, sugar content values, and yeast community profiles were found to be similar for infested and non-infested fruit juices, although fewer yeast colonies were present and identified in non-infested samples (Hamby *et al*., 2023).

Thus, the aim of the present study was to evaluate the microorganisms associated with *Drosophila melanogaster* larvae present in coffee post-harvest processes in Colombia, through the characterization of their kinetic, morphophysiological, and structural behavior, as well as the identification of their geographic origin and the types of yeasts present.

Materials and methods

In this study, coffee "cherries" were collected in the municipalities of Buesaco, Arboleda, and La Unión, in the Department of Nariño. The "cherries" underwent manual pulping, and the epidermis was separated and collected in plastic bags (10 g/bag), which were stored at room temperature (18-20°C). Twenty-four hours post-pulping, the bags were inspected for larvae. Any larvae found were manually collected and placed in 50 ml plastic bottles with 10 ml of distilled water. Subsequently, the bottles were placed in an incubator for 18 h at a temperature of 28°C and a relative air humidity of 85%. For larvae cultivation, a specific culture medium for YGC (Yeast Glucose Chloramphenicol) yeasts was prepared by adding 40 g L^{-1} of water. This culture medium was sterilized along with the Petri dishes and the bacteriological dishes.

Isolation procedure

First, each beetle was individually placed inside YGC agar boxes, allowing it to walk freely across the entire surface. The exterior of the larva was not sterilized in order to keep the insect alive and replicate natural dispersal during decomposition processes. This method increased the probability of isolating yeast from the larvae's mouthparts and digestive systems (Bressani *et al*., 2018). Second, the larvae were macerated and scraped across the entire Petri dish. Third, the residual water from the 50 ml flask was used for further scraping inside the Petri dish.

All Petri dishes were incubated for 24 h at 28°C and a relative air humidity of 85%. After the incubation period, the Petri dishes were examined, and all exhibited microbial growth. Microscopic observation was then conducted, confirming the presence of yeasts.

After the observation, dilutions were prepared in a nutritive broth with the addition of peptone. The purpose was to determine the CFUs, using dilutions ranging from 10^{-1} to 10^{-6} (Tab. 1). For each dilution tube, three replicates were cultured in YGC medium.

Upon incubation, the plates were examined with the following considerations:

1. Colonies of filamentous fungi. All colonies exhibiting a cottony appearance were counted regardless of their size and color;
2. Typical yeast colonies. Creamy, bright colonies of various colors were considered, as these are characteristic of yeasts (Bressani *et al*., 2018).

Based on the criteria above, the colonies on the plates were counted. Two live repetitions were conducted for the same dilution, as shown in Table 1. It is important to highlight that no identification or verification was performed, since this was solely a quantitative sampling of inoculum presence (Mota *et al.*, 2020).

To calculate the number of CFUs per milliliter when working with liquid culture media in test tubes, the following equation was used (Madigan *et al.*, 2018):

$$N = \frac{\Sigma\, CFU}{V(n_1 + 0.1\; n_2)d} \tag{1}$$

where

$N =$ number of colony-forming units/ml;
$V =$ volume of inoculum applied to each plate in ml;
$n_1 =$ number of plates retained in the first dilution;
$n_2 =$ number of plates retained in the second dilution;
$d =$ dilution number corresponding to the dilution retained.

Absorbance measurement in CFU dilutions

To estimate yeast concentrations in suspension, serial decimal dilutions (1:10) were prepared from liquid cultures previously incubated in YGC medium for 24 h at 28°C with constant agitation. Each dilution was subjected to absorbance measurement using a UV-Vis spectrophotometer at a wavelength of 600 nm, with sterile YGC medium used as the blank. Measurements were performed in quartz cuvettes with a volume of 1 ml, and three replicates were recorded per sample. Subsequently, aliquots of each dilution were plated on solid YGC medium for colony-forming unit (CFU) counting after 48 h of incubation at 28°C. The absorbance values obtained were correlated with CFU counts, allowing for the establishment of regression equations that support the relationship between optical density and microbial concentration, as described by Madigan *et al.* (2018).

Staining yeast with methylene blue

The staining of yeast with methylene blue was conducted under sterile conditions to prevent contamination. A slide was sterilized with 5% hypochlorite, and then a drop of sterile water was added to the slide with a dropper. Each time the spoon was used to transfer the sample from the Petri dish to the slide, it was flamed over high heat for 15 s. A sample of the yeasts to be observed was obtained, collecting a small amount of biomass. This sample was deposited into the drop of water on the slide. Then, it was evenly spread across the entire surface to obtain a single layer of cells, ensuring uniformity in cell distribution when observed under the microscope (Marcos-Zambrano *et al.*, 2013). The spoon was flamed again, as it had already been contaminated with the previous sample. After distributing the sample on the slide, a white texture was observed on the upper part. Subsequently, the slide was dried near the top of the burner, avoiding direct contact, until the moisture evaporated. To fix the sample, the slide was passed through the hottest part of the burner to prevent any other type of molecular mass from interfering with its assembly. Then, the sample was stained with methylene blue. The slide was dried by gently heating it near the top of the burner to remove any remaining moisture from the reagent. Finally, the slide was examined under the microscope at 100x magnification (Freydière *et al.*, 2001).

Microscopic morphological criteria of classification

The identification of yeast organisms, which are part of the normal flora of the skin and mucous membranes of insects, was conducted to determine the presence or absence of these microorganisms. However, the repeated isolation of yeasts from various insect samples suggests a potential infection by the isolated microorganism, requiring identification of the species. Identification was based on morphological criteria. In this study, however, the focus was on the identification of macro- and micro- characteristics (Freydière *et al.*, 2001).

For the identification of yeasts, the following means of identification were employed:

- Germinal tube. The filamentous extension of the yeast is evaluated without narrowing at its origin. Its width is usually half that of the progenitor cell, and its length is three or four times greater than that of the mother cell;
- Formation of hyphae, blastoconidia, chlamydospores, and arthrospores. These morphological characteristics are crucial to identify certain yeast species. When hyphal structures are present, it is important to first determine whether these are pseudohyphae or true hyphae. The former result from the formation of blastoconidia and exhibit regular points of narrowing; the latter fragment into arthroconidia;
- Stains. The microscopic examination of yeasts or related microorganisms (genus *Prototheca*) can be performed using staining techniques, including simple staining or Gram staining. With this technique, yeasts usually behave like Gram-positive organisms.

Test of antagonism and synergism in yeast growth

The *in vitro* evaluation of microbial antagonism was conducted by measuring growth inhibition between microorganisms, using the dual plate tests proposed by Zafra *et al.* (2017).

Results

Following the measurements conducted in the laboratory, CFUs were calculated as detailed in this document. The results indicate the concentrations present in relation to the dilutions applied (Tab. 1).

Correlation between colony-forming units (CFUs) and absorbance in coffee-producing municipalities of Nariño

Figures 1, 2, and 3 illustrate the correlation between yeast concentration, expressed as colony forming units per milliliter (CFU/ml), and optical density (OD) measured at 600 nm in samples collected from three coffee-producing municipalities in the department of Nariño: Arboleda, La Unión, and Buesaco. The positive linear relationships observed in each location support the use of spectrophotometric measurements as a complementary method for estimating microbial density in liquid media. This approach is particularly valuable in studies involving yeast populations, which play a critical role in coffee fermentation processes. The regression equations and R^2 values obtained provide evidence of consistency and reliability in the microbial quantification protocol applied.

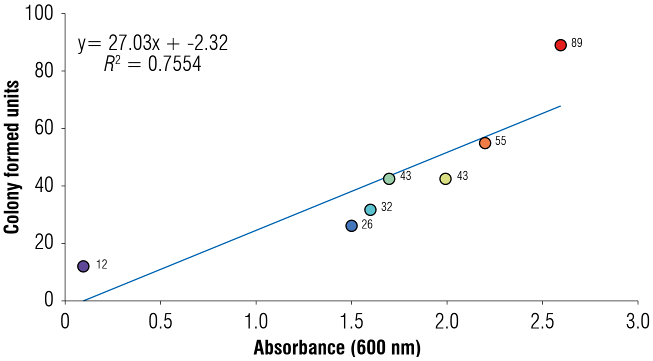

FIGURE 2. Correlation between colony-forming units and absorbance in La Unión.

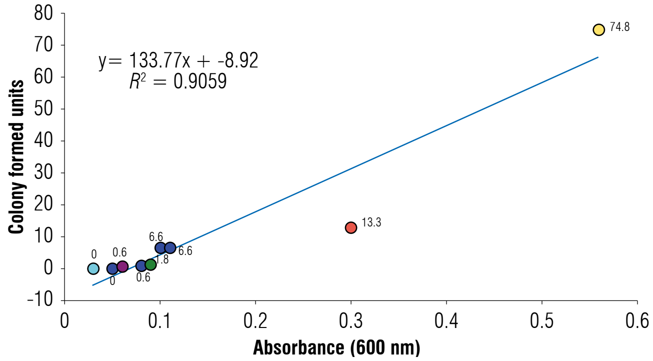

FIGURE 1. Correlation between colony-forming units and absorbance in Arboleda.

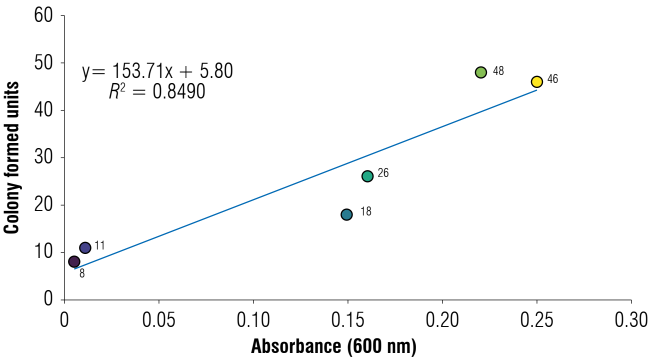

FIGURE 3. Correlation between colony-forming units and absorbance in Buesaco.

TABLE 1. Determination of colony forming units (CFUs) in samples collected from coffee-producing municipalities Arboleda, Buesaco, and La Unión of the department of Nariño.

Dilution	Concentration	Replicate	Filamentous structures	Yeasts structures	Total	Arboleda	Buesaco	La Unión
1	10^1	1	96	76	172	81.90	48	89
		2	77	65	142	67.62	48	88
2	10^2	1	32	24	56	13.33	45	55
		2	34	22	56	13.33	46	55
3	10^3	1	28	18	46	7.30	29	44
		2	20	17	37	5.87	25	43
4	10^4	1	9	3	12	1.43	18	32
		2	10	9	19	2.26	18	33
5	10^5	1	2	5	7	0.67	11	26
		2	2	3	5	0.48	11	26
6	10^6	1	0	0	0	0.00	8	12
		2	0	0	0	0.00	9	10

Test of antagonism and synergism in yeast growth

When performing the culture medium inoculation test with various strains obtained in the laboratory, growth behavior was assessed in the same culture medium. Since insect larvae are common in coffee processing, the yeast Rhodotorula rubra consistently appeared. This yeast was frequently observed in fermentation systems, as previously noted by Reyes Martínez *et al.* (2013). Although their study did not evaluate the distribution of strains across locations, our findings suggest a possible recurrence of similar yeast strains in multiple coffee-processing environments. This finding indicates that this yeast—known to be pathogenic for humans, as it may be the cause of various diseases—is a natural inhabitant of coffee processing environments. Consequently, it needs to be handled with care. The study conducted by Reyes Martínez *et al.* (2013) further revealed that this yeast thrives at a pH value of 4.2, a humidity level of 97%, and environments rich in sugars. Additionally, the highest occurrence of this yeast was observed during fermentation processes between 24 and 48 h.

The genus *Rhodotorula* comprises 34 species, mainly *R. glutinis, R. mucilaginosa*, and *R. minuta*. These red yeast strains are generally healthy, thrive in diverse media, and grow rapidly. *Pseudohyphae* are rare and appear as round or circular cells under the microscope (Jimbo Zapata, 2018). These yeasts produce urease, an enzyme that does not affect food degradation, as reported for *Rhodotorula* species by De Guidi *et al.* (2023). Numerous species of *Rhodotorula* grow in culture media, competing vigorously with other natural inhabitants of fermentation processes, yet without contributing beneficially to such processes. As illustrated in Figure 4, where various strains were cultivated in the same medium, *Rhodotorula* displayed the most extensive spread and inhibited the growth of other yeast colonies, including Saccharomyces, a phenomenon also described by Gomaa (2017) in studies on the antimicrobial activity of *Rhodotorula glutinis.*

FIGURE 4. Results of antagonism and synergism tests between yeast strains isolated from *Drosophila* larvae: white and cream color (*Saccharomyces*), red color (*Rhodotorula*).

The yeast genus examined in this study (*Saccharomyces*) has been associated with sporadic diseases such as endocarditis, meningitis, peritonitis, keratitis, oral ulcers, among others (Tuon & Costa, 2008). According to Reyes Martínez *et al.* (2013), there are no reports of these yeasts being used in biotechnological processes or fermentation. However, they are consistently present in all isolation and purification processes of yeast strains associated with *Drosophila*. Herrera *et al.* (2015) state that these yeasts can be isolated from various natural environments, including air, soil, water, plants, and certain moisture-containing products. These environments are typical of fermentation processes, which may explain the continuous manifestation of this pathogen (Fig. 5). Each microorganism was individually characterized through microscopy to determine its morphophysiological features, as shown in Figure 5. Furthermore, this genus is recognized as an opportunistic fungus and a frequent contaminant in laboratory environments.

A systematic review of *Rhodotorula* infections from the literature revealed that out of 128 cases, 79% were fungemia (103 cases), 7% were ocular infections (9 cases), and 5% (6 cases) were peritoneal dialysis-associated peritonitis. Notably, 87% of *Rhodotorula* infections were associated with underlying immunosuppression or cancer (Tuon & Costa, 2008). This suggests the need for caution when employing this yeast in fermentation processes, as it could serve as a vector for the development of the aforementioned diseases. Furthermore, it was found that *Rhodotorula* was a natural inhabitant in both the larvae and the post-harvest processes of coffee cultivation.

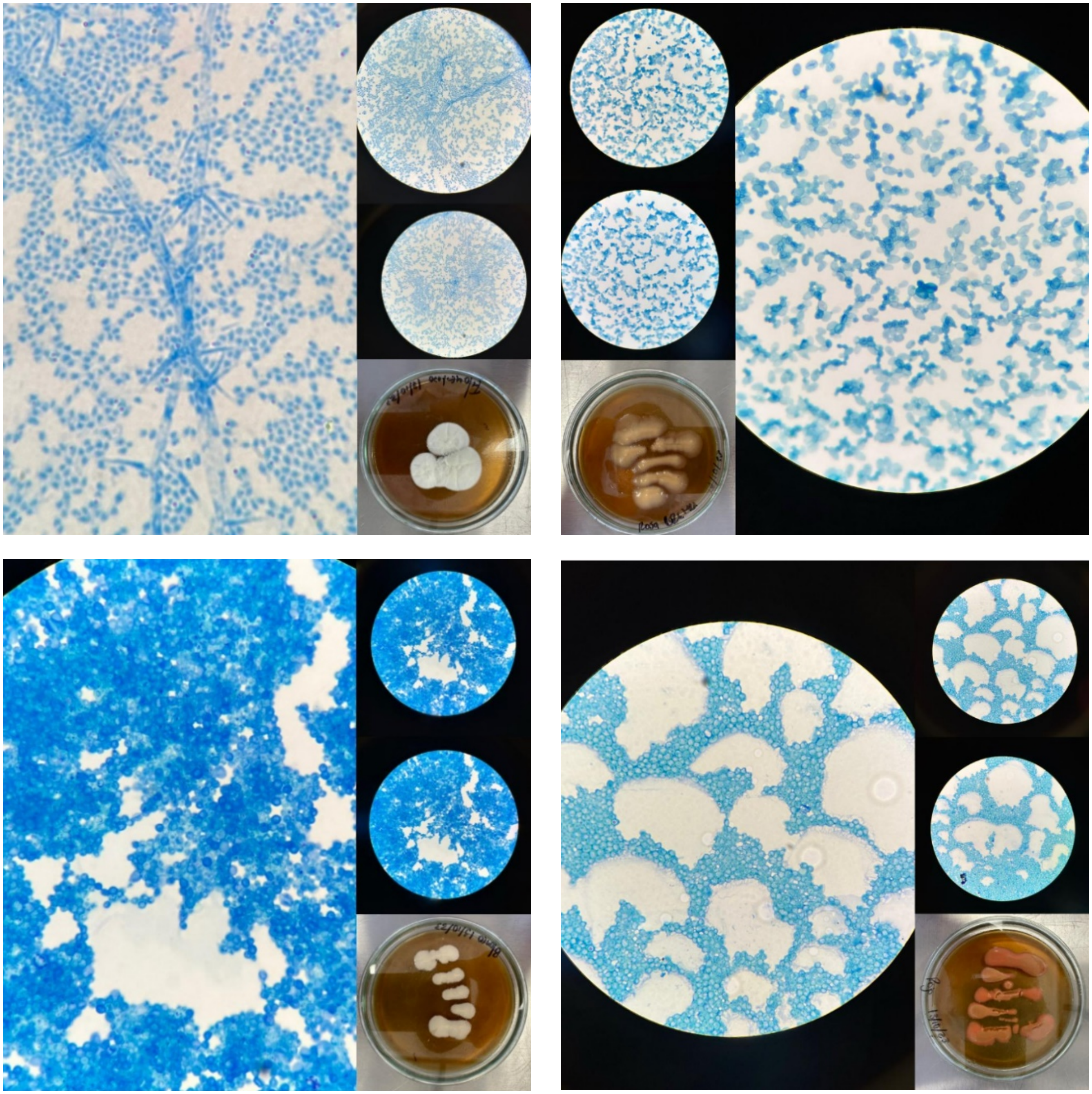

FIGURE 5. Characterization of the isolated strains and the morphophysiological structures observed under a microscope in the different yeast genera.

Discussion

Table 1 illustrates the average colony-forming units (CFUs) across various dilutions. It is important to highlight that the CFUs serve as indicators of the quantity of microorganisms, in this case yeasts, present within a liquid medium (dilution). The values recorded in the columns corresponding to each concentration reflect the number of individual concentrations of cells of this microorganism in liquid medium. These data represent the basis for subsequent analyses related to fermentation hours, which will be conducted in a bioreactor as part of this research project. Considering the coffee post-harvest process, mostly involving liquid fermentation processes, understanding the behavior of these microorganisms under similar conditions is of paramount importance. All observations of the CFUs were evaluated at 12-, 24-, 48- and 96-h post-inoculation in the medium and arranged in Petri dishes for counting. Yeast growth was observed in the Petri dishes as early as the first 12 h, indicating rapid development in the liquid medium when provided with raw material for consumption—in this case, the Sabouraud liquid medium. This medium was essential to determine yeast growth over different time periods, thus allowing for the assessment of yeast concentration and growth, which will indicate its kinetics.

In the context of coffee growing in Nariño – Colombia, there is an evident absence of technological advancements and knowledge regarding the management of coffee processing methods on farms, whether they involve wet or dry techniques, or a mix between the two. This lack of information leads to significant issues, including defects and poor decision-making in the development of protocols for adequate processing of coffee. This situation impacts the quality of the beverage and the final product, resulting in economic losses and missed market opportunities (Federación Nacional de Cafeteros, 2021).

For the reasons explained above, the project must provide the necessary information from a scientific and analytical perspective, using real data to control both the concentration of the inoculum and its kinetics. This approach will enable precise identification of the active hours and conditions under which microorganisms act. With these results, producers in the region can be trained to implement standardized practices that add value to the beverage, meet market demands and increase profitability for local farmers. In this vein, the project offers a clear and reliable alternative for achieving greater control over all the agro-industrial processes involved in coffee bean processing.

Based on the data obtained from the spectrophotometer and cell count observations, a linear regression analysis was performed to compare absorbance against CFUs. This analysis made it possible to determine kinetic parameters, as illustrated in Figures 1, 2, and 3. An analysis of growth over time was subsequently performed, involving the calculation of generation number and doubling time, following the methodology explained by Ortiz (2012). The results concerning CFU growth aligned with those reported by Chalón *et al.* (2013), who examined the growth of yeast strains in media containing glucose and peptone in a specific medium (YGC). They concluded that these substrates offer favorable conditions for yeast growth. In addition, in the present study, various dilutions up to 10^8 were compared using *Saccharomyces cerevisiae* strains, revealing significantly lower values after concentrations of 10^5. Therefore, the results of this trial corroborate the findings reported by recent studies on microbial dynamics in fermentation systems, particularly regarding growth limitations due to substrate availability and inoculum size. Ioannou *et al.* (2019) and Bruyn *et al.* (2017) have described how microbial interactions and initial biomass concentrations significantly influence fermentation efficiency and microbial succession, supporting the importance of controlling these variables in post-harvest coffee processing. Consequently, in the development of fermentation processes involving microorganisms, the substrate represents a limiting factor for growth. The amount of biomass and initial inoculum must be proportional, as these factors influence the entire action of the fermentation process.

The objective of this study was to assess the yeast species linked to *D. melanogaster* larvae in post-harvest coffee processing, to elucidate their contribution to the fermentation dynamics of coffee cherries (Figs. 1-3). It is essential to determine the concentration and dilutions at which the yeasts remain viable in order to set up subsequent experiments in the bioreactors. This approach is supported by Puerta *et al.* (2012), who demonstrated that adding 60 ml of *Saccharomyces* sp. yeast resulted in enhanced fermentation processes and improved quality during sensory evaluation, reducing fermentation time. In their research, it was observed that a 12-h exposure to the microorganism was sufficient to generate enhanced attributes. It is worth highlighting that in certain fungi, the CFU count depends on three main factors: the handling of coffee fruits during harvesting, the conditions under which the cherries are kept throughout the fermentation process, and the antagonism that occurs between microorganisms when trying to proliferate within a limited growth medium.

Certain microscopic characteristics have proved to be useful for the identification of some species. Among the various identification characteristics, the following stand out:

- Germinal tube. This method involves assessing the filamentous extension of the yeast, without narrowing at its origin. Typically, the width is half that of the progenitor cell, and its length is three or four times greater than that of the mother cell. Only certain genera, such as *C. albicans*, are capable of producing true germ tubes. However, other species such as *C. tropicalis* have been reported to produce precocious *pseudohyphae*. These *pseudohyphae* resemble germ tubes but stand out by a characteristic halo and a constriction zone adjacent to the mother cell. This test is useful for differentiating *C. albicans* from other *Candida* spp., as demonstrated in the germ tube test by Moya-Salazar and Rojas (2018);

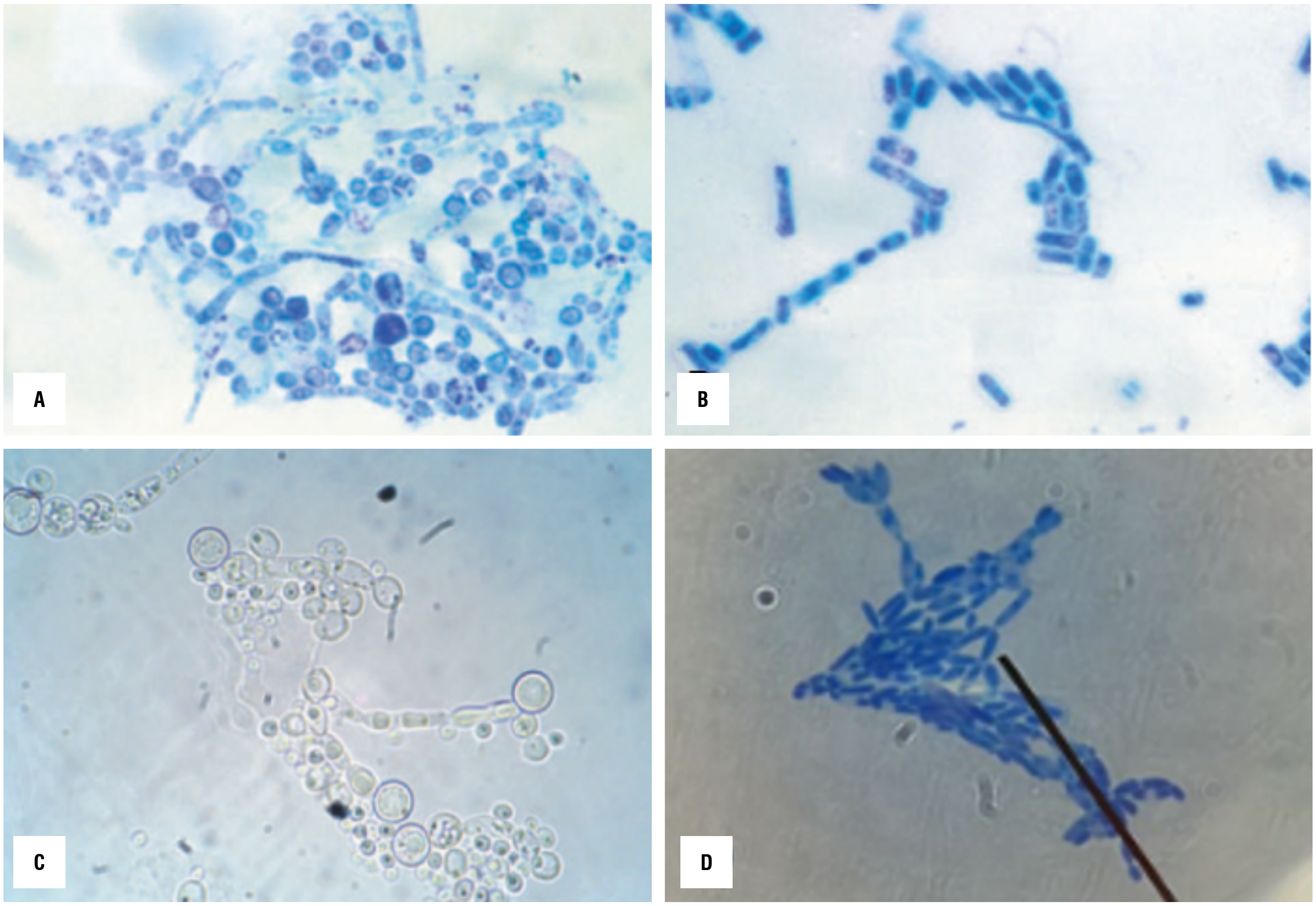

FIGURE 6 (A–D). Morphological diversity of fungal structures observed under light microscopy.

- Formation of Hyphae, Blastoconidia, Chlamydospores, and Arthrospores. The genera *Trichosporon* and *Blastoschizomyces* are characterized by the production of both *Arthroconidia* and *Blastoconidia*. The latter grow from shoots at the angles of the Arthroconidia, acquiring the characteristic rabbit ear shape (Figs. 6A-B). The species *Galactomyces geotrichum* (*G. candidum*) and *Blastoschizomyces capitatus* (*Geotrichum capitatus*) also produce blastoconidia from the angles of the arthroconidia, but in this case, they form a structure known as "hockey stick" (Fig. 6C). Chlamydospores are round or oval, 6-12 µm in diameter, have thick walls, and appear as terminal spores. Chlamydospores are characteristic and diagnostic of *C. albicans* (Fig. 6D), (Navarathna *et al.*, 2016);
- Stains. Methylene blue stain was used to differentiate fungal structures, which were subsequently observed under the microscope, as illustrated in Figure 5. This staining technique yielded positive results when comparing the findings reported by Navarathna *et al.* (2016) and Kauffman *et al.* (2011) (Figs. 6A and 6C) with those obtained in this research (Figs. 6B and 6D). Based on morphological characteristics and staining results, it is possible to affirm that the isolated microorganisms are indeed yeasts.

In the laboratory, most yeasts grow easily across various culture media. However, the YGC medium appeared to be particularly conducive to their development due to its greater specificity. Typically, the majority of yeast colonies are slightly oval or flat with a buttery consistency that can be smooth or rough. Generally, it is uncommon for yeast colonies to develop mycelium. However, there have been cases in which spider-shaped projections can be seen on the periphery of fungal rings. Most white and creamy colonies are identified as yeasts from the genus *Prototheca*, a group of achlorophyllic algae, which develop at an incubation temperature of 28°C (Satoh *et al.*, 2010). That was the case in this research, in which white and creamy colonies, resembling those of the genus *Candida,* were observed.

The identification of the structure of microorganisms in fermentation processes based on morphological characteristics has its limitations. Therefore, it is necessary to characterize their metabolic and molecular profiles for proper classification (Hood, 2013). The characterization of microbiomes in specific environments requires a molecular diagnosis that involves the isolation and extraction of genetic material for sequencing and further bioinformatic analysis (Behjati & Tarpey, 2013). This methodology facilitates the creation of a taxonomic classification of the individuals present in such habitat and their relative abundance within the population (Berg *et al*., 2020).

This study demonstrated that *D. melanogaster* larvae present in coffee post-harvest processes act as vectors of various fermentative yeast species, including *Geotrichum, Galactomyces, Trichosporon, Blastoschizomyces*, and *Rhodotorula*. Through isolation techniques, staining, cultivation on YGC media, and colony-forming unit (CFU) counts, the kinetic and morphophysiological behavior of the strains found in the larvae's oral apparatus and digestive tract was characterized, revealing rapid colonization in liquid media within the first 12 h. Regression analyses between CFUs and absorbance enabled the determination of kinetic parameters essential for designing controlled fermentation processes. Furthermore, in vitro antagonism and synergism assays showed that certain yeasts, especially *Rhodotorula rubra,* can inhibit the growth of other species, which is critical when optimizing microbial interactions in fermentation. These findings highlight the biotechnological potential of these microorganisms to enhance coffee bean quality and the importance of implementing robust morphological and molecular classification methods to ensure their safe and effective application. This research lays the groundwork for future studies aimed at regulating fermentative microbiomes in coffee-growing environments to increase the added value of the final product.

Conflict of interest statement

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

Author´s contributions

JJAM: writing, methodology testing, and project development; AJCF and JGSG: style correction and content revision. All authors approved the final version of the manuscript.

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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.

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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 (,).

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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.

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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.

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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).

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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.

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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.

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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.

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This section should briefly and concisely summarize the most important findings of the research.

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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.

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Published dissertation or thesis references

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Whole book

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P.O. Box 14490, Bogotá-Colombia
(571) 316 5355 / (571) 316 5000 ext. 10265
E-mail: agrocol_fabog@unal.edu.co
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